Enhanced bactericidal activity of silver thin films deposited *via* aerosol-assisted chemical vapour deposition

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Abstract

Silver thin films were deposited on SiO_2 barrier coated float glass, fluorine-doped tin oxide (FTO) glass, Activ™ glass and on a TiO_2 coated float glass *via* AACVD using silver nitrate at 350 °C. The films were annealed at 600 °C and analysed by X-ray powder diffraction (XRD), X-ray photoelectron spectroscopy (XPS), UV/Vis/Near IR spectroscopy and scanning electron microscopy (SEM). All the films were crystalline and the silver was present in its elemental form and of nanometre dimension. The antibacterial activity of these samples was tested against *Escherichia coli* and *Staphylococcus aureus* in the dark and under UV light (365 nm). All Ag-deposited films reduced the numbers of *E. coli* by 99.9% within 6 hours and the numbers of *S. aureus* by 99.9% within only 2 hours. FTO/Ag reduced bacterial numbers of *E. coli* to below the detection limit after 60 minutes and caused a 99.9% reduction of *S. aureus* within only 15 minutes of UV irradiation. Activ™/Ag reduced the numbers of *S. aureus* by 66.6% after 60 minutes and TiO_2/Ag killed 99.9% of *S. aureus* within 60 minutes of UV exposure. More remarkably, we observed a 99.9% reduction in the numbers of *E. coli* within 6 hours and the numbers of *S. aureus* within 4 hours in the dark using our novel TiO_2/Ag system.

Introduction

Significant progress has been made in recent years in tracking healthcare-acquired infections (HCAI), however, there are still many gaps in our knowledge regarding these often life-threatening infections.[1] In order to develop new methods to prevent HCAIs and preserve available antibiotics, we need to evaluate current methods and find new ways of preventing infection.[2] Whilst the incidence

of superbug outbreaks due to *Clostridium difficile* (*C. difficile*) and *methicillin-resistant Staphylococcus aureus* (MRSA) appear to have fallen in the last few years in some European countries, new multiply resistant Gram-negative bacterial pathogens have emerged.[3] In particular, a family of Gram-negative bacteria known as Enterobacteriaceae (such as *E. coli* and *Klebsiella* spp.), which are normally found in the human intestine, are an increasing global problem with strains exhibiting multiple antibiotic resistance.[4]

Several control measures can be implemented to reduce HCAIs, such as patient isolation with appropriate ventilation, improvements in hand washing and using disposable devices and equipment. Furthermore, regular and effective cleaning of hospitals is also required, however, rather than killing bacterial pathogens in the hospital environment, ineffective cleaning may simply spread them around.[5] An alternative approach is to use self-sterilising surfaces in hospitals that do not require regular cleaning to efficiently reduce the load of bacteria associated with HCAIs.[6-8]

Silver thin films have long been considered of interest in applications such as microelectronics, high temperature superconducting ceramics, silver mirrors and bactericidal coatings.[9-10] The mechanism of antibacterial activity of silver films or nanoparticles involves denaturation of cell wall proteins and a consequent decrease in growth rate.[11] Silver ions can be involved in catalytic oxidation reactions between oxygen molecules in cells and hydrogen atoms in thiol groups (forming disulfide bonds through covalent bonding) and lead to blocking of respiration and bacterial cell death.[12] Furthermore, silver nanoparticles can also damage the cell membranes of bacteria by the formation of free radicals.[13]

A variety of methods have been employed to deposit silver films on surfaces including electrostatic deposition, sputtering and evaporation, but the majority of reports have used some form of chemical vapour deposition (e.g., super critical fluid transport,[14] metal organic,[15] low pressure[10] and aerosol-assisted[10, 16]). The deposition of silver thin film *via* CVD has been greatly hampered by the lack of precursors that are stable (most are air and moisture sensitive), volatile[9] and readily available. Precursors previously used include silver halides and carboxylates, organophosphine adducts of silver and more extensively β-diketonates or related mono-thio-β-diketonates and β-diketiminate.[10] These complexes require multi-step synthesis and need to be assessed for their suitability as precursors prior to film deposition[17-18] which can be challenging since they need to have a high vapour pressure as well as react on a heated substrate.[16] Aerosol-assisted CVD (AACVD) is a proven technique for the deposition of films using precursors that are not necessarily volatile and/or thermally stable but only that they are soluble.[19-21] Furthermore, depositions do not require high

pressures and the greater availability of precursors allows more flexibility of the properties of the film makes the technique more versatile and low cost.[19, 22-23]

In this paper, we demonstrate the antibacterial activity of silver thin films deposited *via* AACVD on float glass (SiO_2 barrier coated), fluorine-doped tin oxide (FTO) glass and Activ™ glass (15 nm thick TiO_2 anatase layer) when activated by 365 nm UV radiation. In addition, a TiO_2/Ag film was deposited on float glass for comparison with Pilkington NSG Activ™ glass. To our knowledge this is the first deposition of silver films by AACVD on different substrates. We show that these robust films can be easily made by an efficient up-scalable technique. TiO_2 is a well-known photocatalytic agent that has been extensively used for destroying microorganisms including bacteria, viruses and fungi.[24] These self-sterilising films have potential applications in hospital and other environments where bacterial contamination is problematic, e.g., food processing facilities. Furthermore, the development of metal-modified TiO_2 coatings can enhance the antibacterial activity rendering these films antibacterial even in the dark.

Experimental

Deposition method. Depositions were carried out in a nitrogen atmosphere (99.99%, BOC). The glass substrates (15 cm × 4 cm × 0.3 cm) used were obtained from Pilkington Ltd (UK): SiO_2 barrier coated float (ca. 50 nm thickness; the coating prevents the ions within the glass diffusing to the surface), fluorine-doped tin oxide (FTO) and Activ™. The substrates were cleaned with detergent, propan-2-ol and acetone to remove surface grease. The precursor solution consisting of 0.50 g of silver nitrate (purchased from Sigma-Aldrich) in a 1:5 water to methanol ratio was placed in a glass bubbler. An aerosol was generated using a piezo electric device and carried, in a flow of nitrogen (1 L/min), through into horizontal bed cold-walled reactor (15 cm × 5 cm). The glass substrate, placed on the top and bottom of the reactor, was preheated to 350 ^{o}C on a graphite block containing a Whatman cartridge heater monitored by a Pt-Rh thermocouple. The gap between the top and bottom substrate was 0.5 cm to ensure a laminar flow and the top substrate was 50-70 ^{o}C cooler than the heated bottom glass substrate. Depositions were achieved by the aerosol being carried in a stream of nitrogen through a brass baffle into the reactor. Waste vapours were eliminated through the exhaust. Once the entire precursor solution had been used up the reactor was allowed to cool to room temperature under nitrogen. The films were deposited on the top substrate most likely a result of thermophoresis whereby the particles are unable to diffuse through the diffusion layer at the heated substrate and rebound to adsorb to the top surface.[25] Complete coverage of the substrate was achieved and the films were solvent annealed with methanol at 600 ^{o}C under nitrogen (0.5 L/min) for 2 hours.

A TiO_2 film was deposited onto SiO_2 barrier float glass. The precursor solution consisted of 0.50 g of titanium ethoxide (purchased from Sigma-Aldrich) in 15 mL of toluene (purchased from VWR). The deposition was carried out at 500 ^{o}C under a flow rate of 1.0 L/min nitrogen. The TiO_2/Ag film was produced by first depositing TiO_2 followed by the deposition of silver as described above. Following the deposition of silver, the film was annealed in the same way as the other silver films.

Film analysis. X-ray diffraction (XRD) measurements were obtained using a modified Bruker-Axs D8 diffractometer with parallel beam optics fitted with a PSD LynxEye silicon strip detector to collect diffracted X-ray photons. X-rays were generated using a Cu source with Cu Kα1 and Cu Kα2 radiation of wavelengths 1.54056 and 1.54439 Å, respectively, with an intensity ratio of 2:1 and at 40 kV and 30 mA. The incident beam angle was kept at 1°, and the angular range of the patterns collected was $10° < 2\theta < 66°$ with a step size of 0.05° counted at 0.5 s/step. The patterns were analysed for crystallinity and preferred orientation. Peak positions were compared to patterns from the Inorganic Crystal Structure Database (ICDS).

X-ray photoelectron spectroscopy (XPS) analysis of the films was carried out using a Thermo Scientific K-Alpha spectrometer equipped with a monochromatic Al-Kα source to identify chemical constituents. The peaks were modelled using CasaXPS software with binding energies adjusted to adventitious carbon (284.5 eV) in order to compensate for the effects of charging. Survey scans were collected in the range 0−1350 eV (binding energy) at a pass energy of 40 eV.

UV/Vis/Near IR transmittance and reflectance spectra were produced using the Perkin Elmer Precisely Lambda 950 spectrometer using an air background and recorded between 320-2500 nm.

Scanning electron microscopy (SEM) was used to determine the film morphology from a top-down configuration using a JEOL JSM-6301F Field Emission instrument with accelerating voltages ranging from 3-5 keV on Au-coated samples.

Antibacterial activity. The films were cut into 1 cm^2 sections to evaluate their antibacterial activity. The following samples were tested: float glass (Ag, TiO_2 and TiO_2/Ag), and Ag on Activ^{TM} and FTO. Comparisons were made against the same substrate without modification. The antibacterial activity of these samples were tested against *Escherichia coli* strain ATCC 25922 and *Staphylococcus aureus* 8325-4, which were stored at -70 ^{o}C in Brain-Heart-Infusion broth (BHI, Oxoid) containing 20% (v/v) glycerol and propagated onto MacConkey agar (MAC, Oxoid) for samples exposed to *E. coli*, or Mannitol Salt agar (MSA, Oxoid) for samples exposed to *S. aureus*, for a maximum of two subcultures at intervals of 2 weeks.

BHI broth was inoculated with one bacterial colony and cultured in air at 37 ^{o}C for 18 hours with shaking, at 200 rpm. The bacterial pellet was recovered by centrifugation (20 ^{o}C, 2867.2 *g*, 5 min), washed in PBS (10 mL), centrifuged again to recover the pellet (20 ^{o}C, 2867.2 *g*, 5 min), and the bacteria were finally re-suspended in PBS (10 mL). The washed suspension was diluted 1000-fold to obtain an inoculum of ~10^6 cfu/mL. In each experiment, the inoculum was confirmed by plating 10-fold serial dilutions on agar for viable counts. 25 µL of the inoculum was spread evenly on the surface of each sample in duplicate. The samples were then irradiated for up to 6 hours at room temperature using UVA (Vilber-Lourmat, 2 x 8 W, 365 nm, 0.65 ± 0.23 mW cm^{-2}) radiation and incubated under dark conditions for the same duration. Prior to inoculation, all the samples were sterilised by pre-irradiation overnight using a 365 nm UV light.

Post incubation, the inoculated samples were added to PBS (450 µL) and mixed thoroughly using a vortex mixer (20 s). The neat suspension and 10-fold serial dilutions were plated on agar for viable counts (100 µL). The plates were inoculated aerobically at 37 ^{o}C for 24 hours. Each experiment contained a minimum of two technical replicates and the experiment was repeated three times. The statistical significance of the following comparisons in both the light and dark conditions was analysed using the Mann-Whitney U test: (i) blank glass vs. inoculum; (ii) TiO_2 alone vs glass; (iii) TiO_2/Ag vs TiO_2 alone; (iv) TiO_2/Ag vs Ag alone; (v) Ag alone vs glass or Activ^{TM} or FTO.

Result and Discussion

Analysis of films

Silver thin films were deposited *via* AACVD using silver nitrate in a water/methanol solvent mixture at 350 ^{o}C under a flow of N_2. Films were deposited on SiO_2 barrier coated float glass, fluorine-doped tin oxide (FTO) glass, Activ^{TM} glass and on a TiO_2 coated float glass. TiO_2 was deposited using titanium ethoxide in a toluene solution at 500 ^{o}C under a flow of N_2. The resulting films achieved complete coverage and were subsequently annealed at 600 ^{o}C in N_2 for 2 hours before spectroscopic analysis and evaluation of antimicrobial activity.

XRD showed that all films were crystalline (Fig. 1). The patterns for the silver films confirmed the presence of bulk silver exhibiting reflections in the (111), (200) and (220) planes at 38.1, 44.3 and 64.5 2θ values, respectively, matching well with the standard pattern. Moreover, it was found that the pure Ag films were grown regardless of the substrate indicating that the substrates had no influence on the crystallographic phase as expected.

The TiO_2 pattern has a few suppressed peaks ((103), (004), (112), (105) and (213)) when compared with the standard pattern which suggests that the AACVD grown TiO_2 film has some profound

preferred orientation. TiO_2 was matched to the anatase phase having reflections corresponding to the (101), (200), (211) and (204) planes. However, previous TiO_2 thin films grown *via* AACVD using titanium tetra-isopropoxide in an ethyl acetate solution at 450 °C only showed reflections in the (101), (112) and (211) planes.[22] Hence, deposition conditions such as the temperature and the solvent used have a significant effect on preferential orientation of films grown *via* AACVD. No peaks corresponding to the TiO_2 component were observed from the pattern obtained for Activ™/Ag composite due to the thickness of the Ag layer masking the very thin (15 nm) anatase layer of Activ™ glass. An estimation of the crystallite size that was derived from the XRD data using the Scherrer equation[26] showed the Ag crystallites to be between 30–40 nm and independent of substrate. The AACVD grown TiO_2 film had a similar crystallite size (ca. 30 nm).

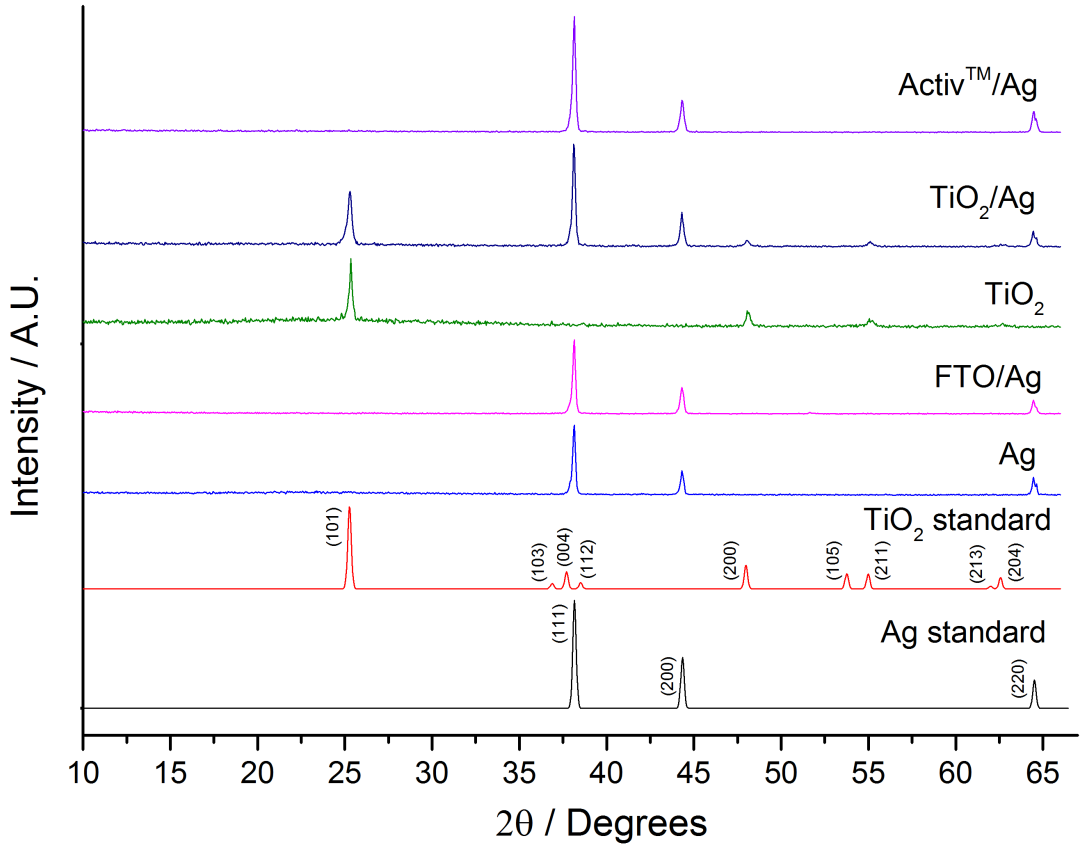

Fig. 1. XRD patterns for the standards[27-28] and for the films deposited on various substrates using AACVD. TiO_2 was deposited at 500 °C using titanium ethoxide in toluene. The silver films were deposited at 350 °C from silver nitrate in a 1:5 water to methanol solution.

Although the XPS binding energies of Ag and Ag_2O are similar,[29] it would not be unreasonable to assume, in light of the XRD patterns, that only Ag is present (Fig. 2) with no Ag_2O contamination. This is advantageous as it shows that there is no detectable surface oxidation, hence maximising the

surface area of silver available for antimicrobial activity. The binding energy of the $3d_{5/2}$ peak were 368.3, 368.3, 368.4 and 368.3 eV for Ag on float glass, FTO, Activ^{TM} and TiO_2, respectively. These values are in close agreement with that reported in the literature: 368.4 eV.[30] The binding energy of the Ti $2p_{3/2}$ was at 458.3 eV and 458.9 eV for TiO_2 and TiO_2/Ag, respectively, corresponding to Ti^{4+}.[22]

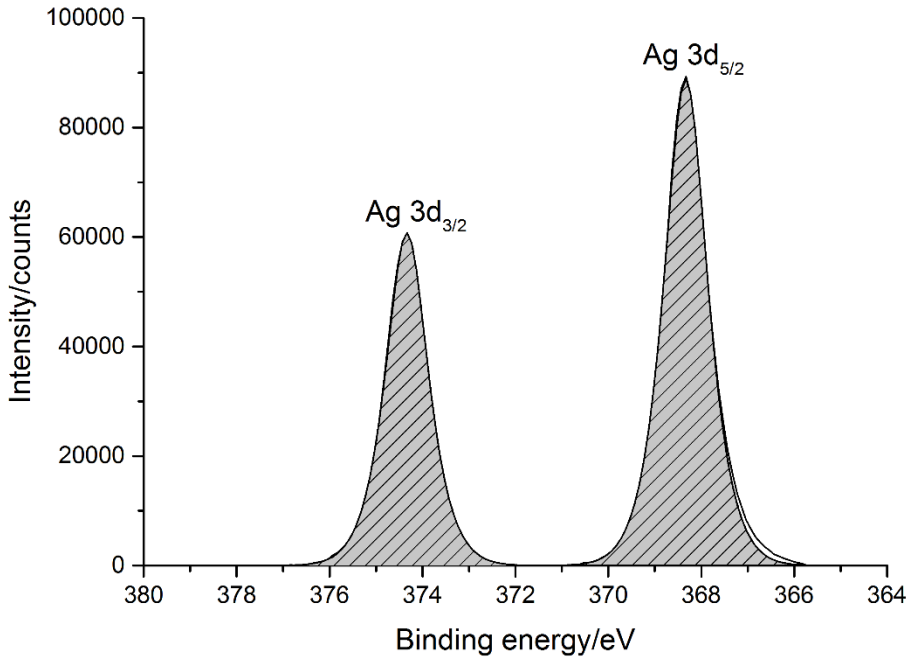

Fig. 2. XPS of silver deposited on SiO_2-barrier coated float glass showing binding energies for the Ag^0 state.

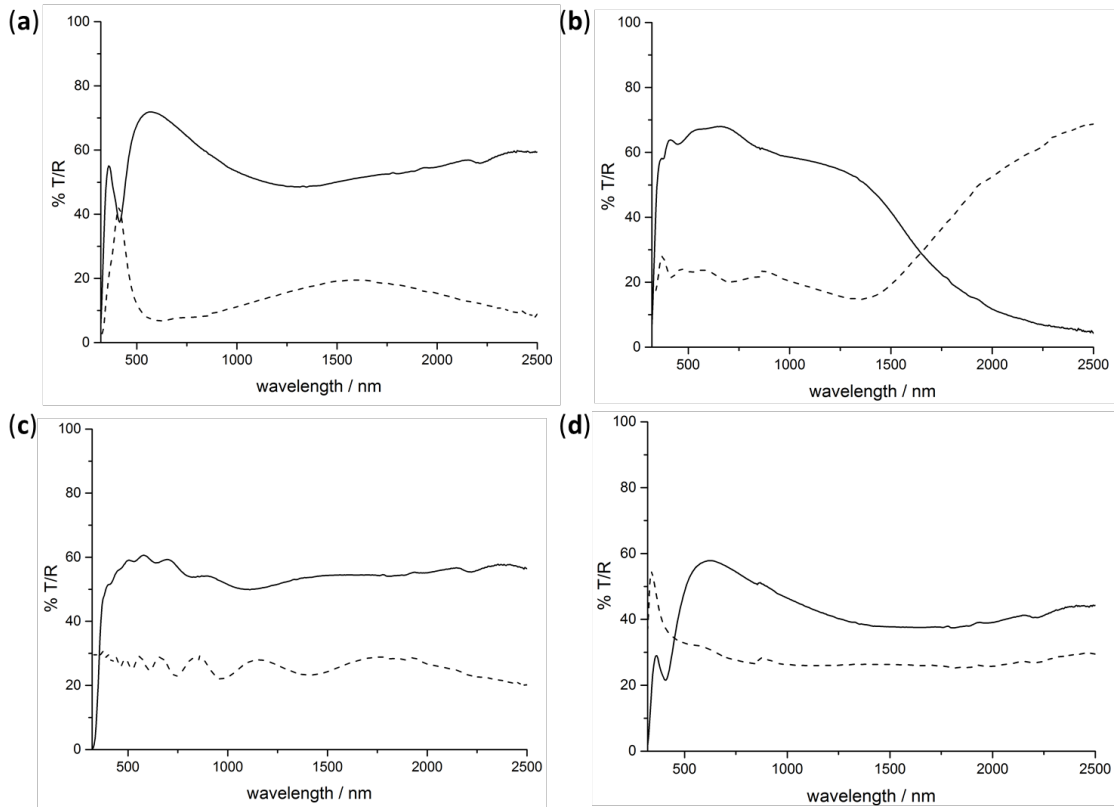

Fig. 3. The transmittance (—) and reflectance (---) properties of the Ag films deposited *via* AACVD on to different glass substrates using silver nitrate in a 1:5 water to methanol solution at 350 ^{o}C. (a) SiO_2 barrier coated float; (b) FTO; (c) TiO_2 and (d) Activ^{TM}.

The transmission and reflectance properties of the films were investigated using UV-vis spectroscopy. The greatest transparency (~70%) was observed with Ag deposited on float glass followed closely by Ag on FTO glass. However, the latter had more than twice the reflectance. The silver coated Activ^{TM} film exhibited the greatest reflectance (~30%).

In an effort to correlate light absorption to antibacterial activity, the absorbance of Ag films at 365 nm was determined from UV-vis data. The results indicated similar absorbance values for Ag deposited on float glass, TiO_2 and Activ™ of 26, 29 and 24%, respectively. These are almost double the absorbance value obtained for Ag deposited on FTO glass, suggesting a lower antibacterial activity after 365 nm UV activation in comparison to other Ag films. However, Ag on FTO glass was found to have potent bactericidal effects against *E. coli* and S. *aureus* (see table 1 and 2).

Figure 4 shows the scanning electron microscope images of the Ag films on different glass substrates: (a) float, (b) FTO, (c) TiO_2 and (d) Activ^{TM}. In general, the particles on the substrates adopt a spherical morphology and are well distributed apart from Ag on FTO, where they appear as if they have coalesced together to form non-spherical entities that are sparsely distributed. Other AACVD studies, which have deposited silver films using different precursors and deposition conditions, have

also observed spherical particles. [31-32] The particles on FTO are much larger at an average width of 7 µm than those found on the other substrates. On float glass and Activ™ the particle sizes vary from a few nanometres to roughly 1.5 µm and 750 nm in diameter, respectively. However, on the AACVD deposited TiO_2 substrate the Ag particles are even smaller, ranging from a few nanometres to 500 nm. This coupled with the dense distribution (substrate almost entirely covered) leads to the exposed Ag surface area on the TiO_2 substrate being the highest. Hence this could be responsible for the enhanced antimicrobial activity (see below) observed on TiO_2 compared to the other substrates.

It is important to note that the particles observed via SEM are made up of a collection of Ag crystallites and hence are of a much larger size than the crystallite sizes estimated by the Scherrer equation.

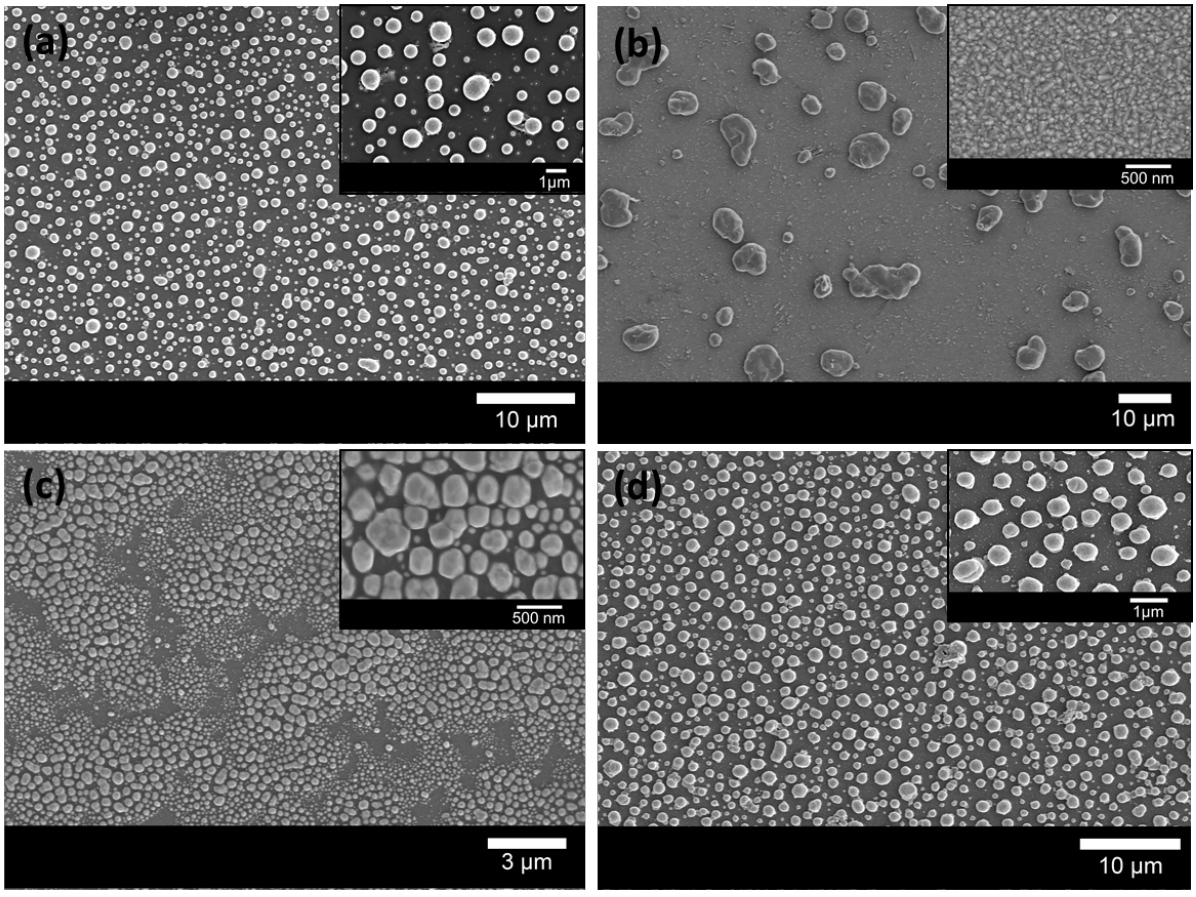

Fig. 4. SEM images showing the morphologies of the films grown on various glass substrates *via* AACVD at 350 °C using silver nitrate in a 1:5 water to methanol solution (a) SiO_2 barrier coated float; (b) FTO; (c) TiO_2 and (d) Activ™.

Antibacterial activity

The antibacterial activity of TiO_2 and Ag thin films on various substrates was tested against *E. coli*, as a representative Gram-negative bacterium and *S. aureus*, as a representative Gram-positive

bacterium. The antibacterial activity was tested using a 365 nm UV light source and the same samples were examined in the dark as a control.

Fig. 5 demonstrates the antibacterial activity of various samples tested in the dark against *E. coli* in 6 hours. Float, FTO, TiO_2 and Activ^{TM} glass did not display any significant antibacterial activity. FTO/Ag and Ag films produced ~1-1.5 log reduction in bacterial numbers (P = 0.001). The TiO_2/Ag film resulted in the greatest kill showing a 99.9% reduction in the numbers of bacteria (~3 log; P = 0.001) following 6 hours of incubation in the dark whereas Activ^{TM}/Ag produced a ~50% reduction in bacterial numbers (P = 0.001). Thus, there is a large difference in the effect of Ag deposited on TiO_2 compared with Ag on Activ^{TM}.

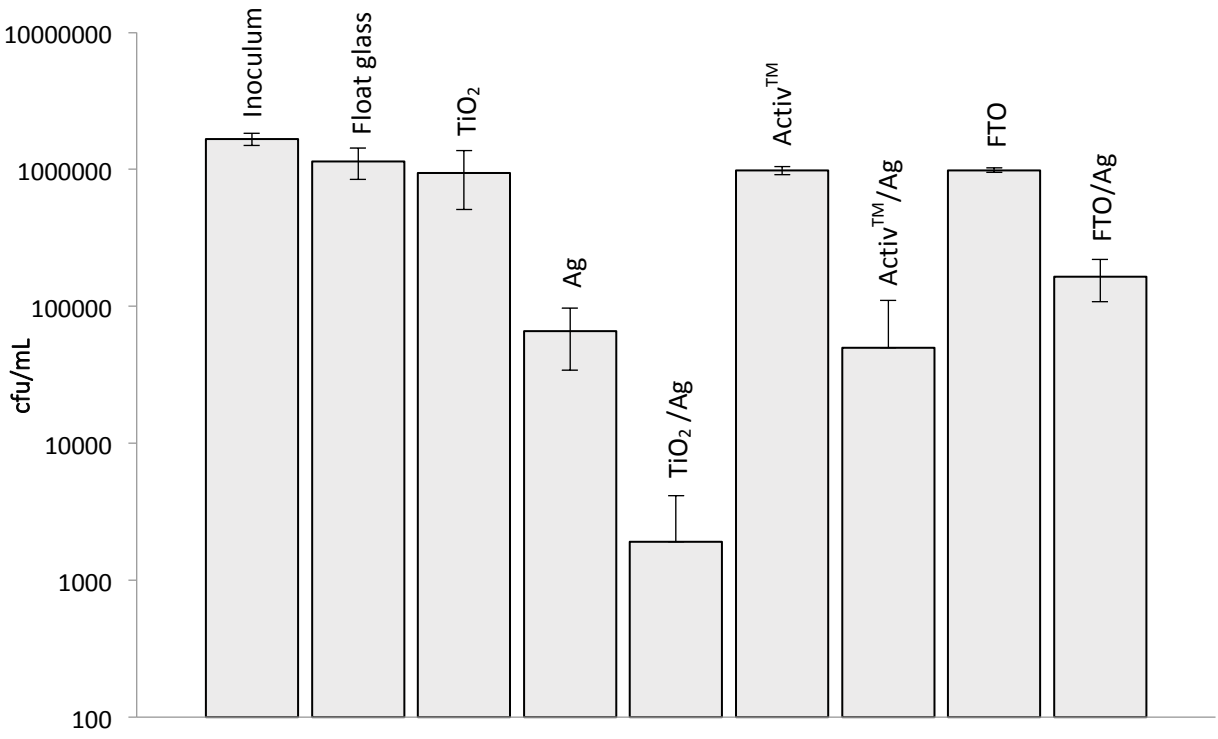

Fig. 5. Viable counts of *E. coli* after incubation on glass substrates incubated at 20^{o}C in the dark for 6 hours.

Fig. 6(a) illustrates the antibacterial activity of the samples against *E. coli* after 365 nm UV light exposure for 6 hours. Activ^{TM}, Activ^{TM}/Ag, FTO and FTO/Ag all achieved complete kill after 6 hours with bacterial numbers reduced to below the detection limit (data not shown). Float glass demonstrated ~1 log reduction in bacterial numbers and the addition of TiO_2 increased this activity

to ~2 log. However, the greatest reduction in bacterial numbers was achieved with Ag and TiO_2/Ag films (≥ 4 log; P = 0.001). Fig 6(b) shows the antibacterial activity of the samples after only 2 hours of light exposure. None of the control samples showed any significant activity. Both Ag and Activ^{TM}/Ag films achieved ~1 log kill of *E. coli*, whereas TiO_2/Ag and FTO/Ag films demonstrated potent antibacterial activity within only 2 hours of UVA light exposure (≥ 4 log; P = 0.001). The antibacterial activity of the TiO_2/Ag and FTO/Ag films were also examined after shorter periods of time; both achieved 99.9% kill of *E. coli* within 60 minutes and a 33.3% reduction after only 30 minutes of UVA irradiation (P = 0.001) (data not shown).

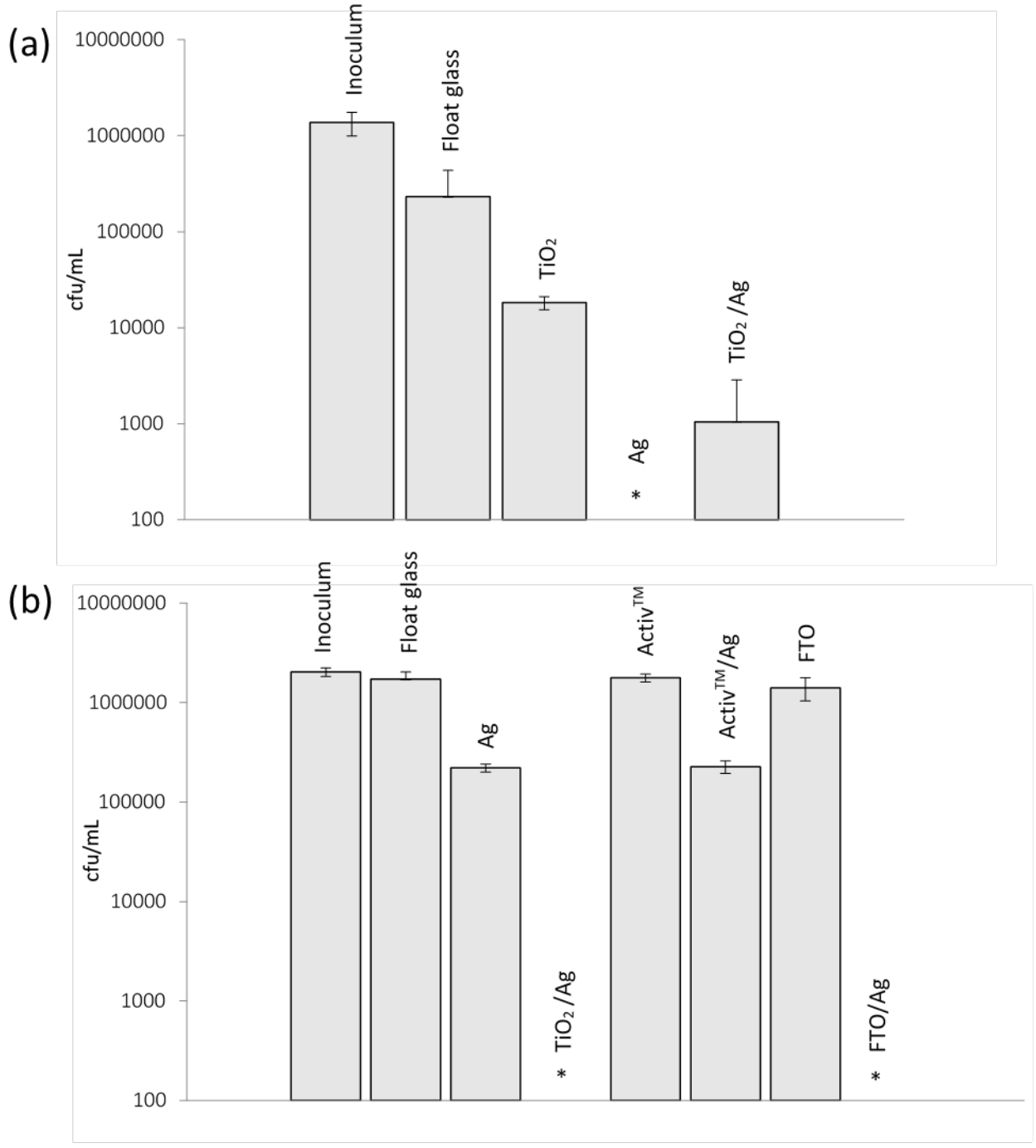

Fig. 6. Viable counts of *E. coli* after incubation on various glass substrates exposed to a 365 nm UV light source for (a) 6 hours and (b) 2 hours. The antibacterial activity was measured at a distance of 16 cm from the samples with an average light intensity of 0.65 ± 0.23 mW cm^{-2}. * indicates that the bacterial numbers were reduced to below the detection limit of 100 cfu/mL.

Table 1. Summary of the antibacterial activity of various glass substrates films against *E. coli* after 2 hours and 6 hours of 365 nm UV radiation and in the dark. [a] indicates that the sample also showed complete kill within 60 minutes of UVA exposure. TiO_2, Ag and TiO_2/Ag were deposited on float glass.

	Antibacterial activity against *E. coli*			
	Dark		**365 nm UV Light**	
Sample	**2 hr**	**6 hr**	**2 hr**	**6 hr**
Float glass	no kill	no kill	no kill	1 log
TiO_2	no kill	no kill	no kill	2 log
Ag	no kill	1.5 log	1 log	≥ 4 log
TiO_2/Ag	1.5 log	3 log	≥ 4 log[a]	≥ 4 log
Activ™	no kill	no kill	no kill	≥ 4 log
Activ™/Ag	no kill	1.5 log	1 log	≥ 4 log
FTO	no kill	no kill	no kill	≥ 4 log
FTO/Ag	no kill	1 log	≥ 4 log[a]	≥ 4 log

Fig. 7 illustrates the bactericidal activity against *S. aureus* when exposed to the various samples in the dark for 4 hours. Float glass, Activ™ and FTO did not show any significant bacterial kill, however, TiO_2, Activ™/Ag and FTO/Ag caused a reduction in bacterial numbers of ~0.5 log. Ag-deposited float produced ~1 log reduction of *S. aureus* and the combination of TiO_2 and Ag on float glass resulted in the greatest kill of *S. aureus* within only 4 hours in the dark (~2.5 log; P = 0.001). Furthermore, FTO/Ag caused ~1 log reduction in bacterial numbers and Activ™/Ag caused ~1.5 reduction of *S. aureus* (P = 0.001). These results illustrate the enhancement of antibacterial activity when TiO_2 and Ag are combined compared to depositing TiO_2 or Ag alone, correlating with the results presented in Fig. 5 against *E. coli*.

Fig. 8 shows the antibacterial activity of the samples when tested against *S. aureus* after 2 hours of 365 nm UV light exposure. Float glass alone did not display significant kill of the bacteria and the addition of TiO_2 showed no enhancement (data not shown). However, Ag-deposited float achieved 99.9% kill of *S. aureus* within only 2 hours of UVA light exposure (P = 0.001). In addition to this, TiO_2/Ag caused a reduction of bacterial numbers to below the detection limit (≥ 4 log; P = 0.001). Activ™ did not show significant reduction of *S. aureus*, but Activ™/Ag also reduced numbers of *S. aureus* to below the detection limit (P = 0.001). Furthermore, FTO alone induced ~1 log reduction in bacterial numbers and the addition of Ag on FTO glass increased the kill to ≥ 4 log within only 2 hours of UVA exposure. The antibacterial activity of TiO_2/Ag, Activ™/Ag and FTO/Ag were tested further at shorter light exposure times. We found that Activ™/Ag caused a 66.6% reduction of bacterial numbers within 60 minutes and TiO_2/Ag displayed a 66.6% reduction of *S. aureus* in 30 minutes and complete kill within 60 minutes of UVA radiation (P = 0.001). Remarkably, FTO/Ag

produced the most significant result, reducing 99.9% of *S. aureus* within only 15 minutes of UVA exposure (data not shown).

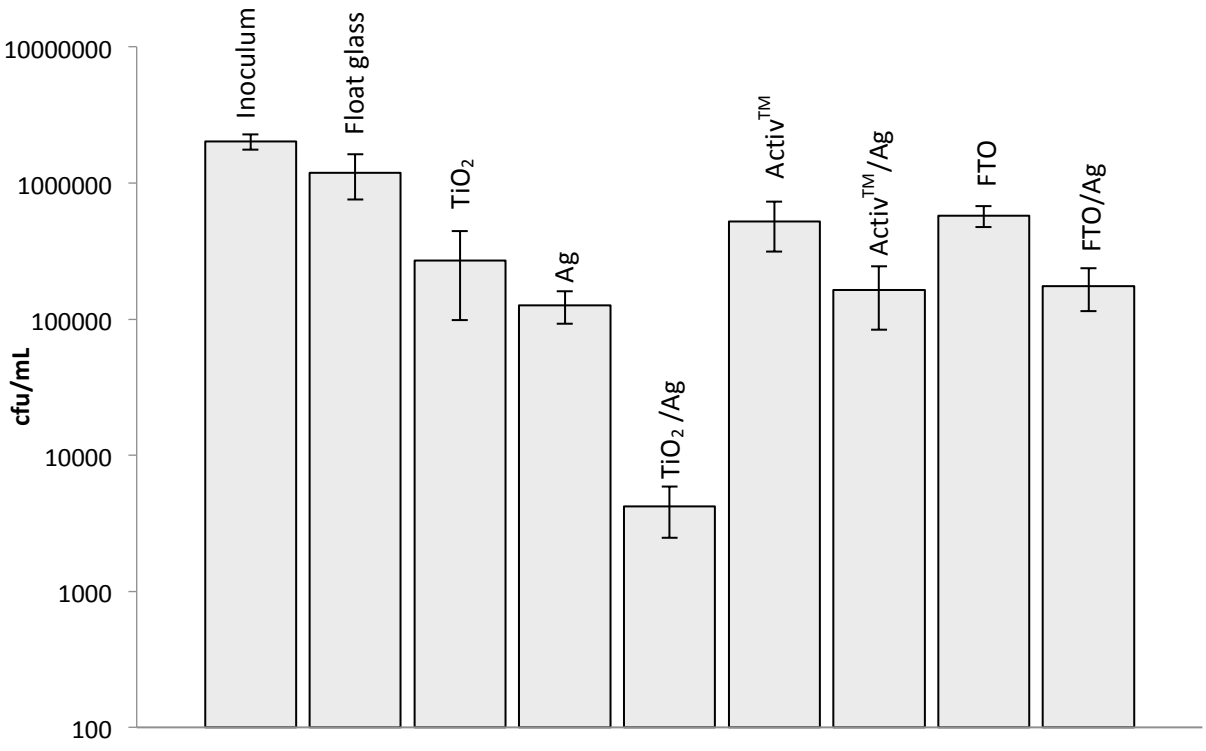

Fig. 7. Viable counts of *S. aureus* after incubation on glass substrates incubated at 20°C in the dark for 4 hours.

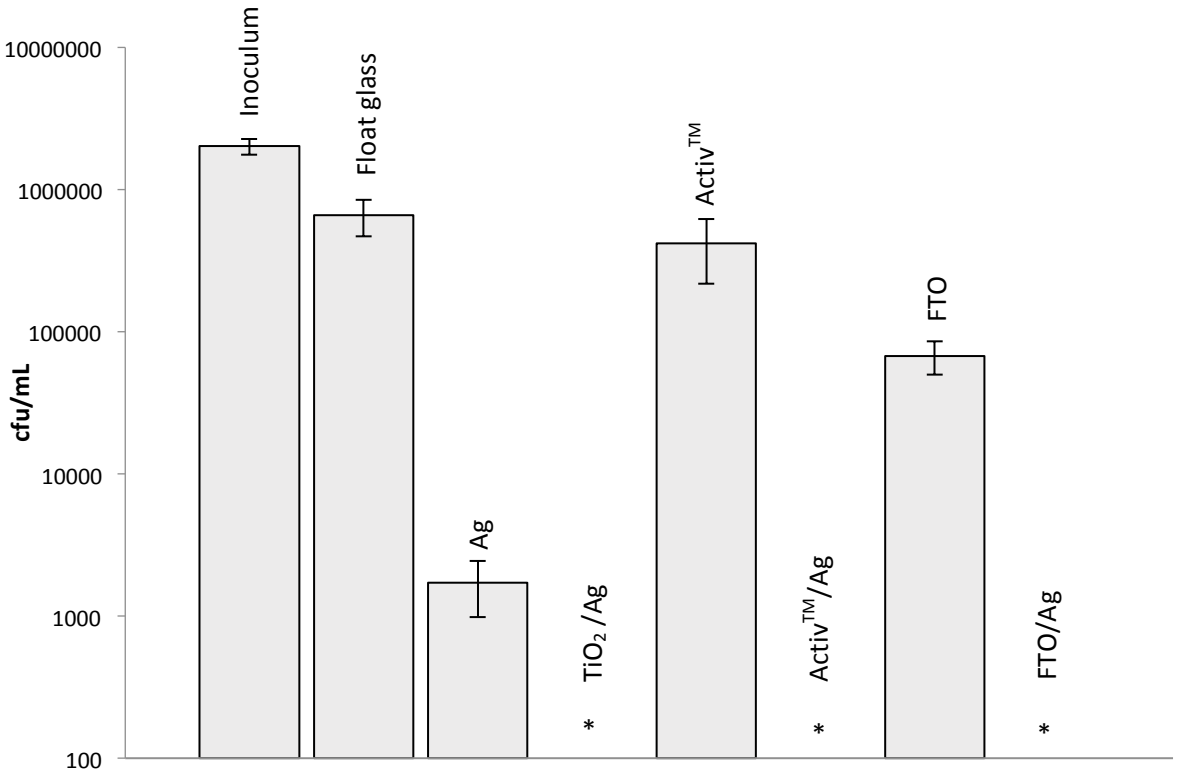

Fig. 8. Viable counts of *S. aureus* after incubation on various glass substrates exposed to a 365 nm UV light source for 2 hours. The antibacterial activity was measured at a distance of 16 cm from the samples with an average light intensity of 0.65 ± 0.23 mW cm^{-2}. * indicates that the bacterial numbers were reduced to below the detection limit of 100 cfu/mL.

Table 2. Summary of the antibacterial activity of various glass substrates films against *S. aureus* after 2 hours and 4 hours of 365 nm UV radiation and in the dark. [a,b] indicates that the sample also showed complete kill within 60 and 15 minutes, respectively, of UVA exposure. TiO_2, Ag and TiO_2/Ag were deposited on float glass.

	Antibacterial activity against *S. aureus*			
	Dark		**365 nm UV Light**	
Sample	**2 hr**	**4 hr**	**2 hr**	**4 hr**
Float glass	no kill	no kill	no kill	2 log
TiO_2	no kill	0.5 log	no kill	2 log
Ag	no kill	1 log	3 log	≥ 4 log
TiO_2/Ag	2 log	2.5 log	≥ 4 log[a]	≥ 4 log
Activ™	no kill	no kill	no kill	≥ 4 log
Activ™/Ag	no kill	0.5 log	≥ 4 log	≥ 4 log
FTO	no kill	no kill	1 log	≥ 4 log
FTO/Ag	no kill	0.5 log	≥ 4 log[b]	≥ 4 log

In this investigation, Ag was deposited onto four different substrates and the antibacterial activity of the materials against *E. coli* and *S. aureus* was compared (summarised in Table 1 and 2). It was clear that there was a large increase in bactericidal activity against both bacteria when Ag was combined with TiO_2 after both UVA light exposure and in the dark. Both the Ag and TiO_2/Ag films achieved bactericidal activity against *E. coli* within 6 hours of UVA exposure, but after 2 hours of UVA radiation and in the dark, the TiO_2/Ag film proved to be more effective. The same trend was demonstrated with Ag and TiO_2/Ag against *S. aureus*, as TiO_2/Ag was more effective at 2 hours than Ag alone. This suggests that there is a synergistic enhancement when TiO_2 and Ag are combined. The Ag nanoparticles could be enhancing the photocatalytic properties of TiO_2 by promoting the formation of $\cdot OH$ and $\cdot O_2^-$ radicals with UVA irradiation. In addition to this, the effective kill of Ag alone under UVA light and not the dark suggests a photogenerated release of Ag ions into the bacteria. Two possible mechanisms could be operating which allow the silver ions to cause denaturisation of proteins in the bacterial cell wall and inhibit bacterial growth.[33] Ag ions could participate in catalytic oxidation reactions between O_2 and hydrogen atoms of thiol groups to produce disulphide bonds, leading to the blocking of respiration and bacterial cell death.[34] Another mechanism of Ag is the production of free radicals that induce oxidative damage of the cell membranes in bacteria.[35]

As summarised in Table 1 and 2, we see that there is a discrepancy between the bactericidal effects of the films against Gram-positive and Gram-negative bacteria, which is most likely due to their differences in cell wall structures.[36] Gram-positive bacteria have a single thick peptidoglycan layer whereas Gram-negative bacteria have a thinner peptidoglycan layer with an inner and outer cell membrane,[37] proving to be less susceptible than Gram-positive bacteria.[38-41] It should be acknowledged that our study has limitations in that only laboratory strains, which are in effect domesticated, were tested. Future work should examine the activity of these materials against wild strains of multidrug-resistant nosocomial pathogens.

We see that the TiO_2/Ag film achieves increased kill of *E.coli* and *S. aureus* compared to the Activ^{TM}/Ag film, which already has a thin layer of TiO_2 on the surface of the glass. It is tempting to speculate that the greater thickness of the home-grown TiO_2 film could account for this difference in the levels of kill achieved (Table 1 and 2). However, it is likely that the difference could be related to the size distribution of the Ag nanoparticles. As shown in Fig. 4, there is a higher concentration of Ag nanoparticles on the TiO_2/Ag surface compared to Activ^{TM}/Ag and they are also smaller in size, giving rise to a greater surface area for bacterial-Ag interactions. This also correlates to the absorbance values of Ag obtained from UV-vis data, as there is a higher absorbance value for TiO_2/Ag than the Activ^{TM}/Ag surface.

Both the TiO_2/Ag and FTO/Ag films achieved rapid bacterial kill (~33.3%) within 30 minutes of UVA exposure against *E. coli*. Activ™/Ag and TiO_2/Ag were highly effective at reducing the numbers of *S. aureus* by 66.6% and 99.9% within 60 minutes, respectively. Our most encouraging result was demonstrated by FTO/Ag, causing complete kill of *S. aureus* within only 15 minutes of UVA radiation. The bactericidal enhancement displayed by FTO compared with Activ™ against both bacteria could suggest that the biocidal properties associated with tin oxide[42] on FTO is causing a bactericidal effect that is more effective than TiO_2 on Activ™ glass. In the dark, the inherent antibacterial properties of TiO_2/Ag are far more effective than any other film against both bacteria. This is promising for hospital environments that do not have optimal lighting, as within 6 hours we achieved a 99.9% reduction in the numbers of *E. coli* and within 4 hours 99.9% kill of *S. aureus*. Activ™/Ag and Ag on float reduced *E. coli* numbers by 50% in the dark, demonstrating that Activ™ glass itself does not possess antibacterial properties.

Conclusion

In this paper we have synthesised crystalline silver films consisting of silver nanoparticles. Films were deposited in an in-house built AACVD rig using easy-to-handle precursors at a relatively low temperature. We report the structural, optical and photocatalytic properties of TiO_2 and TiO_2/Ag films as potential antibacterial surfaces in healthcare environments. TiO_2/Ag films reduced the numbers of *E. coli* by 99.9% within 6 hours in the dark, and TiO_2/Ag and FTO/Ag exhibited potent antimicrobial activity against the bacteria after only 60 minutes of UVA irradiation. TiO_2/Ag was even more effective at reducing the number of *S. aureus* in the dark (99.9% within 4 hours), but FTO/Ag demonstrated the fastest bactericidal activity by achieving 99.9% kill of *S. aureus* within only 15 minutes of UVA exposure. This is the first time, to our knowledge, that Ag has been deposited onto FTO glass and demonstrated such potent antibacterial activity against both Gram-negative and Gram-positive bacterium. Given the current problems in hospitals with multi-drug resistant Gram-negative bacteria our data suggests that these films may be useful in reducing the load of bacteria on hospital surfaces which may in turn lead to a decrease in the spread of HAIs. We conclude that the kill of bacteria on Ag films are substrate specific.

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