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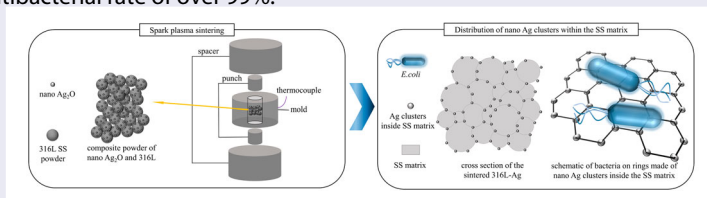
A novel stainless steel with intensive silver nanoparticles showing superior antibacterial property

L. T. Liu^{a,b}, Y. Z. Li^a, K. P. Yu^a, M. Y. Zhu^b, H. Jiang^a, P. Yu^b and M. X. Huang^a

^aDepartment of Mechanical Engineering, The University of Hong Kong, Pokfulam, Hong Kong, People's Republic of China; ^bDepartment of Materials Science and Engineering, Southern University of Science and Technology, Shenzhen, People's Republic of China

ABSTRACT

Stainless steels (SS) are one of the most widely used, affordable materials in public areas, but suffer from a lack of antibacterial property. Unfortunately, traditional steel casting alloying strategy has limits to add Ag due to the extremely low solubility of Ag in SS. The present work demonstrates a novel powder metallurgy technology to fabricate Ag-contained SS with a unique microstructure in which the average distance between Ag particles is similar to the size of a typical bacterium, enabling the direct contact killing mechanism. Consequently, the new Ag-contained SS possesses an excellent and stable antibacterial rate of over 99%.



IMPACT STATEMENT

A novel stainless steel containing intensive Ag particles demonstrates excellent anti-bacterial property.

ARTICLE HISTORY

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KEYWORDS

Stainless steel; antibacterial; nano Ag clusters; powder metallurgy

1. Introduction

As one of the most widely used materials in public hygiene areas, stainless steel (SS) has been investigated extensively because of its numerous excellent properties such as high strength, corrosion resistance, superior workability and good biocompatibility [1–5]. However, SS has no inherent antibacterial property [6], which may result in infection from SS implants or disease transmission via random contact with contaminated SS surfaces [7,8]. Notably, among all these contact transmission-based infections, hospital acquired infections (HAIs) are ranked in the top 10 causes of death in the USA [9]. In 2006, there were about 720,000 HAIs in the USA, of which 74,000 cases were fatal, causing 125 billion extra hospital charges [10]. Furthermore, the rise of antibiotic resistance is becoming more and more threatening for public health worldwide, with a report showing that over

half of the bacteria collected in public areas are multidrug resistant (AMR) in London [7]. Therefore, the development of novel antibacterial SS shall play an important role in combating these public health threats.

The use of inorganic antibiotics such as Ag, Cu, and Zn to modify traditional SS grades represents an attractive research field [11–13] because these inorganic antibiotics are considered free of AMR, while simultaneously having good antibacterial abilities towards a broad bacterial spectrum. In particular, Ag and Cu attract the most research attention due to their strong antibacterial property and low toxicity to animal cells [14,15]. Furthermore, Ag is regarded as the most promising one because it has the strongest antibacterial ability, which is about 100 times stronger than Cu, and a broader antibacterial spectrum [16]. However, the development of Ag-contained antibacterial SS is limited compared with Cu-bearing

CONTACT M. X. Huang ✉ mxhuang@hku.hk Department of Mechanical Engineering, The University of Hong Kong, Pokfulam Road, Pokfulam, Hong Kong, People's Republic of China; P. Yu ✉ yup@sustc.edu.cn Department of Materials Science and Engineering, Southern University of Science and Technology, Shenzhen, People's Republic of China

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antibacterial SS because of the extremely low solubility for Ag in Fe (i.e. 0.002 at%) [17]. Till now, most researches related to Ag-contained antibacterial SS focus on surface engineering, such as coating and ion implantation, by which they cannot maintain their antibacterial property for an extended period due to surface scratching and damage [11,18].

In order to produce Ag-contained antibacterial SS with Ag phases inside the SS matrix, the traditional steel casting method was firstly tried in this paper, and its antibacterial property was evaluated. In addition, the average distance between Ag phases, the size of Ag phases, and the typical size of a bacterium ($1 \sim 5 \mu\text{m}$) are compared and discussed. Yet, due to the extremely low solubility of Ag in SS, a limited antibacterial property was expected for Ag-contained SS fabricated by traditional casting technology. Therefore, the development of a novel method to prepare Ag-contained antibacterial SS with permanently strong antibacterial property is needed.

Powder metallurgy (PM) has been a highlight technology for many years, because it has the advantages of low cost, net-shaping and high material utilization (95%) etc. [19]. In this study, a novel in-situ reaction assisted PM method was developed to prepare a new Ag-contained antibacterial SS. 316L SS powder with the size as a typical bacterium ($1 \sim 5 \mu\text{m}$) was chosen as the raw material, upon which nano-sized Ag-rich particles

were formed uniformly in the SS matrix during sintering. Consequently, the present method produces a new Ag-contained SS with an average distance between these Ag-rich particles similar to the size of a typical bacterium. In addition, the microstructures, antibacterial properties, the Ag ion release behavior, biocompatibility, and mechanical properties of the new Ag-contained antibacterial SS were characterized.

2. Experimental

The Ag-contained 316L SS fabricated by conventional casting, 316L SS and Ag-contained 316L SS produced by the present powder metallurgy are employed for the present study. They are hereafter referred as 316L-Ag-casting, 316L and 316L-Ag-PM, respectively. The detailed information of sample preparing and experimental procedures were presented in the supplementary information.

3. Results

3.1. Powder characterization and phase constitute of different alloys

As shown by the transmission electron microscope (TEM) image of the nano Ag_2O in Figure 1(a), the Ag_2O has a fine particle size of below 80 nm, with its

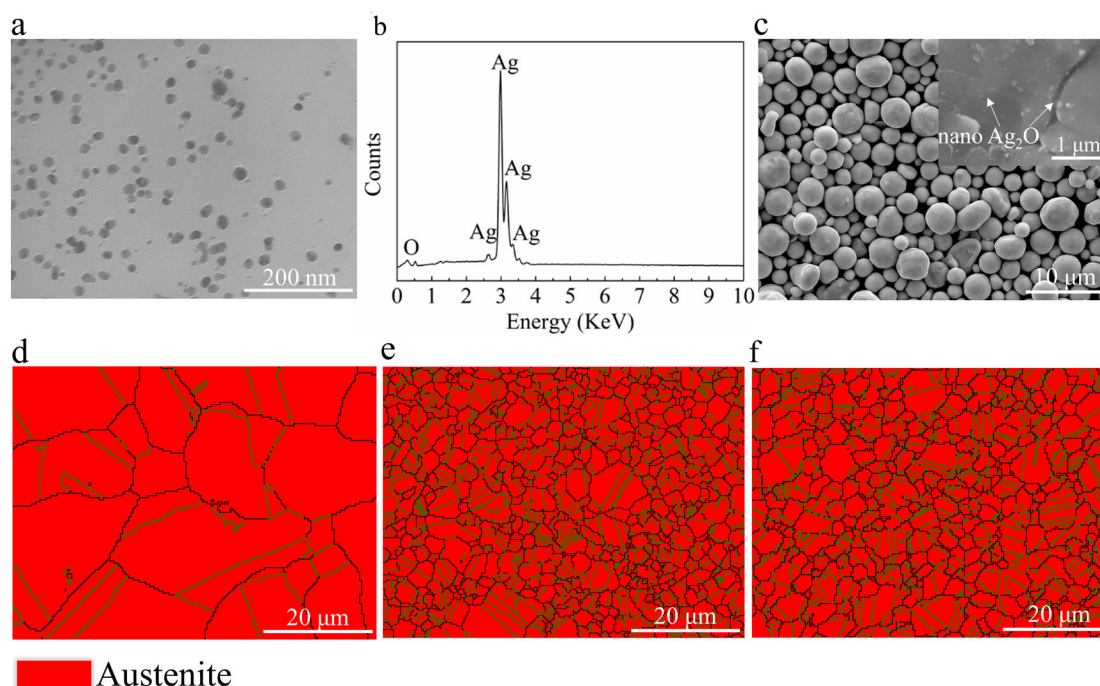


Figure 1. (a) The TEM image, and (b) EDX spectrum of the nano Ag_2O particles. (c) SEM morphology of the mixed powder of 316L SS with nano Ag_2O and its enlarged view; EBSD phase maps (Black lines represent high angle grain boundaries ($\geq 15^\circ$) while green lines are twin boundaries) of the (d) 316L-Ag-casting (grain size: $8.664 \pm 5.51 \mu\text{m}$), (e) 316L-Ag-PM (grain size: $2.445 \pm 0.92 \mu\text{m}$) and (f) 316L (grain size: $2.413 \pm 0.95 \mu\text{m}$).

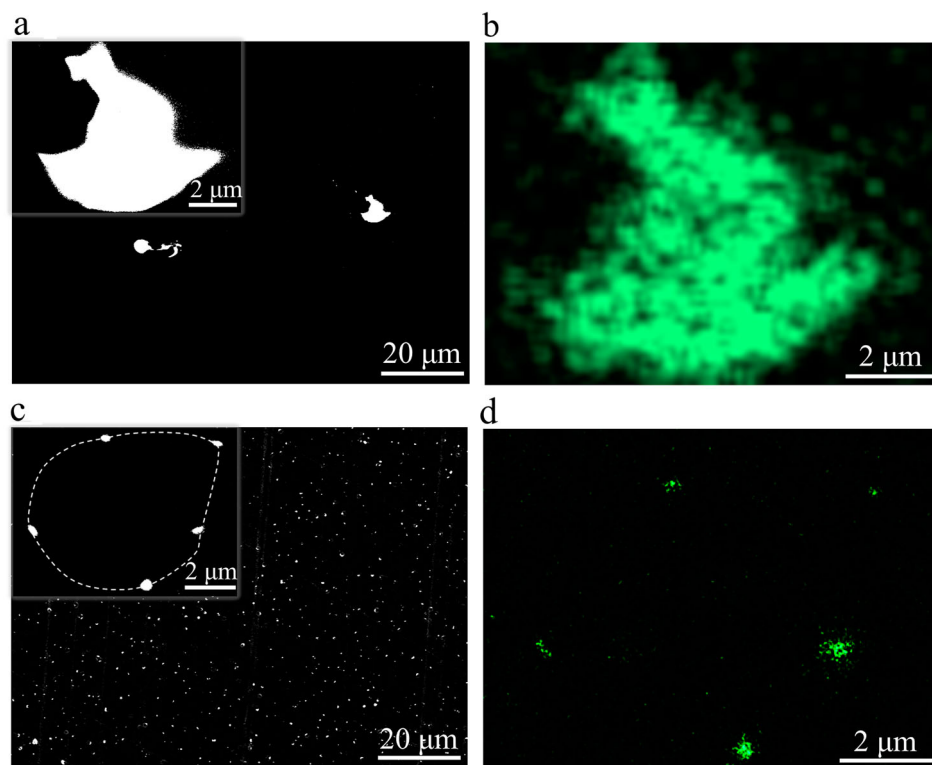


Figure 2. (a) Typical distribution of Ag particles in the 316L-Ag-casting and its enlarged view with, (b) EDX mapping of Ag element; (c) Typical distribution of Ag particles in the 316L-Ag-PM and its enlarged view with (d) EDX mapping of Ag element.

X-ray energy dispersive (EDX) spectrum (Figure 1(b)) confirming its composition of Ag_2O . Figure 1(c) is the scanning electron microscope (SEM) image of the mixed powder of the 316L SS and Ag_2O under low magnification showing that the size of the spherical 316L powder is comparable as a typical bacterium. The picture with higher magnification on the right corner shows the nano Ag_2O distributed uniformly on the surface of the 316L powder.

The typical grain size, phase composition of the 316L-Ag-casting, 316L-Ag-PM, and 316L are shown in the electron backscatter diffraction (EBSD) phase map images in Figure 1(d~f). As exhibited, there are no significant differences in the phase composition of the three typical austenitic SS. However, the grain size of the sintered 316L-Ag-PM and 316L is much smaller than that of the 316L-Ag-casting because they were prepared by rapid sintering technology.

3.2. Characterization of the existence of Ag within the matrix of different alloys

The existence of Ag inside the matrix of the 316L-Ag-casting was characterized firstly. Because the atomic weight of Ag is much larger than that of Fe, the SEM

back-scattering electron images (SEM-BSE) show clearly the Ag-rich areas (white particles), whereas the dark areas are the austenite matrix. As indicated by the SEM-BSE images of the 316L-Ag-casting in Figure 2(a), some white particles were observed within the matrix, with an inserted picture showing a selected area containing white particles at higher magnification. Furthermore, as shown by the EDX mapping result (Figure 2(b)), the white particle (Figure 2(a)) has a high concentration of Ag, which should be Ag-rich particles. However, as exhibited, most of the added Ag exists as coarse Ag-rich particles of several microns. Furthermore, the Ag-rich particles are highly concentrated in some areas, leaving most areas with few Ag particles.

Similarly, the presence of Ag within the matrix of the 316L-Ag-PM was also characterized. As shown in Figure 2(c), tremendous white particles ranging from dozens of nanometers to several hundred nanometers are observed in the 316L-Ag-PM. In addition, the average distance between these nano Ag-rich particles is about 2–5 μm , which is shown by the enlarged view on the upper left corner of Figure 2(c). Besides, as shown in Figure 2(d), the EDX mapping result of the enlarged view of Figure 2(c) also confirms that these nano particles are Ag-rich particles.

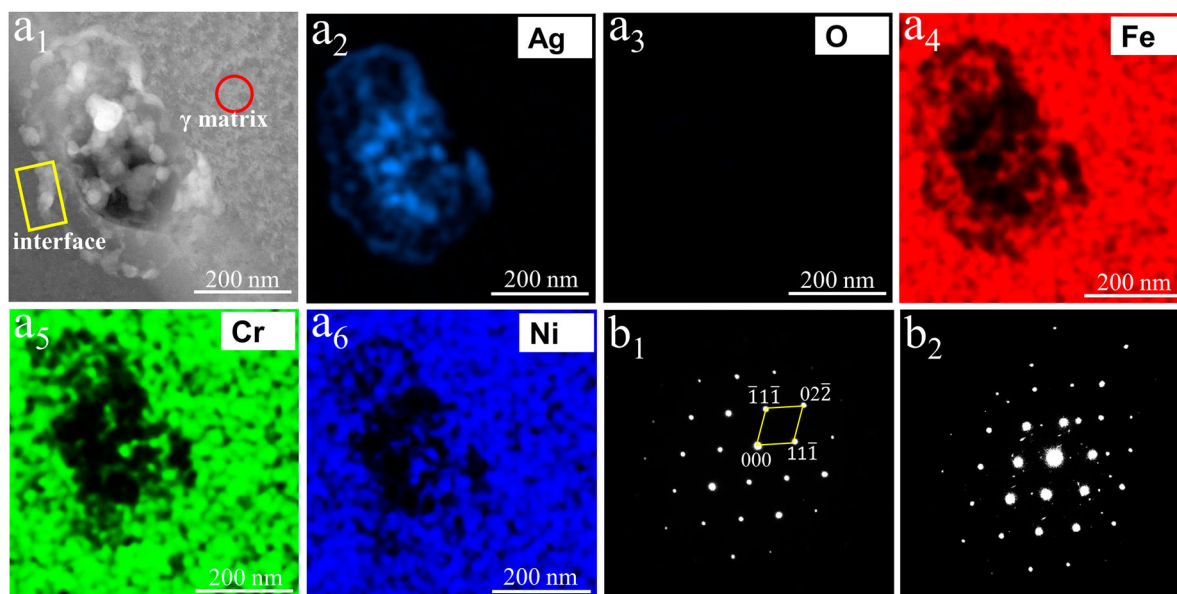


Figure 3. (a1) The TEM image of one Ag cluster in the sintered 316L-Ag-PM and its TEM-EDX mapping of different elements (a₂–a₆). The selected area diffraction patterns of (b₁) the SS matrix and (b₂) its interface with Ag clusters.

3.3. TEM analysis of the Ag-rich particles in the 316L-Ag-PM

The further information about these Ag-rich particles was investigated by talos TEM equipped with high resolution EDX. As shown by the TEM image of an Ag-rich particle under high magnification in Figure 3(a₁), the Ag-rich particle is actually Ag clusters composed of several smaller Ag particles, which is confirmed by the TEM-EDX mapping result in Figure 3(a₂–a₆). Furthermore, there is a very sharp boundary between the Ag clusters and the SS matrix corresponding that Ag has an extremely low solid solubility in SS. Figure 3(b₁) displays a normal [011] selected area diffraction pattern (SADP) taken from the matrix, indicating that the SS matrix are typical γ austenite phase with a face-to-face (FCC) structure and lattice parameter of 0.359 nm. Besides, the SADP taken from the interface of γ matrix and the Ag clusters (Figure 3(b₂)) reveals a diffraction pattern of γ matrix and a lot of unrecognizable diffraction spots, confirming again that the Ag-rich particles are Ag clusters other than a single crystal of Ag.

3.4. Antibacterial performance of different alloys without/with humidity control

Figure 4(a₁ ~ a₃) shows the typical bacterial colonies after 24 h incubation on the surface of 316L-Ag-PM, 316L-Ag-casting, and standard 316L without humidity control, similar to the humidity testing condition reported in the literature [20,21]. As shown in

Figure 4(a₂), there were few bacterial colonies left on the 316L-Ag-casting, indicating an antibacterial rate of about 100%, which was similar to the result reported in the literature [20,21]. Besides, a good antibacterial rate was also observed for the 316L-Ag-PM as exhibited in Figure 4(a₁).

Figure 4(b₁–b₃) displays the testing results of the 316L-Ag-PM, 316L-Ag-casting, and 316L tested under a controlled humidity of 90% as required by the JIS Z2801-2000 testing standard. As shown in Figure 4(b₃), a large number of bacterial colonies can be found on the 316L, consistent with the literature that 316L does not have antibacterial property [6]. On the contrary, few bacterial colonies can be found on the 316L-Ag-PM with an antibacterial rate higher than 99% (Figure 4(b₁)), demonstrating that the 316L-Ag-PM has an excellent antibacterial property.

However, a relatively large number of bacterial colonies were still observed on the 316L-Ag-casting (Figure 4(b₂)). The calculated antibacterial rate of the 316L-Ag-casting is about $87\% \pm 5\%$, indicating that its antibacterial property is mild and cannot satisfy the antibacterial requirement according to the JIS Z2801-2000 standard.

3.5. Ag ion release behavior of different alloys

After 24 h of immersion, no Ag ion was detected for the 316L soaked in phosphate-buffered saline (PBS) and nutrition broth (NB) solution as expected. For the 316L-Ag-PM soaked in PBS, only 0.0013 ± 0.0004 ppm of Ag ion was detected, which is below the detecting capacity

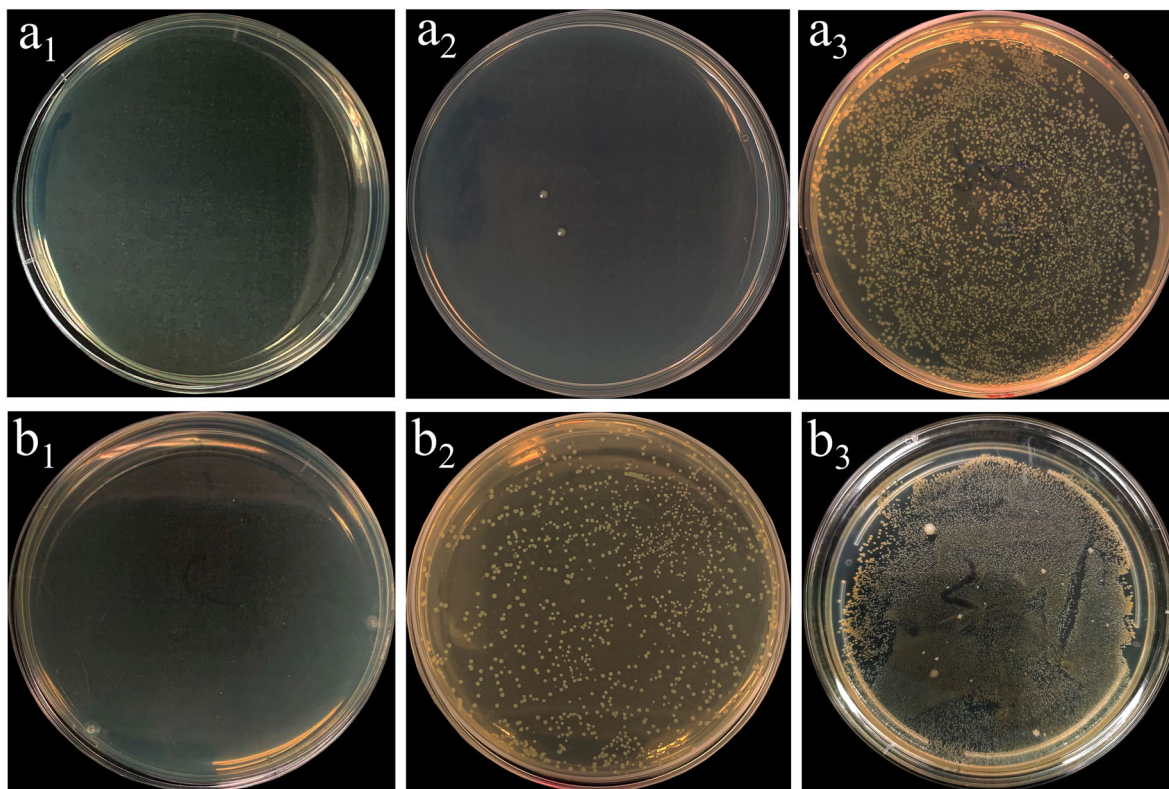


Figure 4. Typical bacterial colonies on the surface of (a₁) 316L-Ag-PM, (a₂) 316L-Ag-casting, and (a₃) 316L under no humidity-controlled condition ($P > 0.05$ for the 316L-Ag-PM and 316L-Ag-casting); Typical bacterial colonies on the surface of (b₁) 316L-Ag-PM, (b₂) 316L-Ag-casting, and (b₃) 316L under a controlled humidity of 90% ($0.01 < P < 0.05$ for the 316L-Ag-PM and 316L-Ag-casting).

of the inductively coupled plasma mass spectrometry (ICP-MS) machine (about 0.005 ppm). For testing samples soaked in NB, 0.0104 ± 0.0004 ppm and 0.0459 ± 0.0039 ppm of Ag ion were detected for the 316L-Ag-PM and 316L-Ag-casting, respectively. The Ag ion release concentration from the 316L-Ag-PM is lower than that of the 316L-Ag-casting, which might be explained by a better repassivation ability of the 316L-Ag-PM (Figure S2).

3.6. Biocompatibility and mechanical properties of the novel Ag-contained SS

Figure 5(a₁ ~ a₃, b₁ ~ b₃) show the fluorescence images of cell morphology of MC3T3-E1 cells cultivated on the 316L-Ag-PM and 316L for 24 h, respectively. After one day incubation, it can be found that the number of cells on the 316L and 316L-Ag-PM has no obvious difference. Moreover, as can be seen, the presence of cytoskeletal components (Actin filaments (F-actin)) and filopodia extending from the cells on both the 316L-Ag-PM and 316L was observed, demonstrating that the cells exhibit a normal cellular activity and good adhesion on both samples [22].

Figure S1 displays the engineering stress-strain curve of the 316L-Ag-PM. As calculated, the 316L-Ag-PM has a yield strength of 492 MPa and an ultimate tensile strength of 733 MPa with an elongation of 30%, demonstrating that the 316L-Ag-PM has good enough mechanical properties for application purpose in terms of PM [23,24].

4. Discussion

Although Ag is a highly promising alloying element to develop antibacterial SS [16], there are very limited applications of Ag-contained SS because of its extremely low solid solubility in SS [17], resulting in macroscopic segregation of Ag in the SS matrix and unstable antibacterial property [11]. As shown in Figure 2(a,b), the distribution of Ag in the 316L-Ag-casting has a typical macroscopic segregation phenomenon. Areas without or with few Ag are much larger than the size of a typical bacterium, which can result in an unstable antibacterial property.

It is interesting to compare the antibacterial property of the 316L-Ag-casting under different humidity conditions. Figure 4(a₂) shows that the 316L-Ag-casting has an excellent antibacterial property when the test condition does not control the humidity. On the contrary,

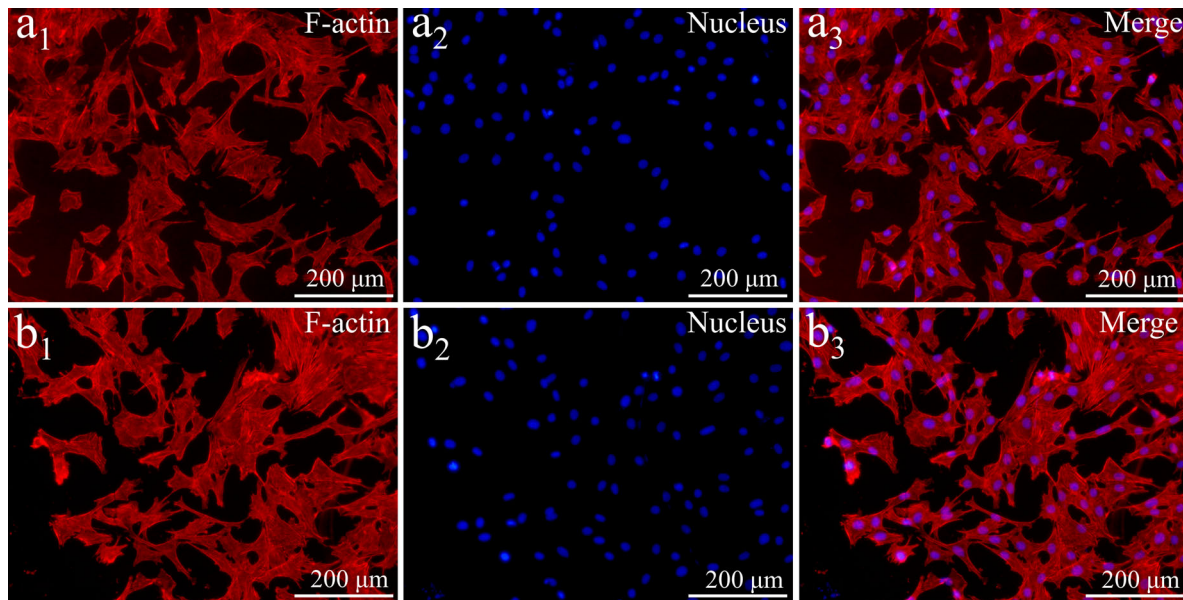


Figure 5. Fluorescence images of cell morphology on the surface of ($a_1 \sim a_3$) the 316L-Ag-PM and ($b_1 \sim b_3$) the standard 316L.

Figure 4(b_2) shows that the 316L-Ag-casting does not display good antibacterial property when it is tested under the controlled humidity of 90%. In other words, the 316L-Ag-casting does not have satisfied antibacterial property under strict testing standards such as JIS Z2801-2000. It is noted that some Ag-contained SS fabricated by casting technology, similar to the present 316L-Ag-casting, has been reported to have excellent antibacterial properties [20,21]. It is expected that these antibacterial tests were performed under no humidity-controlled condition, similar to the result shown in Figure 4(a_2).

It has been demonstrated that the humidity of the culture system can have an obvious effect on the viability of bacteria [25]. Therefore, it is possible that the testing condition can affect the antibacterial rate because there could be evaporation of water from the bacterial suspension during the test, which deteriorates the normal osmotic pressure for some bacteria. Therefore, as required by the JIS Z2801-2000 standard, a proper humidity control should be maintained to eliminate irrelevant factors.

As shown in Figure 4($b_1 \sim b_3$), when all samples were tested with a proper humidity control as specified, the present 316L-Ag-PM (Figure 4(b_1)) reveals an excellent antibacterial rate higher than 99%, which is much better than that of the 316L-Ag-casting (Figure 4(b_2)), though the Ag content is the same.

Although the mechanism is not yet fully understood, researches about the Ag-contained SS proposed that the Ag ion released from the material plays a key role in killing the bacteria. Based on this mechanism, a higher Ag ion concentration leads to a better antibacterial ability. In addition, some reports have shown that the minimum

concentration of Ag ion to inhibit *E. coli* was 60 ppm [26,27], and 39 ppm was needed to produce antibacterial effect against *S. aureus* and *P. aeruginosa* [28]. However, the Ag ion concentration (0.0104 ppm) released from the 316L-Ag-PM after 24 h immersion in NB is much lower than the above-reported value, while it does show an excellent antibacterial property. Therefore, it is possible that the antibacterial mechanism of the present 316L-Ag-PM cannot be fully explained by the total Ag ion release concentration. It is proposed here that the antibacterial property may be induced by the direct contact between the bacteria and the Ag-rich nanoparticles in the 316L-Ag-PM. For the 316L-Ag-casting, the average distance between Ag-rich particles is much larger than the size of a bacterium. Therefore, the probability of direct contact between Ag-rich particles and bacteria in the 316L-Ag-casting is low, resulting in an unstable and mild antibacterial property. Given that the grain size of the 316L-Ag-casting is different from that of the 316L-Ag-PM, the effect of microstructures is also discussed. As shown in Figure S1, all three alloys have no strong textures according to the EBSD grain orientation maps of the 316L-Ag-casting, 316L-Ag-PM, and 316L. Furthermore, literature results have exhibited that the number of bacteria on the surface of 316L SS with grain sizes ranging from 25 nm to 65 μm were basically the same, suggesting that the attachment of bacteria was not affected by grain refinement [29]. Thus, it is proposed that the difference in grain size between the 316L-Ag-casting and the 316L-Ag-PM will not affect the attachment of bacteria.

The cytotoxicity and mechanical properties are also a concerning property for antibacterial SS. There was a

report pointing out that there was no cytotoxicity until the concentration of Ag ion reached 1200 ppm [30], which was far beyond the value (< 0.005 ppm) released from the present 316L-Ag-PM soaked in PBS solution. In addition, the cell morphology analysis and tensile test also indicate a normal cellular function and good tensile properties for the 316L-Ag-PM. Thus, it can be concluded that the 316L-Ag-PM has good cell compatibility and mechanical properties for practical applications. However, *in vivo* biocompatibility of the present 316L-Ag-PM still needs to be evaluated.

5. Conclusion

In summary, a novel Ag-contained antibacterial SS with an average distance between the nano Ag-rich particles similar to the size of a typical bacterium is developed. By this means, the bottle-neck problem that traditional casting Ag-contained SS tends to have a coarse and heterogeneous Ag distribution is solved successfully. Furthermore, the present 316L-Ag-PM SS is suitable for industrial production, which could provide low-cost SS products to combat the public health threatens caused by the pathogen bacteria.

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Disclosure statement

No potential conflict of interest was reported by the author(s).

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