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Mechanisms of the enhanced antibacterial effect of Ag-TiO₂ coatings

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ABSTRACT
It has been demonstrated that Ag-TiO₂ nanocomposite coatings with excellent antimicrobial activity and biocompatibility have the potential to reduce infection problems. However, the mechanism of the synergistic effect of Ag-TiO₂ coatings on antibacterial efficiency is still not well understood. In this study, five types of Ag-TiO₂ nanocomposited coatings with different TiO₂ contents were prepared on a titanium substratum. Leaching tests indicated that the incorporation of TiO₂ nanoparticles into an Ag matrix significantly promoted Ag ion release. Surface energy measurements showed that the addition of TiO₂ nanoparticles also significantly increased the electron donor surface energy of the coatings. Bacterial adhesion assays with *Escherichia coli* and *Staphylococcus aureus* demonstrated that the number of adhered bacteria decreased with increasing electron donor surface energy. The increased Ag ion release rate and the increased electron donor surface energy contributed to an enhanced antibacterial efficiency of the coatings.

1. Introduction
Titanium (Ti) and its alloys are widely used in both orthopedic and dental implants (Jia et al. 2016). However, microbial colonization and biofilm formation on the implanted devices represent an important complication in orthopedic and dental surgery and may result in implant failure (De Giglio et al. 2013). Silver has been known as a broad-spectrum bactericide for centuries. It has been demonstrated that silver nanoparticle coatings on Ti based implants increased their biocompatibility and antibacterial properties (Gyorgyey et al. 2016; Sulej-Chojnacka et al. 2016). Titanium dioxide (TiO₂) has also been known as a broad-spectrum bactericide for a long time. Matsunaga et al. (1985) first reported the microbiocidal effect of TiO₂ photocatalytic reactions. The antibacterial mechanism of TiO₂ has been proven to involve oxidation of all organic compounds in the microorganisms by the generated reactive oxygen species which leads to cell death (Kim et al. 2014; Motlagh et al. 2014). In addition, TiO₂ with moderate hardness and excellent resistance to wear and corrosion can significantly accelerate osteoblast cell growth and improve bone-forming functionality and direct the fate of stem cells in orthopedic-associated implants (Frandsen et al. 2013; Li et al. 2015). This suggests that a Ag-TiO₂ nanocomposite coating may offer a promising solution for improvement of the antibacterial properties of dental and orthopedic implants (Cotolan et al. 2016; Gyorgyey et al. 2016). It has been reported that Ag-TiO₂ nanocomposite coatings exhibit a synergistic effect on antibacterial activity, which made their bactericidal activities stronger than Ag or TiO₂ coatings (Ashkarran et al. 2011; Esfandiari et al. 2014; Motlagh et al. 2014; Prakash et al. 2016). The enhanced antibacterial effect was explained by a contact killing action mechanism (Prakash et al. 2016) and the bactericidal capacity was found to depend on the size characteristics of the Ag-TiO₂ coatings (Esfandiari et al. 2014). It was also reported that Ag-TiO₂ nanoparticles extended the light absorption spectrum toward the visible region and significantly enhanced the inactivation of bacteria under visible light irradiation due to the effect of Ag, by acting as electron traps in the TiO₂ band gap (Ashkarran et al. 2011). However, the mechanism of the enhanced antibacterial effect of the Ag-TiO₂ coatings is still not well understood. The aim of the present work was therefore to investigate the mechanism of the synergistic effect of Ag-TiO₂ coatings on their antibacterial properties. For this purpose, a range of novel Ag-TiO₂ nanocomposite coatings with different TiO₂ content on a titanium substratum were prepared using an electroless plating technique, and the antibacterial...
performance of the coatings was evaluated using two types of bacteria, ie the Gram-negative Escherichia coli and the Gram-positive Staphylococcus aureus.

2. Materials and methods

2.1. Preparation of Ag-TiO₂ coatings

A range of Ag-TiO₂ nanocomposite coatings were prepared on a substratum of pure Ti disks 10 mm in diameter. The disks were first cleaned ultrasonically with acetone, ethyl alcohol and deionized water respectively for 5 min, then they were further treated by alkaline cleaning for 5 min and pickling cleaning for 5 min. The alkaline cleaning solution comprised NaOH 25 g l⁻¹, Na₃PO₄ 30 g l⁻¹, Na₂CO₃ 25 g l⁻¹ and Na₂SiO₃ 10 g l⁻¹, and the pickling solution was HCl 30%: H₂O (1:1 in volume). After this, both sides of the disks were irradiated with UV light at 385 nm for 2 h to activate the inert Ti surfaces, then the disks were immersed in a coupling agent solution for 2 h in order to create the reactive surfaces using the method described previously (Guo et al. 2015). TiO₂ powder with a particle size of 25 nm (Sigma-Aldrich, Dorset, UK) was used. Five types of Ag-TiO₂ coatings with the different TiO₂ concentrations in the plating solution (0.1, 0.3, 0.5, 1.5 and 2 g l⁻¹) were prepared by an electroless plating technique under dark conditions. An Ag coating was also prepared for comparison. The composition of the plating bath and operating conditions are presented in Table 1. All the samples were sterilized in an autoclave at 120°C for 15 min before bacterial adhesion assays.

2.2. Characterization of the Ag-TiO₂ coatings

The surface morphologies of the pure Ti, Ag and Ag-TiO₂ coatings were observed by scanning electron microscopy (SEM) (S-4800, Hitachi, Hitachinaka, Japan). The elemental composition and distribution of Ag and TiO₂ were determined by energy dispersive X-ray analysis (EDX) (EMAX350, Horiba, Kyoto, Japan). The surface topographic and roughness of coatings were observed by atomic force microscopy (AFM) (Multimode 8, Bruker, Karlsruhe, Germany) with tip scanning. Their phase compositions were analyzed by X-ray diffraction XRD (D8 Advance, Bruker, Karlsruhe, Germany). The bonding states of the surface constituents were identified by X-ray photoelectron spectroscopy XPS (ESCALAB 250, Thermo Fisher Scientific, Waltham, MA, USA).

2.3. Ion release

The amounts of Ag and Ti ions released from the coatings were monitored by soaking them in PBS at a pH of 7.3 ± 0.1. The tested samples were immersed in a sealed tank containing 25 ml of PBS and kept at 37°C at 150 rpm. The releases of Ag and Ti ions from the different coatings in PBS were determined by inductively coupled plasma atomic emission spectrometry ICP-AES (Varian 710-ES, Agilent Technologies, Palo Alto, CA, USA) with immersion times of 1 h, 24 h, three days and seven days, respectively, which was the same time as used for bacterial adhesion assays, as described below.

2.4. Contact angle and surface energy

After exposure to UV light, the contact angles of the coatings were measured with a Dataphysics OCA-20 contact angle analyzer (DataPhysics Instruments GmbH, Filderstadt, Germany). The test liquids used for contact angle measurements were distilled water (W), diiodomethane (Di) and ethylene glycol (EG), respectively. The contact angles of living biofilms of E. coli and S. aureus used for bacterial adhesion assays were also measured by the sessile drop method. Details of the contact angle measurements and surface energy calculation are described in previous study (Liu and Zhao 2011). The surface energies of coatings were calculated using the van Oss (1994) approach, including the Lifshitz-van der Waals (LW) apolar component (γ²Lw), the Lewis acid/base polar component (γ²AB), the electron donor component (γ⁺D) and the electron acceptor (γ⁻A) component. The ratio of γ²Lw/γ₂⁻ (also known as the CQ ratio) was also calculated to determine the adhesion strength (Liu and Zhao 2011).

2.5. Bacterial adhesion and removal

E. coli ATCC 8739 and S. aureus ATCC 6538 were used for bacterial adhesion and removal assays on the Ag-TiO₂ coatings. The pure Ti and Ag coatings were also evaluated for comparison. After the frozen bacterial stocks had been defrosted, they were cultured in tryptone soya agar (TSA) plates in an incubator overnight at 37°C. A single colony was taken into 10 ml of tryptone soy broth (TSB) and grown statically overnight at 37°C. Then, 500 μl of each bacterial suspension were transferred into 100 ml of the corresponding medium in a conical flask and cultured.
in a shaker-incubator (LUXI SZX-IS1A, Beijing, China) at 150 rpm at 37°C until the bacteria had grown to mid-exponential phase. Then, the suspensions with 10^7 CFU ml^{-1} of each type of bacterium were prepared. For bacterial adhesion assays, each sample was immersed in a tank containing 25 ml of bacterial suspension at 37°C for 1 h, 24 h, three days and seven days, respectively. After that, each sample was moved up and down vertically for 25 s in a glass tank containing 50 ml of distilled water at a constant speed 0.028 m s^{-1} with a corresponding shear stress of 0.014 N m^{-2} in order to remove adhered bacteria using a home-made dipping device. Then, the total number of adhered bacteria and the bacterial removal rate for each sample were determined on TSA plates by a viable plate counting method. Details of the bacterial adhesion assay are described in a previous study (Liu and Zhao 2011).

2.6. Statistical analysis

For the bacterial adhesion assay five replicate samples of each coating were tested, and for each sample three measurements were performed (N = 15). One-way analysis of variance (ANOVA) was used to determine whether there were any statistically significant differences between the means of two or more independent groups. If p < 0.05 they are considered to be significantly different.

3. Results

3.1. Surface characterizations

Figure 1a shows a typical SEM image of pure Ti with gullied surface and Figure 1b shows the morphology of an Ag coating with typical polygonal rounded hillock units. The morphology of Ag-TiO_2 coating has a similar structure to the Ag coating in Figure 1c, which was produced with 1.5 g l^{-1} TiO_2 in the plating solution. Clearly, the TiO_2 particles were homogenously incorporated in the Ag matrix.

The Ag and TiO_2 contents on the coatings were determined by EDX analysis based on their Ti, O and Ag contents. Figure 2a and b show the EDX images of the Ag coating and the Ag-TiO_2 coating (1.5 g l^{-1} TiO_2), respectively. The detailed EDX results for all the coatings are given in Table 2, which indicates that TiO_2 was embedded into the Ag-based matrix. Furthermore, the results demonstrate that the TiO_2 content in the Ag-TiO_2 coatings increased with increasing TiO_2 concentration in the plating solution, while the Ag content on the Ag-TiO_2 coatings decreased with increasing TiO_2 concentration in the plating solution.

Figure 3 shows the surface morphology of the pure Ti, and Ag coatings and the Ag-TiO_2 coating (1.5 g l^{-1} TiO_2, see Table 2) by AFM, respectively. The roughness for each surface was an average of six measurements at different position with the surface area of 10 × 10 μm^2. The surface roughness values (Ra) of the Ag and Ag-TiO_2 coatings were in the range of 102–125 (±14) nm, while the Ra values for the pure Ti were ~202 ± 23 nm (see Table 2). The AFM images indicated that the roughness of the pure Ti disk was higher than that of the Ag coating and the Ag-TiO_2 coatings.

Figure 4 shows the typical XRD spectra of the pure Ti, Ag and Ag-TiO_2 coatings (TiO_2 1.5 g l^{-1}). It can be seen that the pure Ti surface only contained the Ti phase, while the pattern of the Ag coating contained the silver phase and small peaks of Ti from the substrate. The Ag-TiO_2 coating showed Ag and anatase TiO_2 peaks, and some small peaks of the rutile phase, which is consistent with the results of Prakash et al. (2016). Clearly the Ag-TiO_2 coating was composed of Ag and TiO_2 phases.

Further XPS studies revealed that Ag, Ti and O peaks were found for all the Ag-TiO_2 coatings. Figure 5 shows the results for the typical Ag-TiO_2 (TiO_2 0.5 g l^{-1}) coating. The peaks of Ag 3d, Ti 2p and O1s were clearly observed (Figure 5a). The C1s signal was caused by surface contamination. The XPS spectra of elemental Ag from the coating shows the energies of Ag 3d peaks at 368.2 eV and 374.2 eV, respectively, which fits with Ag^0 in the coating (Figure 5b). The bonding energy of the Ti 2p peak is located at 458.8 eV and 464.6 eV (Figure 5c) and O1s peak is located at 529.9 eV (Figure 5d), which are attributed to the chemical bonding of TiO_2. The Ti 2p and O1s peak structures become broader and much slighter in asymmetries due to the amount of Ag in the coatings. All the XPS binding energies of the Ag 3d, Ti 2p and O1s photoelectrons for the coatings are consistent with the results of Kuo et al. (2007). The results
3.2. Ion release

The amounts of Ag ion released from the Ag-TiO₂ coatings (with TiO₂ 0.1, 0.3, 0.5, 1.5 and 2.0 g l⁻¹, respectively) in PBS are given in Table 3. The amount of Ag ion released from the Ag coatings increased steadily to...
in general the amounts of Ag ion released from all the Ag-TiO$_2$ coatings were much higher than that released from the Ag coating. Clearly, the incorporation of TiO$_2$ nanoparticles into the Ag coating promoted Ag ion release.

0.5811 ± 0.0155 ppm cm$^{-2}$ from 0.1276 ± 0.0010 ppm cm$^{-2}$ during the 7 days while the amount of Ag ion released from Ag-TiO$_2$ coatings increased from 0.2404±0.0011 ppm cm$^{-2}$ to 0.6281±0.0104 ppm cm$^{-2}$. The results indicate that in general the amounts of Ag ion released from all the Ag-TiO$_2$ coatings were much higher than that released from the Ag coating. Clearly, the incorporation of TiO$_2$ nanoparticles into the Ag coating promoted Ag ion release.
3.3. Contact angles and surface energy

Table 4 shows the contact angles and surface energies of the coatings used in this study, including pure Ti, the Ag coating and the Ag-TiO₂ coatings with different TiO₂ contents. The results show that the water contact angle of the Ag-TiO₂ coatings was slightly lower than that of Ti or Ag coatings due to the incorporation of TiO₂ nanoparticles. Previous studies reported that the water contact angle on TiO₂-coated surfaces significantly decreased after exposure to UV light (Li and Logan 2005; Liu and Zhao 2011). Table 4 indicates that, in general, the electron donor surface energy (γ₂) of the Ag-TiO₂ coatings increased with increasing TiO₂ concentrations in the plating solution or the TiO₂ contents in the coatings. It was found that the ratio of LW apolar to the electron donor surface energy components of the substrata (γ_LW/γ₂ or CQ ratio) significantly decreased with increasing TiO₂ concentration in the plating solution. However, no correlation was observed between other surface energy components of the coatings (γ_LW, γ⁺, γ⁻ or γTOT) and TiO₂ concentration in the plating solution. The contact angles and surface energy of the E. coli and S. aureus used in this study are also given in Table 4. S. aureus exhibits a more hydrophilic surface and a higher value of electron donor surface energy (γ₂), compared to E. coli.

3.4. Bacterial adhesion assays

In order to compare the antibacterial properties of the Ti surface, the Ag coating and the Ag-TiO₂ coatings, E. coli and S. aureus were used for adhesion assays with contact times of 1, 24, 72 and 168 h, respectively. The Ag-TiO₂ coatings performed best against bacterial adhesion compared with the Ti surface and the Ag coating at different contact times. Figure 6 shows typical results for the contact time 168 h. The Ag-TiO₂ coatings reduced adhesion of E. coli by up to 52.4% (p < 0.05) and 39.8% (p < 0.05), respectively, compared with the Ti surface and the Ag coating (Figure 6a). The Ag-TiO₂ coatings reduced the adhesion of S. aureus by up to 45.1% (p < 0.05) and 41.2%

Table 3. Comparison of the amount of Ag ion released (ppm cm⁻²).

| Time (h) | Ag coating       | Ag-TiO₂ 1 | Ag-TiO₂ 2 | Ag-TiO₂ 3 | Ag-TiO₂ 4 | Ag-TiO₂ 5 |
|---------|------------------|-----------|-----------|-----------|-----------|-----------|
| 1       | 0.1276 ± 0.0010  | 0.2404 ± 0.0008 | 0.2404 ± 0.0009 | 0.2404 ± 0.0003 | 0.2404 ± 0.0005 | 0.2404 ± 0.0011 |
| 24      | 0.2893 ± 0.0057  | 0.3474 ± 0.0029 | 0.2590 ± 0.0067 | 0.2712 ± 0.0127 | 0.3030 ± 0.0162 | 0.3243 ± 0.0199 |
| 48      | 0.3562 ± 0.0112  | 0.3896 ± 0.0220 | 0.3498 ± 0.0322 | 0.3775 ± 0.0188 | 0.4167 ± 0.0130 | 0.3880 ± 0.0244 |
| 72      | 0.4341 ± 0.0125  | 0.4656 ± 0.0176 | 0.4568 ± 0.0193 | 0.4584 ± 0.0096 | 0.4804 ± 0.0141 | 0.4819 ± 0.0081 |
| 168     | 0.5811 ± 0.0155  | 0.6281 ± 0.0104 | 0.5951 ± 0.0174 | 0.6021 ± 0.0099 | 0.6244 ± 0.0081 | 0.6263 ± 0.0110 |

Table 4. Contact angle and surface energy components of the coating samples and bacteria.

| Sample   | TiO₂ concentration in solution (g l⁻¹) | Contact angle, θ (°) | Surface free energy (mJ m⁻²) | CQ ratio γ_LW/γ₂ |
|----------|----------------------------------------|----------------------|------------------------------|-----------------|
| Ti       | 79.2 ± 1.5 | 60.0 ± 0.3 | 37.1 ± 0.5 | 28.58 | 2.92 | 3.64 | 6.52 | 35.09 | 7.85 |
| Ag       | 73.7 ± 1.2 | 48.5 ± 0.6 | 46.9 ± 1.1 | 35.11 | 0.36 | 9.93 | 3.79 | 38.90 | 3.53 |
| Ag-TiO₂ 1 | 62.8 ± 2.2 | 42.4 ± 1.0 | 38.9 ± 1.2 | 36.31 | 0.09 | 16.15 | 2.47 | 38.78 | 2.52 |
| Ag-TiO₂ 2 | 68.1 ± 3.1 | 46.3 ± 1.4 | 47.8 ± 2.1 | 36.31 | 0.05 | 28.04 | 2.38 | 38.91 | 1.77 |
| Ag-TiO₂ 4 | 70.7 ± 2.5 | 54.0 ± 0.9 | 57.0 ± 0.6 | 32.02 | 0.01 | 18.12 | 0.87 | 32.89 | 1.30 |
| Ag-TiO₂ 5 | 61.4 ± 0.5 | 45.9 ± 0.2 | 53.3 ± 1.5 | 36.53 | 0.05 | 28.04 | 2.38 | 38.91 | 1.14 |
| E. coli  | 57.8 ± 1.4 | 63.3 ± 0.5 | 32.0 ± 0.3 | 26.68 | 2.06 | 23.42 | 13.88 | 40.56 | 4.57 |
| S. aureus| 54.2 ± 4.0 | 40.6 ± 2.4 | 20.4 ± 0.8 | 37.37 | 0.88 | 21.33 | 8.68 | 46.05 | 2.25 |

Figure 6. Comparison of the antibacterial performance of a Ti surface, an Ag coating and an Ag-TiO₂ coating with (a) E. coli and (b) S. aureus (N=15, error bars = SE).
(p < 0.05), respectively, compared with the Ti surface and the Ag coating (Figure 6b).

In order to observe bacterial adhesion behavior on the different substrata, the Ti disks and the Ag-TiO₂ coatings were observed with SEM after immersion in the suspensions of *E. coli* and *S. aureus* for 24 h. Figure 7a and b show typical SEM images of *E. coli* adhered on the pure Ti disk and the Ag-TiO₂ coating, respectively. A large number of the rod-shaped *E. coli* cells formed on the Ti surface with a size of ~4 μm × 0.3 μm (Figure 7a); while a few *E. coli* cells formed on the Ag-TiO₂ surface with a size of ~1 μm × 0.3 μm (Figure 7b). Figure 7c shows that a large number of spherical *S. aureus* cells formed on the pure Ti and Figure 7d shows a few *S. aureus* cells formed on the Ag-TiO₂ coating. These results indicate that the cells did not prefer to attach to the Ag-TiO₂ coatings compared to the Ti surface.

### 3.5. Bacterial removal assays

The experimental results showed that the removal rates of *E. coli* and *S. aureus* from the Ag and the Ag-TiO₂ coatings were higher than that from the pure Ti surface (p < 0.05) due to the bactericidal properties of the Ag and the Ag-TiO₂ coatings. Figure 8 shows a comparison of the Ti, the Ag and the Ag-TiO₂ coatings (TiO₂: 1.5 g l⁻¹) on bacterial removal rates at the contact times of 1, 24, 72 and 168 h, respectively. Figure 8a also indicates that the *E. coli* removal rate increased to the highest amount (89.0% ± 6.5) when the contact time reached 72 h (p < 0.05). Similar results were obtained for *S. aureus* (Figure 8b).

### 3.6. Effect of surface energy on bacterial adhesion and removal

Figure 9a and c show the influence of the electron donor surface energy (γ₉) of the seven surfaces (see Table 4) on the adhesion of *E. coli* and *S. aureus* cells for the contact times of 1, 24, 72 and 168 h, respectively. The number of adhered bacteria decreased with increasing the γ₉ surface energy of the coatings. The correlation coefficient R² values were in the range of 0.71–0.85. Figure 9b and d show that the number of adhered bacteria increased with increasing the γ₉/LW/γ₉ ratio (CQ ratio). The results indicate that there was a good correlation between bacterial adhesion and the γ₉/LW/γ₉ ratio. The correlation coefficient R² values were in the range of 0.72–0.91.

Figure 10a and c show the effect of the γ₉ values of the coatings on the removal of *E. coli* and *S. aureus* biofilms.
were much higher than that from the Ag coating. This finding explains why the Ag-TiO$_2$ coatings performed better than the Ag coating against bacterial adhesion, which was consistent with the results of Motlagh et al. (2014). In addition, the surface energy also plays an important role in bacterial adhesion (Yeo et al. 2012). In general, bacteria are negatively charged, and the larger the electron donor component $\gamma_2^-$ of a surface, the more negatively charged the surface (Liu and Zhao 2011). Therefore, bacterial adhesion should decrease with increasing electron donor $\gamma_2^-$ values of the coatings if other parameters that affect bacterial adhesion are identical (Liu and Zhao 2011). Table 4 indicates that the $\gamma_2^-$ value of the Ag-TiO$_2$ coatings significantly increased due to the incorporation of TiO$_2$ nanoparticles for contact times of 1, 24, 72 and 168 h, respectively. The bacterial removal percentage increased with increasing the $\gamma_2^-$ values of the coatings. Figure 10b and d show that the removal percentage of E. coli biofilm and S. aureus biofilm decreased with increasing the $\gamma_2^{LW}/\gamma_2^-$ ratio. Again there was a good correlation between bacterial removal and the $\gamma_2^{LW}/\gamma_2^-$ ratio.

4. Discussion

In general the antibacterial efficiency of Ag-based coatings depends mainly on the release rate of Ag ions (Fordham et al. 2014). The experimental results clearly indicate that the amounts of Ag ion release from all the Ag-TiO$_2$ coatings were much higher than that from the Ag coating. This finding explains why the Ag-TiO$_2$ coatings performed better than the Ag coating against bacterial adhesion, which was consistent with the results of Motlagh et al. (2014). In addition, the surface energy also plays an important role in bacterial adhesion (Yeo et al. 2012). In general, bacteria are negatively charged, and the larger the electron donor component $\gamma_2^-$ of a surface, the more negatively charged the surface (Liu and Zhao 2011). Therefore, bacterial adhesion should decrease with increasing electron donor $\gamma_2^-$ values of the coatings if other parameters that affect bacterial adhesion are identical (Liu and Zhao 2011). Table 4 indicates that the $\gamma_2^-$ value of the Ag-TiO$_2$ coatings significantly increased due to the incorporation of TiO$_2$ nanoparticles.
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prepared using an electroless plating technique. The TiO$_2$ particles were homogenously incorporated in the Ag matrix. The TiO$_2$ content in the coatings increased with increasing TiO$_2$ concentration in the plating solution. This work demonstrated for the first time the mechanism of the synergistic effect of Ag-TiO$_2$ coatings on antibacterial efficiency. The incorporation of TiO$_2$ nanoparticles into an Ag matrix promoted Ag ion release and increased the electron donor (\(\gamma_e^{-2}\)) surface energy of the coatings, leading to the enhanced antibacterial effect of the Ag-TiO$_2$ coatings. The experimental results indicate that bacterial adhesion or the percentage removal has strong correlation with \(\gamma_e^{-2}\) or the CQ (\(\gamma_e^{-2}/\gamma_L^2\)) ratio. The number of adhered bacteria decreased with an increasing \(\gamma_e^{-2}\) value or with a decreasing the CQ ratio. The results give a clear direction for the design of antibacterial coatings for orthopedic and dental application by using the highest \(\gamma_e^{-2}\) value approach or the lowest CQ ratio approach.

5. Conclusions

A range of Ag-TiO$_2$ nanocomposite coatings with different TiO$_2$ contents on a Ti substratum were successfully prepared using an electroless plating technique. The TiO$_2$ nanoparticles increases the negative charge of the Ag-TiO$_2$ coatings. As both E. coli and S. aureus cells are negatively charged, this further explains why the Ag-TiO$_2$ coatings were repellent to the bacteria and performed much better than the Ag coating or the Ti surface against bacterial adhesion. Figure 6 also indicates that the number of adhered E. coli cells was much higher than S. aureus cells. It has been reported that different surface energies cause different bacterial adhesion due to bacteria being repelled from the surface by charge similarities when coming closer to the surface (Liu et al. 2015). Table 4 shows that the \(\gamma_e^{-2}\) value of S. aureus (21.33 mJ m$^{-2}$) is much higher than that of E. coli (10.31 mJ m$^{-2}$). This explains why the S. aureus is relatively more repellent to the coatings. Liu and Zhao (2011) demonstrated that the ratio of LW apolar to electron donor surface energy components of substrata (\(\gamma_L^2/\gamma_e^{-2}\), the CQ ratio) controls bacterial attachment and removal using the extended DLVO theory. Figures 9 and 10 further demonstrate the relationship experimentally.

Figure 10. Effect of the surface energy components \(\gamma_e^{-2}\) and \(\gamma_L^2/\gamma_e^{-2}\) on the removal of E. coli and S. aureus biofilms (\(N=15\), error bars = SE).

Disclosure statement

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