

LETTER

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Study on micro fabricated stainless steel surface to anti-biofouling using electrochemical fabrication

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Abstract

Biofilm formed on the surface of the object by the microorganism resulting in fouling organisms. This has led to many problems in daily life, medicine, health and industrial community. In this study, we tried to prevent biofilm formation on the stainless steel (SS304) sheet surface with micro fabricated structure. After then forming the microscale colloid patterns on the surface of stainless steel by using an electrochemical etching forming a pattern by using a FeCl_3 etching was further increase the surface roughness. Culturing the *Pseudomonas aeruginosa* on the stainless steel fabricated with a micro structure on the surface was observed a relationship between the surface roughness and the biological fouling of the micro structure. As a result, the stainless steel surface with a micro structure was confirmed to be the biological fouling occurs less. We expect to be able to solve the problems caused by biological fouling in various fields such as medicine, engineering, using this research.

Keywords: Anti-biofouling, Micro pattern, Stainless, Electrochemical

Background

Biofilm is formed in a thin film form on a microorganism. This is three-dimensional structure formed in a self-secreting oligomer substrate (polymeric matrix) on a various surface. Biofilm by the microorganism can be formed from almost any type of tissue of the solid surface and the living organisms [1]. In particular, the biofilm formed in water pipes, water purifiers and water quality monitoring sensors can give damage to the industry and daily life. Biofilm is difficult to remove, it is strongly attached to the surface, it continues to release the microorganism from the surface [1, 2]. Biofilm will cause a very large problem in public health because it acts as a repository for microorganisms. Biofilm formed in the detection section of the sensor requiring high sensitivity and high accuracy degrades the detection performance of the sensor.

Biofilm formation prevention or removal methods because of these problems has been developed. Up to date, Biofilm prevention coating or removal method has a

problem that affects not only biofilm but also a device or surface. Physical methods like sand-blasting for removing biofilm on vessel surface or instrument surface require constant management by thinning the thickness of protective coating such as paint on the surface. On the other hand, Wrinkle-like micropatterns formed on the skin surface of the whale or on the shells of many shellfishes and leaves of lotus are effective in preventing the biofilm formation that easily occurs in the underwater environment [3–8].

Microstructure was formed using an electrochemical etching (ECF) and FeCl_3 etching solution on the surface of stainless steel (SS304) which is widely used in medical, industrial purpose [9–11]. After the microstructures formed on the surface, *Pseudomonas aeruginosa* Pa14 were cultured and evaluate biofilm formation tendency stained with crystal violet dye by gram staining.

Methods

Fabrication of micro structure

Stainless steel microstructure was fabricated. 6-in. stainless steel (type 304 ss) was used for this study (with a thickness of 100 μm , horizontal 9 cm, vertical 11 cm). The microstructures were prepared by photolithography

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and ECF, and then wet etching with FeCl_3 solution to form smaller random pattern. The fabrication process is shown in Fig. 1. In the fabrication process, the stainless steel wafer prepared in the first step was cleaned with acetone for 20 min using an ultrasonic washing machine. Next, HMDS (Hexamethyldisilazane) was spin-coated on a stainless steel wafer, and AZ-1512 photo-resist was spin-coated to a thickness of 1–2 μm . The coated photoresist was subjected to a soft bake on a hot plate at 120 $^\circ\text{C}$ for 2 min, followed by an exposure process and a development process under appropriate conditions. Thereafter, a hard bake process was performed at 160 $^\circ\text{C}$ for 20 min. Next, a microstructure with a depth of 10 μm was formed through an ECF process. A bath for electrolytic solution which has a capacity of 2-l was prepared for the ECF process and was designed to automatically circulate the electrolyte. The electrolytic solution was prepared by mixing sulfuric acid (H_2SO_4 , 97%), phosphoric acid (H_3PO_4 , 50%) and DI water. Volume ratio of H_2SO_4 , H_3PO_4 and DI water is 30:60:10.

The process conditions for etching was at 0.1 mA for 20 min (Fig. 2). After the etching process, the photoresist was removed and the surface roughness was increased by FeCl_3 solution at 80 $^\circ\text{C}$ for 15 s wet etching process

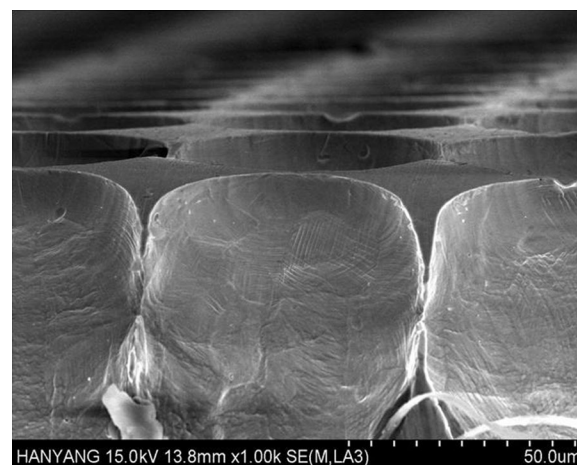
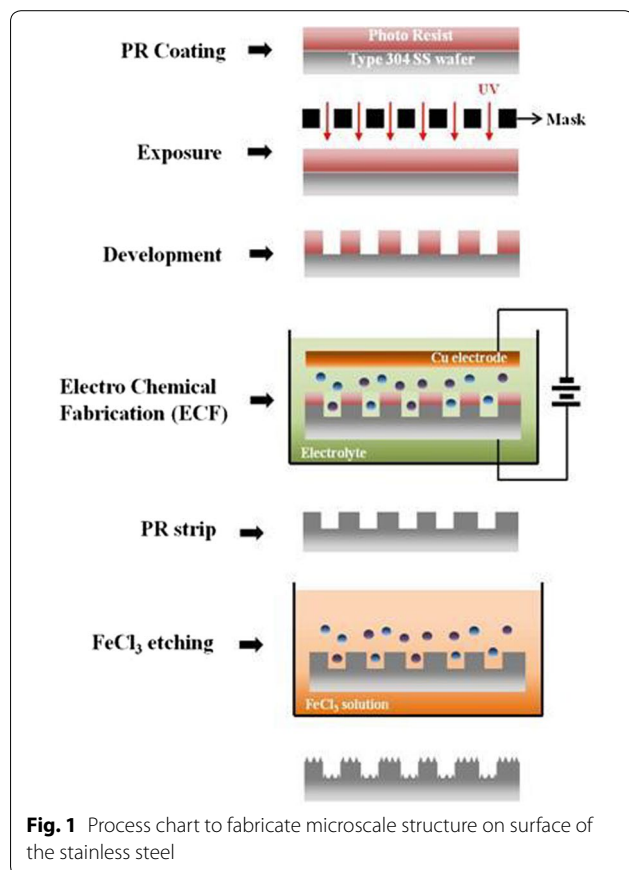


Fig. 2 Fabricated microscale structure using ECF

(Fig. 3). After the process was completed, ultrasonic washing was performed for 20 min with acetone to prevent contamination.

Surface properties of microscale structure formed surface

The fabricated microstructures are shown in Table 1. The microstructures were circular pore structures with a width of 20 μm and a depth of 10 μm , and the spacing between structures was 30 μm . The patterned stainless steel surface after ECF etching showed a contact angle of 74.5°. A few micrometer sized pores were then formed using random etching through FeCl_3 solution to form additional patterns of smaller size. The degree of random pattern formation was controlled by adjusting the etching time using FeCl_3 . The contact angle of the etched surface and the surface roughness were measured using an optical three-dimensional surface meter. As the etching time was longer, the surface with more random patterns increased the surface roughness, and the contact angle

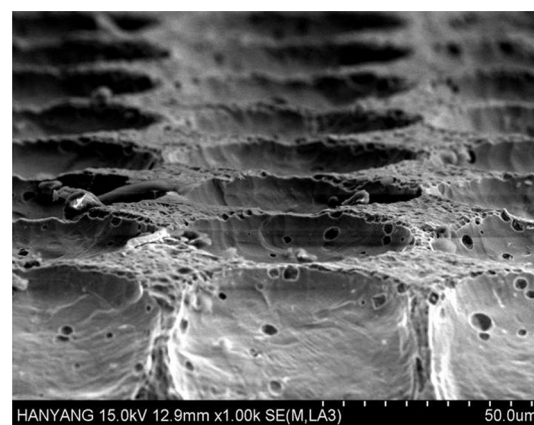
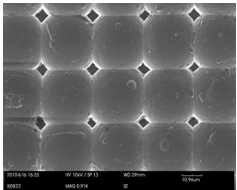

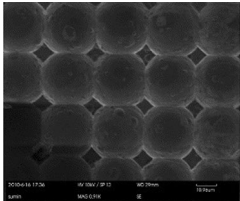

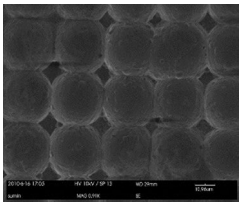



Fig. 3 Microstructures formed by etching FeCl_3 after ECF

Table 1 FeCl₃ etching time of the contact angle and surface roughness

FeCl ₃ etching (min)	SEM image	Wettability	Contact angle (°)	Roughness, Ra (nm)
1			74.2	98
3			49.8	142
5			22.6	178

became lower, indicating hydrophilicity. It is considered that the micro-sized pores formed by FeCl₃ etching increase the non-uniformity on the surface, which causes the surface tension on the water droplet and stainless steel interface to be lowered, resulting in a wetting phenomenon due to a lower contact angle.

Microbial culture in a stainless steel surface

Pseudomonas aeruginosa PA14 was cultured in stainless steel with microstructures and stainless steel without microstructures in order to investigate the presence of microstructures and biofilm formation tendency. Two experiments were conducted under the same conditions to see if the culture results had the same tendency. The biofilm formation was carried out using the P63 strain of *Pseudomonas aeruginosa* and the M63 minimal medium [M63 salt 12 g/l KH₂PO₄, 28 g/l K₂HPO₄, 8 g/l (NH₄) SO₄, 1 mM MgSO₄, 0.5%] 150 µl of M63 liquid medium was dispensed into two 12-well plates, and the seed culture of the clinical cultures cultured for 24 h was inoculated at 2% and cultured at 30 °C for 72 h. The plate was inverted and the culture solution was discarded, washed twice with water, and then added with 180 µl of 0.1% crystal violet solution as shown in Fig. 4. After 200 µl of absolute ethanol was added to the plate, the plate was flicked for 30 min to dissolve the crystal violet, and the absorbance was measured at 600 nm. In order to compensate for the error due to the amount of cells, this absorbance was

divided by the optical density value measured previously, and the degree of biofilm formation was suggested.

Results

Biofilm formation tendency of the microstructure fabricated surface

Biofilm formation on the surface is compared between fabricated microstructure surface of the stainless wafer and bare stainless wafer. Based on the biofilm formed on the bare stainless wafer, crystal violet was measured biofilm

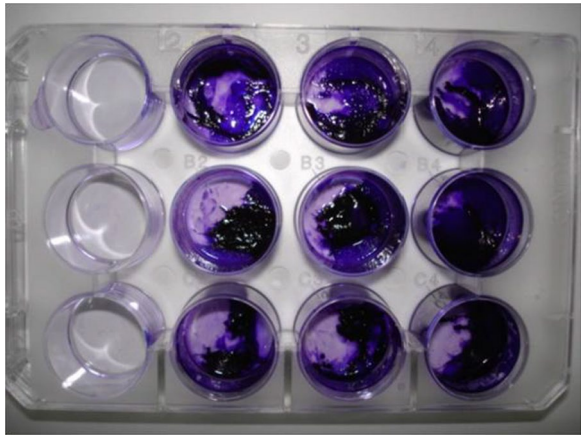
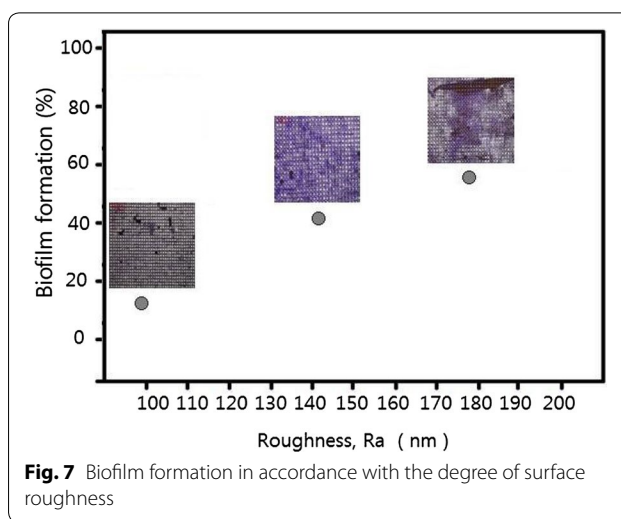
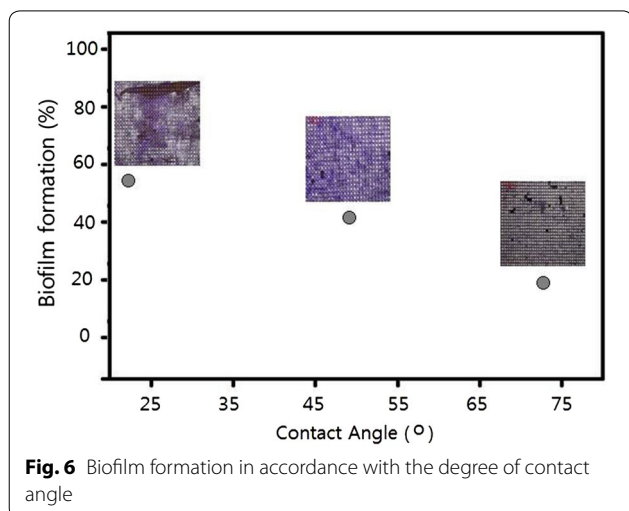
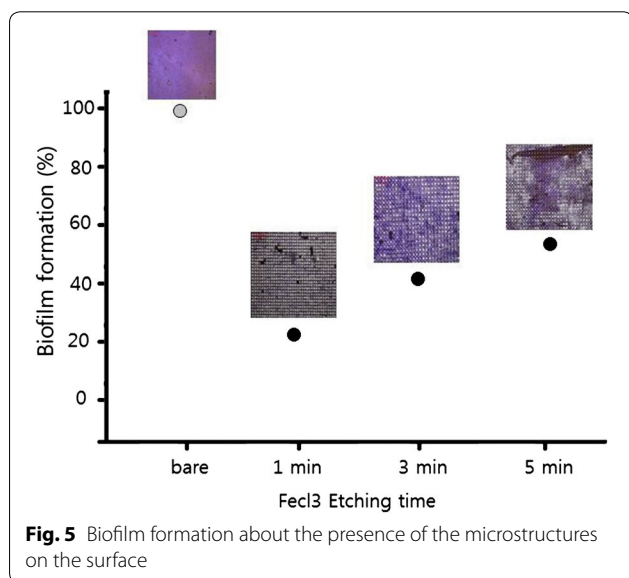


Fig. 4 Gram stained stainless steel wafer after culturing the microorganism

formation degree relatively. Gray dot is the sample without microstructure and Black dots are the surface samples with microstructure at Fig. 5. Relative to the microstructure fabricated surface shows that the formation of biofilm less.

Biofilm formation tendency of the roughness and the contact angle of the surface

After the microstructure formation, the contact angle and the surface roughness of the surface can be controlled by controlling the etching time of FeCl_3 . As shown in Fig. 6, by observing the tendency to form biofilm in accordance with the change in the contact angle, It is observed that the biofilm formation is inhibited at high contact angle. Conversely, Fig. 7 shows that smaller the surface roughness affect that the biofilm formation is inhibited.



Discussion

In this study, after the microstructure of the stainless steel fabrication, biofilm formation was analyzed in accordance with the contact angle and the surface roughness changes. Microstructure was formed by using the photolithography and etching method for the electrochemical. The contact angle and the surface roughness was adjusted using FeCl_3 solution through the etching process.

The biofilm formation on the stainless steel surface, on which pore-type microstructures were formed through ECF, was considerably lower than that on the stainless steel surface without patterning. However, it can be seen that the formation of smaller pores on the surface by increasing the FeCl_3 etching treatment time tends to increase the formation of biofilm again. This results in the formation of a smaller pore pattern on the surface, which increases the roughness of the surface and increases the hydrophilicity of the interface between the surface and the culture fluid. An environment that can be easily attached to the surface due to increased hydrophilicity promotes biofilm formation, with some structures appearing to play the same role as a framework of thicker biofilm formation.

Research on biofilm formation control has been continuously carried out to clarify the mechanism of biofilm formation. The difference in biofilm formation depending on the interface between liquid and surface is expected to be applied to the future research on pollution prevention surface and high cultured media for bacteria. It could be used to reduce the damage caused by fouling organisms.

Authors' contributions

BJ carried out the experiment and drafted the manuscript. SH participated in the design of the study and performed the analysis. BJ and SH conceived of the study, and participated in its design and coordination. Both authors read and approved the final manuscript.

Competing interests

The authors declare that they have no competing interests.

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