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Evaluation of biofouling in stainless microfluidic channels for implantable multilayered dialysis device

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An implantable artificial kidney can markedly improve the quality of life of renal disease patients. Our group has developed an implantable multilayered dialysis system consisting of microfluidic channels and dialysis membranes. Long-term evaluation is necessary for implant devices where biofouling is a critical factor, culminating in the deterioration of dialysis performance. Our previous work revealed that surface conditions, which depend on the manufacturing process, determine the amount of biofouling, and that electrolytic etching is the most suitable technique for forming a channel wall free of biofouling. In this study, we investigated the electrolytic etching conditions in detail. We conducted in vitro experiments for 7 d and evaluated the adhesion of biomaterials by scanning electron microscopy. The experiments revealed that a surface mirror-finished by electrolytic etching effectively prevents biofouling. © 2017 The Japan Society of Applied Physics

1. Introduction

The kidney plays a significant role in maintaining our health. It adjusts the ionic concentration of electrolytes, maintains essential proteins and nutrition, and removes internal wastes from our blood.1-3 When we lose these functions, we suffer from renal diseases, and end-stage patients must receive dialysis treatment or transplant. Dialysis treatment is well developed and highly reliable. However, patients must visit hospitals every two days and stay there for 4 h, which limits the patients’ daily activities. Dialysis treatment for 12 h a week is rather intensive compared with 24 h of 7 d dialysis conducted by healthy kidneys.2

Implantable dialysis systems can solve these problems and improve the patients’ quality of life.3-6 Our group has been developing an implantable dialysis device that is composed of microfluidic channels and nanoporous dialysis membranes.7-9 The dialysis membranes selectively allow low-molecular-weight molecules, such as sodium, potassium, urea, and creatinine, to be filtered from blood while maintaining high-molecular-weight molecules, such as albumin and red blood cells, in blood, as shown in Fig. 1.10 The device is designed to work under blood pressure and not to use dialysate, which leads to the miniaturization of the dialysis system since a storage tank with a pump for the dialysate is not necessary. The dialysis system with poly(ether sulfone) (PES) membranes, whose formation process is described in Fig. 1, successfully suppresses the increase in the creatinine level of a rat by 92.6% for 5 h in in vivo experiments.8 For practical applications, it is

Fig. 1. (Color online) Implantable micro-dialysis device and its filtration phenomena. Method and process used to form the dialysis membrane.
necessary for this device to maintain the performance for a long time, even years. We conducted 24 d in vitro experiments to evaluate the long-term performance of a PES membrane.11) The dialysis performance decreased by one third after 7 d before it plateaued. This decrease is considered to be caused by biofouling on the surface of the membrane. Biofouling processes, such as thrombus, coagulation, and protein adhesion, cause the decline in the water permeability of the PES membrane, the clogging of the channels, and, in the worst-case scenario, the formation and release of thrombus, which may lead to cerebral infarction.12–14)

Biofouling occurs not only on the surface of a nanoporous membrane but also on the sidewall of the channels. Thus, it is essential to investigate the clogging property of the flow path with respect to the channel geometry that determines blood flow. The surface conditions are also considered to affect biofouling. We conducted preliminary in vitro experiments using microfluidic channels made of SUS316L, which is highly biocompatible and is practically used as medical materials.15) It was experimentally revealed that the channels should not have sharp turns with small radii of curvature, which caused the stagnation of blood and the highly likely adhesion of biomaterials on the wall surface. Surface roughness was found to affect biofouling, where the channels processed by electrical discharge machining showed large surface roughnesses and severe biofouling even with post finishing with a mechanical grinder. The surface treatment by electrolytic etching could successfully prevent biofouling, which may originate from the small surface roughness and/or the formed passive state layer.

In this study, we conduct in vitro experiments for 7 d to verify the efficiency of electrolytic etching. We prepare two samples by electrolytic etching under different process conditions and, therefore, under different surface roughness. We observed the growth of a biofilm on a surface by scanning electron microscopy (SEM) after 3, 5, and 7 d, and extract the dominant parameter for biofouling.

2. Materials and methods

2.1 PES membrane

Our dialysis membrane is composed of PES (Sumitomo Chemical Grade 4800P), poly(ethylene glycol) (PEG; molecular weight of 1000, Wako Pure Chemical Industries), and N,N-dimethylacetamide (DMAc; Wako Pure Chemical Industries). The casting solution is a blend of PES (16.0 g), PEG (13.3 g), and DMAc (62.1 g), and is stored at room temperature after mixing to become transparent.11,16–20) Subsequently, to obtain a flat membrane with a thickness of 100 µm, the casting solution is spin-coated at 300 rpm on a glass substrate and soaked in deionized water to remove additives. The PES membrane is gelatinized, forming a nanoporous membrane. The membrane forming process is illustrated in detail in Fig. 1.

2.2 Microfluidic channel process and etching

The device consists of dialysis membranes and microfluidic channels made of the biocompatible metal SUS316L (Nilaco) with a thickness of 200 µm. The channels are wound to have a sufficient filtration area and prevent the soft membrane from deformation. From the result of a previous work,15) the channels have a radius of curvature of 2.5 mm, as shown in Fig. 2.

Fig. 2. (Color online) Design of microfluidic channel (radius of curvature: 2.5 mm) and preliminary research design (radii of curvature: 0 and 1.0 mm).15)

The channels are first formed by wire electrolyte discharge machining (wire-EDM). Second, the work surface is polished by electrolytic etching, as shown in Fig. 3. Wire-EDM creates altered layers on the surfaces and large surface roughnesses.21,22) Electrolytic etching produces mirror-polished surfaces of SUS316L. During the etching, convex parts are preferentially dissolved, resulting in small surface roughnesses.23,24) In addition, a passive layer is formed on
the processed surface. Ethylene glycol (300 mL; Wako Pure Chemical Industries), pure water (300 ml), sodium chloride (60 g; Taisei Chemical) and citric acid monohydrate (40 g; Wako Pure Chemical Industries) are used as the electrolyte. A masking layer is placed on the material surface with a tape and cut along the channel shape; only the sidewall surface is etched for 30 s. The voltage determines the surface properties. Figure 4 shows SEM images of the (a) nonprocessed side wall and the side walls processed by electrolytic etching at (b) 7–10, (c) 15–20, and (d) 20–22 V. The electrolytic etching forms on etching pit where the low voltage results in a large surface roughness.

2.3 Experimental methods

The metal layers and dialysis membranes are stacked to form a six-layered device. In this experiment, we compare samples (c) and (d) to evaluate protein adhesion. Samples (a) and (b) have large roughnesses enough to cause poor adhesion (Fig. 4). As shown in Fig. 5, a peristaltic pump is used to circulate human whole blood (Kohjin Bio A blood type) at 130 mmHg. The temperature of the blood is controlled to be 309 K, while the blood is stirred using a magnetic stirrer. The experiments are continued for 7 d, during which the blood is replaced every 2 d to prevent the deterioration of the blood caused by hemolysis and contact with air. The surface is evaluated after 3, 5, and 7 d to observe the growth of biofouling.

2.4 Evaluation methods

We investigate the roughnesses of the processed and polished surfaces using a laser microscope (Keyence VK-X 100) with respect to the calculated average roughness $R_a$ and the maximum height $R_z$ in sampling areas of 50 and 10 µm². We follow JIS B 0601 2001 in the measurement.

The formation of biomaterials on the surface is observed by SEM according to ISO 10993-1 and JIS T 0993-1, the regulations concerning the development of medical devices. After the in vitro experiment, we conduct cell fixation immediately using the following the steps: (1) washing with phosphate-buffered saline (PBS; Wako Pure Chemical Industries), (2) first fixation with glutaraldehyde (GA; Wako Pure Chemical Industries) aqueous solution (GA: 2.5%; PBS: 50%; pure water: 47.5%) for 1 d, (3) second fixation with GA aqueous solution (GA: 100%) for 1 h, (4) washing with ethanol aqueous solution (Wako Pure Chemical Industries; 20, 40, 60, 80, and 100%) for 15 min each, and (5) coating with osmium (thickness of 3 nm).

To evaluate the adhesion quantitatively, the cell adhesion area is derived from the SEM images using Adobe Photoshop. Ten different points in the sampling area are investigated and the average, maximum, and minimum values are plotted.

3. Results

3.1 Surface roughness

Table I shows the $R_a$ and $R_z$ values of the surfaces processed by wire-EDM, mechanical etching and electrolytic etching at different voltages. By comparing the sampling areas of, 50 × 50 and 10 × 10 µm², those processes were found to reduce both the small jagged and large unevenness values.

![Fig. 4.](Color online) SEM images of work surface before treatment (a) and surfaces polished by electrolytic etching: (b) 7–15, (c) 15–20, and (d) 20–22 V.

![Fig. 5.](Color online) Blood loop system for in vitro experiment and device construction.

<table>
<thead>
<tr>
<th>Measurement area (µm²)</th>
<th>EDM</th>
<th>Etching (15–20 V)</th>
<th>Etching (20–22 V)</th>
</tr>
</thead>
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<tr>
<td>50 × 50</td>
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<td>$&lt;1$</td>
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<td></td>
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<td></td>
<td>$R_z$4&lt;</td>
<td>$10&lt;</td>
<td>$&lt;1.5$</td>
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</tbody>
</table>

![Table I.](Roughnesses (in µm) of work surfaces before and after polishing ($R_a$ and $R_z$).)

![Fig. 6(a).](Color online) SEM images of preliminary research results indicating the relationship between surface roughness and protein adhesion.

3.2 SEM analysis of the biofouling

Figure 6(a) shows SEM images of the preliminary research results indicating the relationship between surface roughness
and biofouling. The images reveal that surface roughness and radius of curvature affect biofouling. Thus, we selected only the polished channel with a high voltage.15)

Figure 6(b) shows SEM images of the surfaces processed by wire-EDM before and 3, 5, and 7 d after the in vitro experiments. The surfaces treated by electrolytic etching at low voltages were found to be covered by biomaterials more than those treated by electrolytic etching at high voltages. According to previous works, electrolytic etching at a high voltage forms a mirror-polished passive layer on a surface.25,31) The surface roughness decreases with the voltage. Although the passive layer was considered to be formed in both cases, the surfaces etched at a voltage higher than 20 V exhibited a small adhesion. However, a small adhesion was also observed on the low specular surface. Therefore, the surface roughness is more dominant than the formation of the passive layer in biofouling.

Figure 7 quantitatively shows the expansion of biofouling in the adhesion area. It is clearly observed that a completely mirror-polished surface can prevent protein adhesion in comparison with a low specular surface. The adhesion markedly increased between 3 and 5 d.

4. Discussion
There are many reports on the voltage and surface roughness of electrolytic etching.23–25) Since the current greatly changes even at the same voltage depending on the distance between the electrodes and the exposed area of etched materials, it was necessary to investigate the etching time and voltage in this research. In addition, a passive layer can improve the corrosion resistance by preventing the elution of metal ions and is more highly biocompatible than the other metals used in medical equipment.32)

In our previous research, it has been confirmed that thrombus adherence to our microfluidic channel is very large when the flow velocity drops markedly and when the stagnation point is formed.15) Generally, as surface roughness increases, cells clearly tend to adhere: However, proliferation becomes difficult.33) Thrombus formation could be considerably reduced in this in vitro experiment, although the detailed mechanisms of thrombus formation and blood clotting have not been elucidated, and thus further long-term in vitro and in vivo experiments are required.

5. Conclusions
We experimentally revealed that the postfinishing of microchannel walls by electrolytic etching at voltages higher than 20 V could effectively prevent biofouling. Surface roughness was found to be more dominant than the formation of the passive layer of SUS316L. Through this work, we believe that we could determine the requirements in terms of wall surface conditions for medical devices that are in contact with blood for a long period.
Acknowledgment
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