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Exploring the Anti-Biofilm Properties of Austenitic Stainless Steel: A Study on Material Surface Characteristics and Biofilm Resistance via Selective Laser Sintering

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INTRODUCTION

Biofilms, structured communities of bacteria that adhere to and proliferate on medical surfaces, can become sanctuaries for bacteria against antibiotics, mainly when formed on implants and medical devices. This often leads to chronic infections and hospital-acquired infections, making the development of effective countermeasures critical. Given that biofilms result from the interaction between bacteria and material surfaces, manipulating material properties could offer a viable strategy for mitigation. This study investigates the anti-biofilm properties of austenitic stainless steel produced with varying grain sizes using Selective Laser Sintering (SLS). By examining the relationship between surface characteristics of the materials and their resistance to biofilm formation, the research aims to identify key material attributes that inhibit biofilm development, potentially leading to the design of more hygienic medical devices.

EXPERIMENTAL

Sample Preparation:

Austenitic stainless steel (SUS 316) was selected as the test material for evaluating anti-biofilm properties. The samples were fabricated using Selective Laser Sintering (SLS) with varying grain sizes. The SLS process was carried out using a ProX200 machine from 3D Systems, equipped with a fiber laser with a maximum output of 300 W. The powder used was a combination of water atomized and gas atomized powders. The detailed conditions for laser processing were as follows: a scanning pitch of 0.1 mm, a layer thickness of 0.03 mm, laser output ranging from 60 W to 250 W, and a scanning speed between 380 mm/s and 2700 mm/s in a nitrogen atmosphere with a residual oxygen concentration of about 0.5%. The sample geometry was 10 mm × 10 mm × 10 mm, ensuring consistent material properties across the samples. The surface roughness of the fabricated SUS

316 steel was measured with a stylus surface profilometer (Bruker Nano Dektak XT-A). The contact profile measuring method has a tactile pressure accuracy range of 0.03-15 mg, a maximum scan length of 55 mm, a step measurement repeatability of 0.5 nm and a height measurement range of Max. 1 mm.

Biofilm Evaluation:

The anti-biofilm properties of the prepared SUS 316 samples were evaluated according to the ISO 4768:2023 standard method. Biofilm formation was induced using the bacterial strain *Staphylococcus epidermidis*. The bacteria were pre-cultured in a 1/5 Tryptic Soy Broth (TSB) medium, and the culture was adjusted to a concentration of 10^3 CFU/mL. The specimens were then immersed in the bacterial suspension for 48 hours for biofilm formation. After incubation, the biofilm formed on the test specimens was stained with 0.1% crystal violet solution. Subsequently, the stained biofilm was solubilized using sodium dodecyl sulfate (SDS) solution, and the absorbance was measured at 590 nm. Crystal violet is an electrolyte composed of a triphenylmethane group cation and a chloride anion; the cation binds to the negatively ionized part of the macromolecule and gives off a blue-violet color. This method uses its oversized absorption of around 590 nm to evaluate the colored areas quantitatively. The higher the absorbance, the more likely biofilms are formed. The grain size was varied by changing the scanning speed; Fig. 1 shows the apparatus's appearance and the sample's appearance.

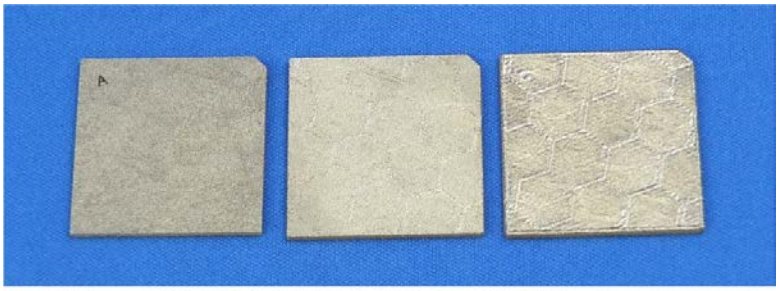

Fig1. Appearances of the apparatus and specimens.

Specimen A→Specimen B → Specimen C: coarsely to densely

RESULTS AND DISCUSSION

Fig.2 shows the surface roughness of specimens. As already mentioned, surface roughness and density were performed by varying the scanning speed. Specimen A, with the roughest surface, was produced at 2700 mm/s, Specimen B at 1400 mm/s and Specimen C at 760 mm/s.

Biofilm evaluation tests were conducted on these samples. All experiments were performed at N = 3 and absorbance was measured using the prescribed method. The results are shown in Fig. 3. The results show the average value for each sample. More biofilm was formed in the coarser sample A. Biofilm formation was suppressed in sample B, which had a denser surface. It was expected that biofilm suppression would be further enhanced in the denser sample C, but contrary to expectations, biofilm formation was enhanced in the densest sample.

The relationship between surface roughness and biofilm formation can be understood through a nuanced interaction between bacteria and the material surface. On rough surfaces, numerous micro-irregularities act as "hideouts" that facilitate bacterial adhesion, making it easier for bacteria to attach and subsequently form biofilms. Additionally, these surface irregularities can function as a physical shield, protecting the bacteria from antimicrobial agents and immune responses. On surfaces with moderate roughness, while there are sufficient irregularities to allow initial bacterial adhesion, the surface is smooth enough to make it challenging for bacteria to establish stable colonies. Furthermore, the reduced roughness allows antimicrobial agents and immune responses to more effectively act on the bacteria, potentially inhibiting biofilm formation. Conversely, on very fine surfaces, the irregularities are so minute that they may lose their "anchor" effect, making bacterial adhesion less stable. However, fine surfaces tend to have higher surface energy, which could promote bacterial attachment. Moreover, on these fine surfaces, attached bacteria are more likely to come into close contact with each other, which might facilitate the rapid initial formation of biofilms. Overall, these findings suggest that surface roughness has a nonlinear effect on biofilm formation. While extremely rough surfaces make it easy for bacteria to attach, excessively fine surfaces, due to high surface

energy and close bacterial contact, may also promote adhesion. On the other hand, moderate surface roughness appears to strike an optimal balance, potentially offering the best conditions for inhibiting bacterial adhesion and subsequent biofilm formation.

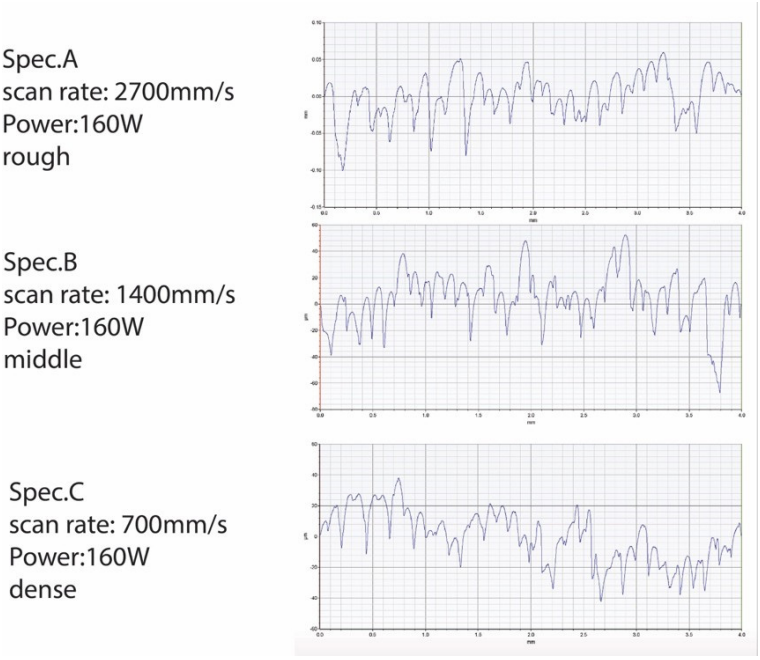

Fig2. Surface roughness of the fabricated SUS 316 steel.

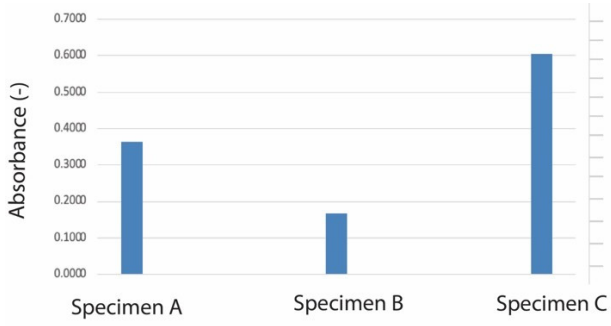

Fig.3 The results of Biofilm Evaluation test according to ISO4768.

CONCLUSION

This study explored the anti-biofilm properties of austenitic stainless steel (SUS 316) fabricated using Selective Laser Sintering (SLS), with a focus on understanding the relationship between surface roughness and biofilm formation. The findings indicate that surface roughness has a significant but nonlinear impact on biofilm formation. Specifically, it was observed that coarser surfaces facilitated biofilm formation due to the presence of micro-irregularities that support bacterial adhesion. In contrast, surfaces with moderate roughness were more effective in inhibiting biofilm development, likely due to a balance between sufficient irregularities for initial adhesion and a smoothness that impedes the establishment of stable bacterial colonies. However, as the surface became finer, biofilm formation increased again, potentially due to higher surface energy and closer bacterial contact, which might promote rapid initial biofilm formation. These insights suggest that optimizing surface roughness could be a viable strategy for enhancing the hygienic properties of medical devices, paving the way for further research and development in this field.

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