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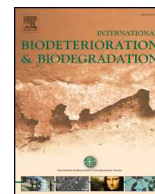
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Effectiveness of titanium nitride silver coatings against *Staphylococcus spp.* in the presence of BSA and whole blood conditioning agents

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ABSTRACT

Implanted medical devices are at risk of developing an infection at the surgical site. Once a medical implant is inserted, it initially becomes coated by a conditioning film, followed by bacterial retention. In the present study, medical grade stainless steel substrata were coated with titanium nitride (TiN) or titanium nitride/silver (TiN/14.94 at.%Ag or TiN/19.04 at.%Ag). Surface analysis determined that with increased silver concentration, silver nanoparticles were heterogeneously distributed throughout the coatings. The effect of bovine serum albumin or whole blood conditioning agents on the antimicrobial activity and microbial retention were determined using *Staphylococcus aureus* or *Staphylococcus epidermidis*. The presence of the conditioning agents reduced the antimicrobial effect of the surfaces against *S. aureus*. When the cells and conditioning agents were applied together, a reduction in bacterial retention and conditioning film was observed. These results suggest that the impact of conditioning agents should be considered since conditioning films may reduce bacterial retention but may also decrease the antimicrobial properties of the surface coatings.

1. Introduction

External fixations are essential components of modern orthopaedic surgery. For example, orthopaedic devices such as fine-wire fixators and external fixators are commonly used for the treatment of long bone fractures and pelvic fractures for both adults and children (Ktistakis et al., 2015). However, external fixations are associated with a high incidence of pin tract infection rates (Ktistakis et al., 2015; Schalamon et al., 2007). Indeed, the surface of medical devices and implants provides an artificial interface on which bacteria can aggregate to form a biofilm (Gristina, 1987; Lindsay and von Holy, 2006). Biofilm infections are associated with chronic infection, which are recalcitrant to traditional antibiotic therapy (Costerton et al., 1999). The excessive use of antibiotics has led to the emergence of antibiotic-resistant pathogens (Neu, 1992) and is why alternative strategies to combat bacterial growth and transmission have been investigated. Zander and Becker (2018), stated that the two main categories of strategies currently employed for preventing the infection of biomedical devices are either antimicrobial or antifouling. Some pathogenic strains of common skin

microbiota species, such as *Staphylococcus aureus* and *Staphylococcus epidermidis*, can grow in these biofilms and also be involved in pin tract infections. For example, Schalamon et al. (2007) found that among 37 external fixations placed on 30 children, 19 (52%) led to at least one infection. *S. aureus* and *S. epidermidis* were found in 33% and 22% of the paediatric pin tract infections respectively.

Some metals are known for their antimicrobial properties (Cyphert and von Recum, 2017). Therefore, the use of metal coatings for preventing infection of surfaces has been previously considered. Indeed, studies have shown that coating some metals, such as stainless steel with titanium can reduce bacterial attachment/retention, and/or have antimicrobial properties (Whitehead et al., 2015). Stainless steel and titanium have been considered in depth since they are the most common materials used to produce pins or wires used in bone fixing (Galanakos et al., 2009). It has been suggested that infection rates seem to be higher for stainless steel alone, compared to titanium alloys (Veerachamy et al., 2014). Previous research has demonstrated that silver coated pins decreased bacterial colonisation and pin tract infection both *in vitro* and *in vivo* (Bosetti et al., 2002). However, some silver

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impregnated structures have also demonstrated stronger bacterial adhesion, whilst still presenting an increased incidence of dead cells (Whitehead et al., 2011).

Thus, there is some debate as to which substrata provide the most beneficial surfaces. Following insertion of the implant, a conditioning film forms rapidly on the surface, as proteins such as fibrinogen are adsorbed onto the substratum of the device (Hohmann et al., 2015). The exact format of the conditioning film is dependent on the surface properties of the implanted biomaterial, such as hydrophobicity and topography (Whitehead and Verran, 2015). Organic films may also modify the impact of the coatings on microbial attachment/retention and may alter their antimicrobial activities. To the authors' knowledge, the effect of a conditioning film on bacterial retention and antimicrobial activity on TiNAg coatings, i.e., nanocomposite coatings containing silver particles in a titanium nitride matrix, has not been previously described. For this study, two conditioning agents were used; bovine serum albumin (BSA) and whole blood (WB). BSA was used since it is representative of plasma proteins. Whole blood proteins are involved in conditioning film formation on implant surfaces (Hohmann et al., 2015).

The aim of this work was to determine the effect of two conditioning agents on the retention and antimicrobial activity of a range of surfaces (medical grade stainless steel, titanium nitride (TiN), TiN/14.94 at.% Ag and TiN/19.04 at.% Ag). This information may help to determine if such coatings have the potential to be used to reduce infections in bone fixation devices.

2. Materials and methods

2.1. Substrata

The surfaces were prepared according to Whitehead et al. (2010a,b). In brief, using a guillotine, 10 mm × 10 mm coupons of stainless steel (SS) were cut. Coatings were deposited onto the stainless steel coupons, (titanium nitride (TiN), titanium nitride with 14.94% silver (TiN/14.94 at.%Ag) and 19.04% (TiN/19.04 at.%Ag) using an adapted magnetron sputtering method (Whitehead et al., 2010a,b).

2.2. Energy Dispersive X-ray Spectroscopy (EDX)

To determine the presence of an element in the sample the EDX spectra were compared with known characteristic X-ray energy values. Between approximately 0.1 to a few atoms percent can be detected using this method depending on the sample matrix and the element (Whitehead et al., 2011b). The samples were attached to stainless steel stubs using carbon tabs (Agar Scientific, UK).

Scanning electron microscopy was used to image the surfaces using a Zeiss Supra VP40 field emission gun scanning electron microscope. EDX was performed on the samples to determine the chemical composition of the coatings (EDAX Trident) using an EDAX Sapphire Si (Li) detector, and the data was quantified using a standardless ZAF algorithm. The chemical composition was calculated as percentage weight ($n = 3$) (Vaidya et al., 2018).

2.3. Atomic force microscopy

An atomic force microscope (AFM) (Explorer, Veeco Instruments, UK) was used in contact mode using pyramidal shaped, silicon nitride tips to obtain the images using a scan rate of $20.03 \mu\text{m s}^{-1}$ with 300-pixel resolution. Cantilever spring constants of 0.05 N m^{-1} were defined by the manufacturer ($n = 15$).

2.4. Bacterial preparation

The microorganisms *Staphylococcus aureus* NCTC 8532 and *Staphylococcus epidermidis* NCTC 11047 were used throughout this

study. Stock cultures were stored at -80°C and when required the cultures were thawed and inoculated onto Nutrient Agar (NA) media (Oxoid, UK) and incubated for 24 h at 37°C . The inoculated agar plates were kept refrigerated at 4°C and replaced every four weeks to maintain the genotype.

Sterile nutrient Nutrient Broths (NB) (Oxoid, UK) (10 mL) were inoculated with *S. aureus* or *S. epidermidis* and incubated overnight in an orbital incubator (200 rpm) at 37°C for 24 h. The cultures were removed from incubation and the cells were washed in sterilized, membrane filtered water (MilliporeElix, USA) (10 mL) by centrifuging at 540 g for 10 min. The supernatant was removed and the cells were re-suspended in sterile distilled water. The cells were diluted to an optical density (OD) of 1.0 ± 0.05 at 540 nm using a spectrophotometer (Jenway 6305, UK), calibrated against sterile distilled water. Cell numbers were determined in colony forming units/mL (CFU/mL) using serial dilutions and were determined to be $9.72 \pm 1.3 \times 10^7$ cells for *S. epidermidis* and $1.2 \pm 0.2 \times 10^8$ cells for *S. aureus*.

2.5. Conditioning agents preparation

A 10% v/v concentration of conditioning film was chosen since previous work in our laboratories demonstrated that this was the optimal concentration to obtain detection of such proteins on the surfaces without producing an excessively thick conditioning film layer (Whitehead et al., 2010a,b). Powdered bovine serum albumin (BSA) (Sigma, UK) was dissolved in sterile dH_2O to obtain a 10% (w/v) solution which was filter sterilized (PALL[®] Acrodisc[®] 32 mm syringe filter, $0.2 \mu\text{m}$ Supor membrane[®]). Sterile horse blood, donated as whole blood (WB) (TCS Biosciences, UK) was diluted to 10% (v/v) using sterile dH_2O .

2.6. Retention assays

Twenty five millilitres of cell suspension alone or mixed with conditioning agent was gently poured over the coupons which had been placed into glass Petri dishes. To obtain the cell suspension with conditioning agent, 12.5 mL of the OD of 1.0 bacterial suspension and 12.5 mL of either 10% BSA or 10% blood plasma solution was mixed together and incubated without agitation for 1 h at 37°C . Following incubation, the coupons were rinsed once for 5 s using a drip lock bottle at a 45° angle with sterile dH_2O and air dried in a microbiological class 2 hood. The numbers of cells retained was determined by epifluorescence, taking into account the dilution effect of the conditioning agent.

A 1:1 ratio of Rhodamine B (0.1 mg/L) and 4',6-diamidino-2-phenylindole (DAPI) (0.1 mg/L) was prepared (Whitehead et al., 2009a) and 10 μL of the mix was spread across the coupons. Following staining, the coupons were viewed using either DAPI 330–380 nm or rhodamine B 590–650 nm filters (Nikon Eclipse E600, UK with F view-II black and white digital camera, Soft Imaging System, UK). The percentage coverage of cells was calculated (Cell F software, UK) and recorded ($n = 60$).

2.7. Microbial adhesion to solvents (MATS) assays

In order to determine the relative hydrophobicity of the microbial cells, MATS assays were carried out (based on an assay by Bellon-Fontaine et al., 1996). Bacteria were centrifuged at 540 g for 10 min, then washed 3 times using pH 7.1 PUM buffer ($22.2 \text{ g L}^{-1} \text{K}_2\text{HPO}_4 \cdot 3\text{H}_2\text{O}$, $7.26 \text{ g L}^{-1} \text{KH}_2\text{PO}_4$, $.8 \text{ g L}^{-1}$ urea and $0.2 \text{ g L}^{-1} \text{MgSO}_4 \cdot 7\text{H}_2\text{O}$). Cells were re-suspended to an OD 1.0 at 400 nm. To a round bottomed test tube 15 mm in diameter, 1.5 mL volume of washed cells suspended in PUM buffer was added. Two hundred and 50 μL of one of the test chemicals, decane (BDH, UK), hexadecane (Sigma, UK), ethyl acetate (Sigma, UK) or chloroform (Sigma, UK) was added to the suspension which was incubated at 37°C for 10 min. Following

vortexing for 2 min the mixture was incubated again at 37 °C for 30 min. The optical density of the lower aqueous phase was determined (400 nm). To determine the cell surface adhesion to the solvent:

$$Adhesion = \left(1 - \frac{A}{A_0}\right) \times 100 \quad [1]$$

where *A* was the optical density measured at 400 nm of the extracted lower aqueous phase; *A*₀ was the optical density of the microbial suspension (*n* = 3).

2.8. MATS assay in the presence of a conditioning agent

The microbial adhesion to solvents assay was followed with the following modifications. Prior to testing, 7.5 mL of standardised bacterial suspension (OD 1.0 at 540 nm) and 3.25 mL of 10% bovine serum albumin (Sigma, UK) was vortexed for 1 min. The mixture was centrifuged at 540 g for 10 min and rinsed once with 10 mL PUM buffer, and was re-centrifuged. The final pellet was diluted to an OD of 1.0 at 400 nm in PUM buffer before testing (*n* = 3). This assay was not carried out using whole blood, since the presence of the blood cells interfered with the results.

2.9. Zone of inhibition assays (ZoI)

S. aureus or *S. epidermidis* (100 µL) was spread across the surface. For the ZoI with conditioning agents only, 10 µL of 10% conditioning agent (BSA or WB) was spread onto the coupon surfaces using a sterile pipette tip and dried in a microbiological class II flow hood for 1 h. The substrata with or without conditioning agent was placed surface down on to the bacterial lawn. The agar plates were incubated overnight at 37 °C. The presence of bacterial clearance around the coupons was measured with callipers correct to 0.01 mm (*n* = 6).

2.10. Statistical analysis

Statistics were carried out using two tailed T-tests using Excel. The results were reported as a mean ± standard error. Variance seen within the data was considered significant if *p* < 0.05.

3. Results

3.1. Surface characterisation

EDX analysis of the surfaces demonstrated the chemical composition of the coatings (% weight) including the concentration of the silver which was determined to be 14.94%at.wt and 19.04%at.wt (Table 1). AFM images showed that with increased silver concentration, the silver nanoparticles were heterogeneously distributed throughout the TiN matrix, thus demonstrating that the surface coatings were not chemically homogenous in composition (Fig. 1c and d). In order to quantitatively assess the topographical heterogeneity of the surfaces, line profiles were taken from the AFM images (Fig. 1). The addition of the TiN and TiNAg coatings changed the nanotopography of the surface of the stainless steel, resulting in higher/wider peaks and deeper valleys than was found on the stainless steel. The stainless steel demonstrated the smallest width (0.8 nm–8.86 nm) and depth of the valleys

(0.52 nm–7.3 nm) and the smallest peak widths (4.43 nm–17.72 nm) and peak heights (2.4 nm–8 nm) (Fig. 2a). This was followed by the TiN19.04 at.%Ag which demonstrated valley widths of 23 nm–104.23 nm, valley depths of 18 nm–26.53 nm (Fig. 2b), peak heights of 9.87 nm–61.75 nm and peak widths of 60 nm–271.91 nm (Fig. 2a). The TiN14.94 at.%Ag surface demonstrated the greatest valley widths of 4.86 nm–418.25 nm (Fig. 2). The TiN demonstrated the largest surface features for all the parameters tested (valley depth 17.5 nm–128.7 nm, peak width 4.33 nm–316.09 nm and peak height 3.58 nm–139.43 nm), with the exception of the valley width parameter (8.67 nm–95.13 nm).

3.2. Retention assays

Retention assays were carried out in the presence of the bacteria or conditioning agents alone, or in bacterial – conditioning agent combinations (Fig. 3). In the absence of conditioning agent, bacterial numbers were greater for *S. aureus* retained on the different coatings (range 4.29%–6.11%), than for *S. epidermidis* (range 1.75%–3.14%) although only the *S. epidermidis* alone was statistically significant on the surfaces when compared to the *S. aureus* (*p* < 0.05). The conditioning agents retained in greatest amounts were found to be statistically significant (*p* < 0.05) on the stainless steel surfaces (BSA = 34.80 ± 6.54%, WB = 17.28 ± 3.95%), but in lower amounts on the titanium coatings with silver (BSA = 4.23 ± 0.11%, WB = 6.24 ± 1.55%, and BSA = 3.32 ± 0.53%, WB = 11.38 ± 2.25%, for TiN/14.94 at.%Ag and TiN/19.04 at.%Ag respectively). On the TiN coating without silver no conditioning film was detected. Interestingly, when the cells and conditioning agents were tested together, the percentage coverage of both types of cells was not significantly different (*p* > 0.05) on the TiN and TiNAg surfaces for both conditioning agents (*S. aureus* and *S. epidermidis* between 0.88 ± 0.04%–0.14 ± 0.01% and 0.88 ± 0.04–0.16 ± 0.01% respectively) but was significantly lower than when the cells were tested alone, with the exception of *S. epidermidis* in the presence of BSA on the SS surface (4.22 ± 0.82%) (*p* > 0.05). Overall, most fouling, except for the TiN coatings, occurred when the conditioning agents were used alone.

3.3. Microbial adhesion to solvent (MATS) assays

The MATS assays was used to determine the physicochemistry of the bacteria in the presence and absence of BSA. This assay was unable to be carried out in the presence of whole blood since the presence of the red blood cells interfered with the results (Fig. 4). In the absence of conditioning agents, both species demonstrated the greatest adhesion to the acidic polar solvent chloroform (94.82 ± 1.25% and 92.05 ± 5.27% for *S. aureus* and *S. epidermidis* respectively). Both species also demonstrated high adhesion to the apolar n-alkanes decane (94.27 ± 1.22% and 85.78 ± 5.85%) and hexadecane (89.37 ± 4.46% and 90.21 ± 3.33%) (*S. aureus* and *S. epidermidis* respectively). The affinity of both species to adhere to the non-polar hydrocarbons decane and hexadecane was high (> 55%), demonstrating that both bacterial species were highly hydrophobic and were strong electron donors (Fig. 4). However, the electron status of the organisms could be argued to be both donating and accepting, since both organisms adhered to the acidic solvent chloroform in greater

Table 1
EDX analysis of the metal coupons in percentage weight ± standard error.

	C	O	Si	Cr	Fe	Ni	Ti	N	Ag
SS	5.91 ± 0.66	2.86 ± 0.18	0.61 ± 0.08	16.60 ± 0.63	63.51 ± 0.92	10.51 ± 0.04			
TiN							62.94 ± 0.91	37.06 ± 0.91	
TiN/14.94 at.%Ag							58.34 ± 0.17	26.72 ± 0.23	14.94 ± 0.14
TiN/19.04 at.%Ag							42.97 ± 1.76	37.99 ± 1.52	19.04 ± 0.24

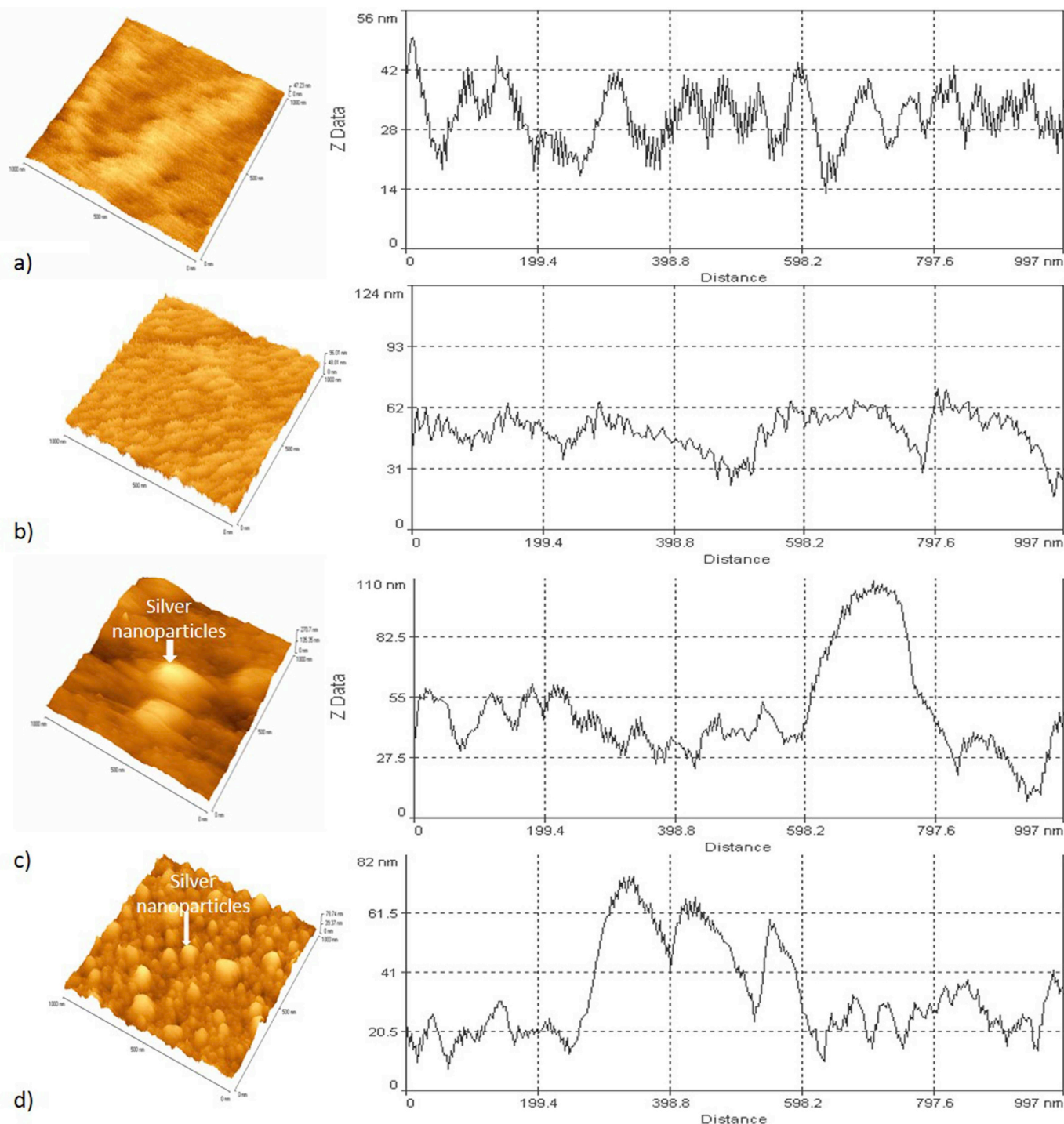


Fig. 1. Atomic force microscopy and line profiles of a) stainless steel, b) titanium nitride, c) TiN/14.94 at.%Ag and d) TiN/19.04 at.%Ag demonstrating surface microtopographies, and shape of the surface features.

numbers than to the basic solvent, ethyl acetate, demonstrating a higher likelihood of donating electrons rather than accepting them, thus the microorganisms were likely to be capable of exhibiting both properties. The hydrophobicity of the organisms was also demonstrated by the higher combined affinity to the non-polar hydrocarbons (decane and hexadecane) than to the polar solvents (chloroform and ethyl acetate).

When both species were exposed to a sterile 10% BSA solution prior to performing the MATS assay (Fig. 4), they demonstrated significant reductions in the adherence to chloroform ($46.08 \pm 11.96\%$ and

$21.65 \pm 3.95\%$ for *S. aureus* and *S. epidermidis* respectively), decane ($29.78 \pm 8.71\%$ and $15.93 \pm 2.33\%$) and hexadecane ($46.87 \pm 13.00\%$ and $17.22 \pm 3.33\%$). However, the adherence to ethyl acetate decreased only in the case of the *S. epidermidis* strain. These results therefore demonstrated both a reduction of the hydrophobicity and a reduction in the ability to donate electrons, for both strains. Further, the combined adherence to the polar solvents (chloroform and ethyl acetate) exceeded that to the non-polar hydrocarbons (decane and hexadecane), confirming an increase in the hydrophilicity for both strains.

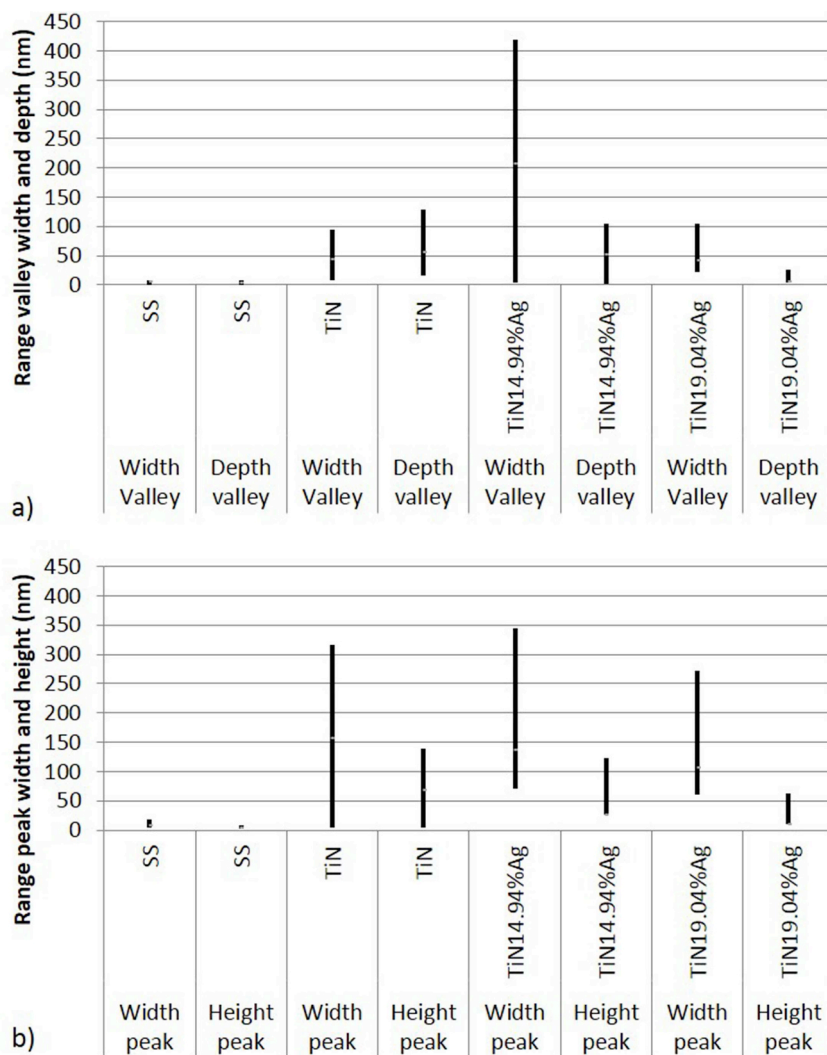


Fig. 2. Dimensions of surface a) peak widths and heights and b) valley depth and widths.

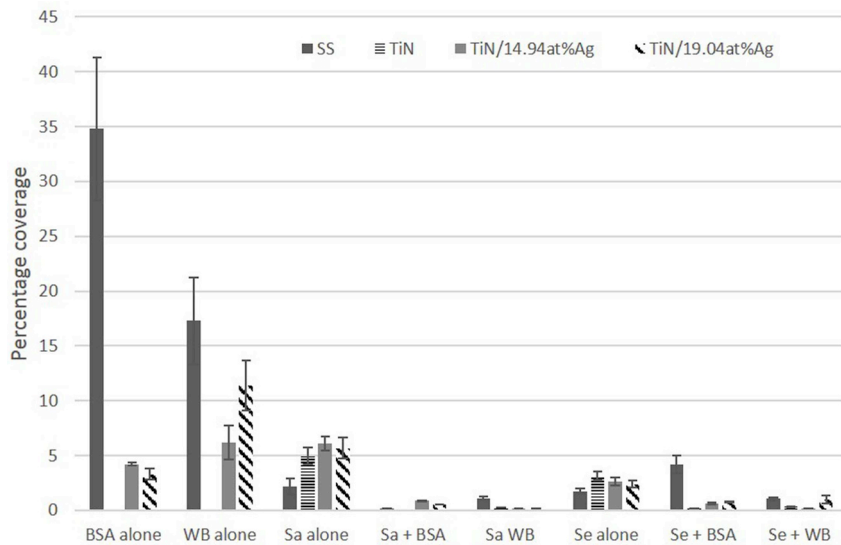


Fig. 3. Percentage coverage of cells and/or conditioning agent retained on the surfaces. *S. aureus* (Sa) or *S. epidermidis* (Se) without conditioning agent, in the presence of BSA, or in the presence of whole blood (WB), or BSA or WB conditioning agent alone.

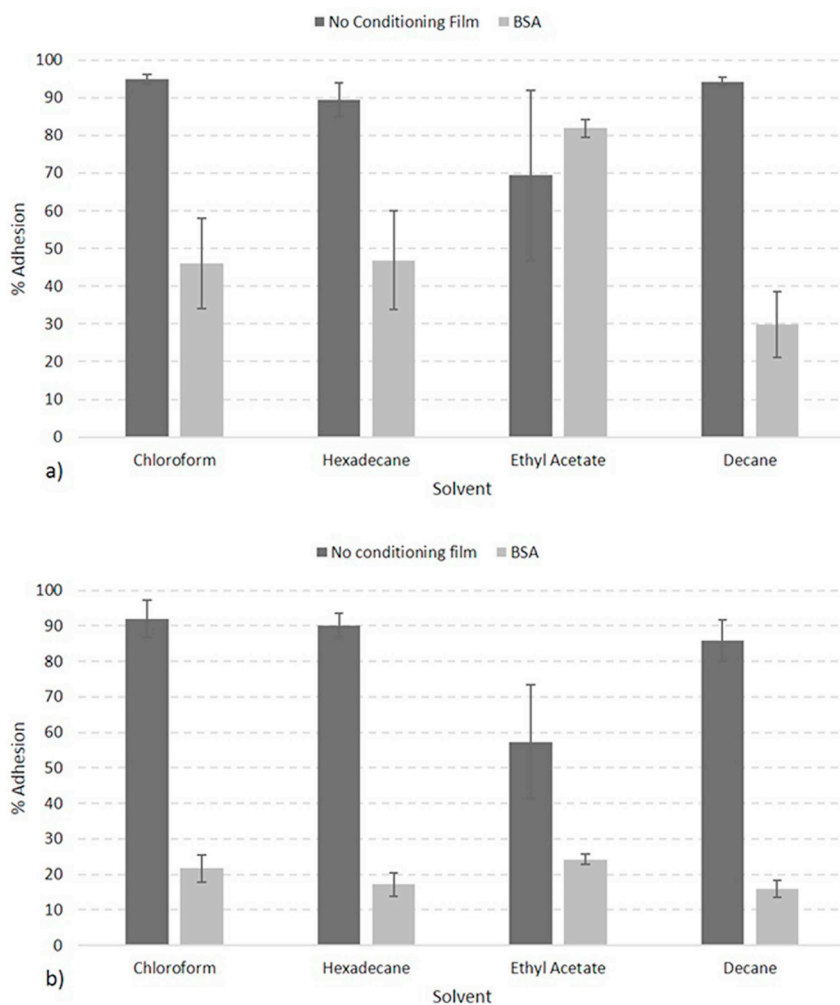


Fig. 4. MATS assays for a) *S. aureus* and b) *S. epidermidis* in the presence of BSA.

3.4. Antimicrobial activity

Zones of inhibition were carried out to determine the antimicrobial activity of the surfaces in the presence and absence of conditioning agents (Fig. 5). Stainless steel and titanium nitride coupons did not demonstrate antibacterial properties against the bacteria in the

presence or absence of conditioning agent (Fig. 5). In the absence of conditioning agents, the TiN/19.04 at.%Ag (0.31 ± 0.02 mm) coating demonstrated a significantly more pronounced effect than the TiN/14.94 at.%Ag coating (0.06 ± 0.003 mm) against *S. epidermidis* ($p < 0.05$), but not in the case of *S. aureus* ($p > 0.05$). In the presence of WB or BSA, there was a negligible ZoI effect demonstrated for the

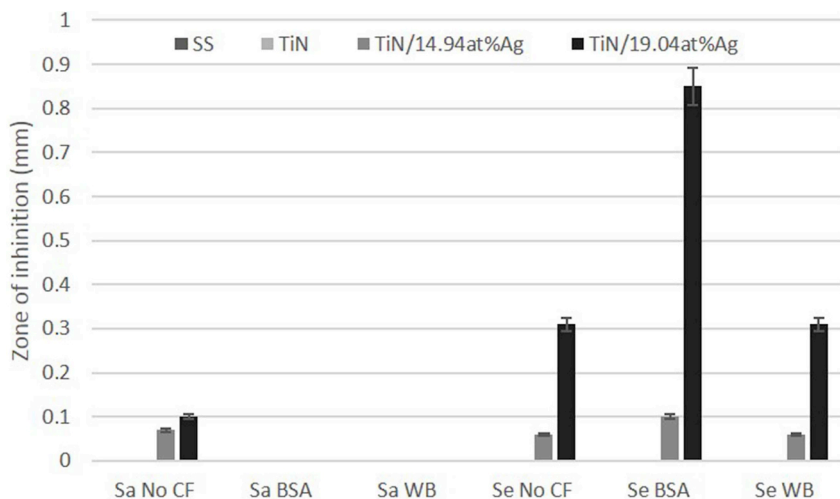


Fig. 5. Zone of inhibition assays of the surfaces against *S. aureus* (Sa) or *S. epidermidis* (Se) without conditioning agent, in the presence of BSA, or in the presence of whole blood (WB).

TiN/14.94 at.%Ag and TiN/19.04 at.%Ag coatings against *S. aureus* (0.1 ± 0.005 mm on TiN/14.94 at.%Ag; 0.07 ± 0.004 mm on TiN/19.04 at.%Ag). However, in contrast, in the presence of conditioning agents, the BSA conditioning agent did have an enhanced antimicrobial effect against *S. epidermidis*. On the TiN/14.94 at.%Ag coating, in the presence of BSA (0.1 ± 0.005 mm), the results demonstrated similar ZoI to when BSA was not present (0.06 ± 0.003 mm). On the TiN/19.04 at.%Ag coating, when BSA was present, a significantly greater ZoI was demonstrated (0.85 ± 0.04 mm) ($p < 0.05$). In the presence of WB, similar ZoI were demonstrated on both the TiN/14.94 at.%Ag (0.06 ± 0.003) and TiN/19.04 at.%Ag (0.31 ± 0.016) coatings to those without WB present for *S. epidermidis*. Thus, overall the addition of the conditioning agents decreased the antimicrobial activity against *S. aureus*, but did not affect it (WB) or improved it (BSA) against *S. epidermidis*.

4. Discussion

The use of external fixators are common for the treatment of some fractures, such as long bone fractures and pelvic fractures, and infections related to the use of these biomedical devices have been recorded (Ktistakis et al., 2015; Schalamon et al., 2007). Preventing bacterial colonisation of biomedical devices is a key concept to reduce infection incidence after orthopaedic surgery operations. However, it is important to determine the effect of conditioning films that may be retained on coatings or on surfaces that could be used to produce biomedical devices, since they may alter the antimicrobial properties of the surfaces, and increase/decrease bacterial retention. In this study, the retention and antimicrobial capabilities of stainless steel, TiN or TiN coated with different amounts of silver, in the presence and absence of conditioning agents and in the presence/absence of microorganisms were determined.

4.1. The effect of surface properties on biofouling

Overall, it was demonstrated that the low surface roughness of the TiN surface in comparison to the other surfaces used in this study may have influenced the reduced amount of conditioning film attachment. The addition of the conditioning agent and bacteria together to the surfaces reduced the number of bacteria and the amount of conditioning agent retained. This effect could not be attributed to the surface topography but may be in part attributed to the changes in the physicochemistry demonstrated when the bacteria were subjected to the conditioning agent. Further, the addition of the conditioning agent reduced the antimicrobial activity of the silver containing surfaces against *S. aureus* but not against *S. epidermidis* suggesting that a component of the conditioning agents may have protected the *S. aureus* against the antimicrobial action of the surfaces. However, further work is necessary to determine the mode of action of these biochemical processes. The reduction in the antimicrobial activity of the surfaces against *S. aureus* and the decrease in the numbers of cells retained on the surfaces demonstrated that novel coatings should be tested in the presence of a conditioning agent to determine their effect on the retention of the bacteria and to ensure that the antimicrobial efficacy of the surface is maintained.

4.2. The effect of physicochemistry on surface biofouling

In previous studies it has been shown that the presence or absence of a conditioning film could increase, decrease, or even have no impact on bacterial retention (Linnes et al., 2012). One explanation might be that conditioning film and cell retention are influenced, at least in this study, by the effects that the conditioning film has on the physicochemistry of the surface and the cells. In the presence of a conditioning film, the surfaces could become more wettable (Whitehead et al., 2009b). This may in part explain why the presence of

conditioning agents reduced the bacterial adhesion to the surfaces. Therefore, rather than encourage microbial adhesion, the presence of conditioning agent proteins marginally reduced cellular adhesion to a surface, and this interesting factor should be taken into consideration when selecting materials for use.

Bacterial retention might also rely on hydrophobic properties of wall cell proteins, which were possibly modified by the conditioning agent components. Our results showed that before the addition of the conditioning agent, both bacterial species were highly hydrophobic. When both species were exposed to the BSA conditioning agent, they became more hydrophilic and electron accepting. Fewer cells were retained on the surfaces in presence of BSA. This may be due to the conformation of the proteins on the metallic surfaces and on the cells; if they exhibit similar properties when exposed to BSA they may repel one other.

Another explanation for these results is that the conditioning agent components and *Staphylococcus* cell wall proteins might compete with each other for binding sites on the surfaces. Indeed, cells and conditioning agents interact with surfaces with both specific (ligand-receptor) and non-specific interactions (van der Waals, electrostatic, and hydrophobic interactions) (Senaratne et al., 2005). Albumin has been shown to suppress initial bacterial adhesion to surfaces, which has been suggested to be due to the lack of specific interactions between the albumin and the bacteria (Linnes et al., 2012). Kinnari et al. (2005) demonstrated that binding of *S. aureus* on human serum albumin-coated surfaces was significantly inhibited (from 82 to 95% depending on concentration). Xu et al. (2008) reported that BSA adsorption to either fibronectin-coated substrata or *S. aureus* cell surfaces reduced *S. aureus* bacterial adhesion on fibronectin, and suggested that BSA blocked both nonspecific and specific adhesion/adsorption sites. Grześkowiak et al. (2011) also suggested that mechanisms other than hydrophobic interactions were involved in the binding process between bacteria and BSA, which led to the inhibition of bacterial adhesion to this protein. Similarly, other proteins found in WB, such as fibronectin and fibrinogen, can bind to some metallic surfaces and/or bacterial surfaces, which can lead to an increase or a decrease of bacterial adhesion on the surface (An and Friedman, 1998). Indeed, fibronectin and fibrinogen deposited on silicon catheters in similar concentrations found in plasma resulted in decreased retention of *S. epidermidis*, but increased retention of *S. aureus* (Espersen et al., 1990).

4.3. Conditioning agent effects on the antimicrobial properties of the coatings

In addition to the retention capability, the antimicrobial properties of surfaces play a key role in reducing surface contamination (Cyphert and von Recum, 2017). Following the ZoI assays it was demonstrated that stainless steel and TiN did not display antimicrobial activity against the bacteria. However, an antimicrobial effect was observed when the TiN was incorporated with silver, and the higher silver concentration (TiN/19.04 at.%Ag) displayed a higher antimicrobial activity when compared with a lower silver concentration (TiN/14.94 at.%Ag). Previous studies have demonstrated that a concentration of silver higher than 4.6% in TiN/Ag coatings significantly reduced the amount of viable *Pseudomonas aeruginosa* and *Staphylococcus aureus* cells compared with TiN coatings without silver (Kelly et al., 2009). In this study, the presence of BSA increased the antimicrobial activity of the TiN/19.04 at.%Ag 3-fold compared with the absence of a conditioning agent or presence of whole blood against *S. aureus*. This suggests that the BSA may have resulted in an adjuvant effect on the action of silver against *S. epidermidis*. In the presence of conditioning agents, no antimicrobial effect was demonstrated on *S. aureus*. This may suggest a specific protective effect from the conditioning agent on the *S. aureus* bacteria, suggesting that each strain may act differently in the presence of conditioning agents and thus they need independent consideration.

5. Conclusion

The presence of the conditioning agents resulted in differences in the antimicrobial effect of the surfaces, and even though the bacteria used in this work were both *Staphylococcus* spp. The conditioning agents also interacted with the bacteria in different ways, resulting in differences in retention. This is important since the addition of the conditioning agent on the surfaces clearly affects the cell surface properties which in turn affects the amount of bacterial retention, in this case deterring it. These results suggest that the impact of conditioning films should be considered when designing new surfaces since conditioning agents may either enhance or impair bacterial initial adhesion and the antimicrobial properties of surface coatings.

Declaration of interest

None.

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Appendix A. Supplementary data

Supplementary data related to this article can be found at <https://doi.org/10.1016/j.ibiod.2018.06.016>.

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