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ARTICLE TYPE

Antimicrobial Activity of Copper and Copper(I) Oxide Thin Films Deposited via Aerosol-Assisted CVD

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Hospital acquired infections are prevalent in many hospitals and contaminated touch surfaces are a route of transmission. To find a solution for this, copper and copper oxide thin films were deposited via aerosol assisted chemical vapour deposition using copper nitrate as the precursor and the films were characterised by a range of techniques including powder X-ray diffraction and scanning electron microscopy. The antimicrobial activity of the deposited copper and copper oxide films were investigated against *Staphylococcus aureus* (*S. aureus*) and *Escherichia coli* (*E. coli*). A 5-$\log_{10}$ reduction in the viable counts of *E. coli* was observed on the copper thin films after 30 minutes while a 2-$\log_{10}$ reduction was observed on copper oxide films after 1 hour. In the case of *S. aureus* both copper and copper oxide films exhibited 4-$\log_{10}$ reduction after 1 hour. The high antimicrobial efficacy of the Cu_2O films, approaching that of the pure copper films, suggests that oxide formation on copper objects should not significantly impair their antimicrobial activity.

1 Introduction

There is a great need to deal with hospital acquired infections (HAI), which have become a major problem in hospitals across the world. Statistics from the UK Department of Health have shown 300,000 cases of HAI costing the NHS approximately £1 billion annually.[1,2] The Centres for Disease Control have estimated that 1 in 20 hospitalised patients contract a HAI costing US hospitals over $30 billion.[3] The overreliance on and inappropriate use of antibiotics has led to the development and maintenance of multi-drug resistant bacteria which is an integral part of the HAI problem.[4]

Throughout history metals, such as copper, have been used as antimicrobial agents. The oldest recorded use of copper's antimicrobial ability were seen in Egyptian medical texts where copper was used to sterilise water and treating wounds dating between 2200 and 2600 BC.[5,6]

Copper is an essential metal needed for many functions in organisms although in large concentration it can be toxic.[7] For example, there are many copper containing proteins, present in microbes where copper acts as an electron donor/acceptor due to its ability to switch between copper(II) and copper(I) ions.[8] Although the exact mechanism of the antimicrobial activity of copper is not known many investigations have pointed to reactive oxygen species produced through Fenton-type reactions leading to DNA damage.[9] The release of copper ions causing inactivation of enzymes has also been attributed to its toxicity.[10] Given the antimicrobial activity of copper it may be possible to use it on surfaces in hygiene sensitive areas, such as in hospitals to reduce microbial contamination.

In vitro experiments testing bioactivity of copper materials were first conducted over 30 years ago when it was noted that stainless steel doorknobs and stainless steels strips did not have antimicrobial activity while brass and copper strips did have antimicrobial activity.[11] More recent studies have also shown that copper has antimicrobial activity against a wide range of bacteria including *Staphylococcus aureus*[12] (*S. aureus*) and *Escherichia coli*[13] (*E. coli*).

In situ studies in a busy ward of a UK hospital have also been reported. In this study items described as frequently touched fixtures and fittings were replaced with copper and copper alloys containing a minimum of 60% copper. This ten week study found that all copper containing items on average had between 90 – 100% lower microbial contamination when compared to control fixtures and fittings.[14,15]

In this paper, we report the antimicrobial activity of copper and copper oxide deposited via a solution based technique called aerosol assisted chemical vapour deposition (AACVD). Copper films have been deposited via Metal-Organic (MO)CVD,[16,17] from copper(II) or copper(I) precursors, such as $[Cu(hfac)_2]$ or [Cu(hfac)L] (where hfac = hexafluroroacetylacetonate and L = neutral soft donor). However, these precursors have a low thermal stability which causes them to have a short self-life.[18,19] Improvements on copper precursors have been investigated[20,21] and copper alkoxides,[22,23] such as $[Cu(OCHMeCH_2NR_2)_2]$ (where R = Et or Me) have been used to deposit copper successfully and have the potential for low budget synthesis. In addition, the copper(I) amidinate $[Cu\{(^iPrN)_2CMe)\}]_2$ has been used to deposit copper on to a range of substrates.[24] Relatively high temperatures were needed for these depositions resulting in the formation of copper oxide which needed to be reduced to form the desired copper films.

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AACVD is a variation on the conventional CVD process and overcomes the need for precursors to be volatile since this technique depends on the solubility rather than the volatility of the precursor. This has increased the number of precursors that can be explored.[25,26] AACVD uses a nebulizer to form the aerosol droplets of precursor so that it does not rely on the evaporation of precursor solution when a carrier gas passes over it. This simplifies the delivery and vaporisation of the precursors, which would reduce the cost. There is a need for the carrier gas to have enough pressure to be able to transport the aerosol to the CVD reactor.[27,28]

This investigation describes the deposition of copper metal and cuprous oxide via a single step AACVD, and assessment of the antimicrobial activity of the resulting films.[29] Copper oxide was investigated along with copper since it has been reported that the antimicrobial activity of silver occurs when silver is oxidised.[30] Furthermore, the high antimicrobial efficacy of copper oxide has been shown to approach that of pure copper.[31] Previous investigations have shown the bioactivity of copper oxide thin films using an alternative method of CVD known as Flame assisted CVD.[32] However, there are many limitations in depositing uniform thin films.[33] AACVD is a simple and industrially scalable process and has low maintenance and set up costs involved in scaling up the process.[27,34] The AACVD process is able to deposit films onto a large variety of substrates for different applications including antimicrobial surfaces. Alternative antimicrobial thin films such as titanium dioxide have previously been investigated.[35,36] Copper and copper(I) oxide have an advantage over these photo-induced thin films as there is no need for light activation and they are active in the light and dark.

2 Experimental

All chemicals used in this report were purchased from Sigma-Aldrich Chemical Co. Nitrogen (99.99%) was obtained from BOC and was used as supplied. Deposition was on microscope slides or 150 x 45 x 45 mm SiO_2 coated float-glass (the SiO_2 acts as a barrier layer preventing diffusion of ions from within the glass into the deposition film) which has been supplied by Pilkington Glass plc. Prior to use the glass substrate was monitored using a Pt-Rh thermocouple.

Nitrogen was passed through a two-way tap, which was used to divert the nitrogen carrier gas through a glass bubbler. All deposition experiments were conducted by heating the cold-wall horizontal-bed reactor to the required temperature before diverting the nitrogen line through the aerosol into the reactor. The aerosol was carried into the reactor in a stream of nitrogen gas through a brass baffle to obtain a laminar flow. The gas flow was continued until all of the precursor mix had passed through the reactor, typically 1 hr. The glass substrate was allowed to cool with the graphite block under a flow of nitrogen until it reached room temperature before it was removed.

Copper nitrate trihydrate ($[Cu(NO_3)_2.3H_2O]$, 0.5g) was added to 40 mL of methanol solvent. After addition the solid was allowed to dissolve and used immediately for AACVD deposition. An aerosol was generated at room temperature using a PIFCO ultrasonic humidifier. A graphite heating block under the glass substrate heated the CVD reactor to 350 ^oC. Once the solution had finished, the films were allowed to cool under nitrogen to form copper films. To form copper oxide films the experimental procedure was the same as for the AACVD reaction to form copper but the film was allowed to cool in air instead of nitrogen.

Characterisation

The resulting copper and copper oxide films were handled and stored in air. The coated glass substrates were used for powder X-ray diffraction (XRD) and then were cut into 1 cm x 1 cm squares for subsequent analysis by Scanning Electron Microscopy (SEM), on a JEOL 6301 filament scanning electron microscope. A Perkin-Elmer Lambda 25 UV-Vis Spectrometer was used to measure the UV-Vis absorption spectra of copper(I) oxide samples.

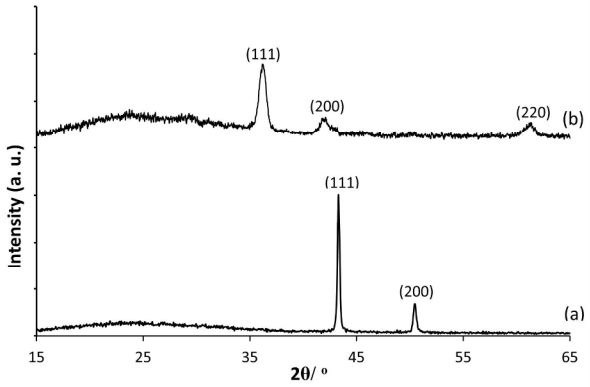

Figure 1. Powder XRD pattern for (a) a Cu film deposited from $[Cu(NO_3)_2.3H_2O]$ in methanol at 350 ˚C and cooling under N_2 and (b) a Cu_2O film deposited from $[Cu(NO_3)_2.3H_2O]$ in methanol at 350 ˚C and cooling in air.

Antimicrobial activity

E. coli strain (ATCC 25922) and *S. aureus* (8325-4) were maintained by weekly subculture on Brain Heart Infusion (BHI) agar (Oxoid, Basingstoke, UK). One bacterial colony of either *E. coli* or *S. aureus* was used to inoculate 10 mL of sterile BHI broth (Oxoid, Basingstoke, UK) and incubated aerobically at 37 ^oC for 24 hours. Bacteria from the overnight culture were harvested by centrifugation at 13,000 xg for 1 minute. Bacteria were then re-suspended in phosphate-buffered saline (PBS) (Oxoid, Basingstoke, UK) and again centrifuged at 13,000 xg for 1 minute. Finally the bacterial pellet was re-suspended in PBS before use. The turbidity of the bacterial cell suspension was measured at 600 nm using a spectrophotometer and was adjusted to an optical density which corresponded to approximately 10^6 colony forming units (cfu) per 25 µL aliquot.

Prior to use, the copper and copper(I) oxide slides were cut into 1x1 cm sections. A humidity chamber was created to ensure that the suspensions did not dry out. For each exposure time, triplicate samples were analysed and uncoated glass microscope slides were used as a control. Each exposure time was also repeated on three separate occasions. A 25 µL aliquot of the bacterial cell suspension was spread evenly on the surface of each slide and incubated at room temperature (21 ± 2 ^oC) for the allocated exposure time. After incubation the slides were aseptically transferred to 225 µL PBS and vortexed for 30 seconds to release the bacteria into the solution. Serial dilutions of the resulting bacterial suspensions were prepared in PBS and 25 µL from each dilution was spread on to MacConkey Agar (Oxoid, Basingstoke, UK) for *E. coli* and BHI agar (Oxoid, Basingstoke, UK) for *S. aureus*. Plates were incubating aerobically at 37 ^oC for 24 hours. After incubation, any bacterial colonies were counted and viable counts of bacteria were calculated. Mann-Whitney U test was used to determine the significance of the activity of copper or copper(I) oxide slides compared to the control glass slide.

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The longevity of the copper and copper(I) oxide was investigated over 7 days. The experiment was repeated, as described above, however the samples were kept and not placed into the 225 µL PBS and were not vortexed. Instead only the aliquot placed on the sections were vortexed. Serial dilutions of the resulting bacterial suspensions were prepared in PBS and 25 µL from each dilution was spread on to MacConkey Agar (Oxoid, Basingstoke, UK) for *E. coli* and BHI agar (Oxoid, Basingstoke, UK) for *S. aureus*. Plates were incubated aerobically at 37 °C for 24 hours. The sections of copper and copper(I) oxide were cleaned with alcohol (70%) and repeated on day 4 and day 7. Each test was done in triplicate, duplicated and compared to glass controls.

3 Results

Deposition of Copper and Copper oxide films

AACVD was used to deposit copper onto glass substrates at a range of temperatures. AACVD of $[Cu(NO_3)_2.3H_2O]$ in methanol at 350 °C resulted in the formation of copper films. However, after deposition the copper film formed is unstable and sensitive to oxygen. Pure copper films are formed if the films are allowed to cool to room temperature under nitrogen (Eqn 1). The films formed had a uniform coverage and were free from pin-hole defects. The films appeared to have a copper (brown) metallic colour. Powder X-ray diffraction (XRD) was performed (Figure 1a), which identified the presence of polycrystalline copper with reflections corresponding to the (111) and (200) planes.

$$[Cu(NO_3)_2 . 3H_2O] + MeOH \xrightarrow[N_2]{350\ ^oC} Cu \xrightarrow[\text{Cool in } N_2]{} Cu \quad (1)$$

$$[Cu(NO_3)_2 . 3H_2O] + MeOH \xrightarrow[N_2]{350\ ^oC} Cu \xrightarrow[\text{Cool in air}]{} Cu_2O \quad (2)$$

To form copper oxide films, the same reagents were used as for the deposition of Cu (Eqn 1), however the films were allowed to cool in air instead of nitrogen (Eqn 2). The films deposited were uniform and had a yellow transparent colour. Powder XRD confirmed the presence of polycrystalline Cu_2O (Figure 1b) reflections corresponding to the (111), (200) and (220) planes.

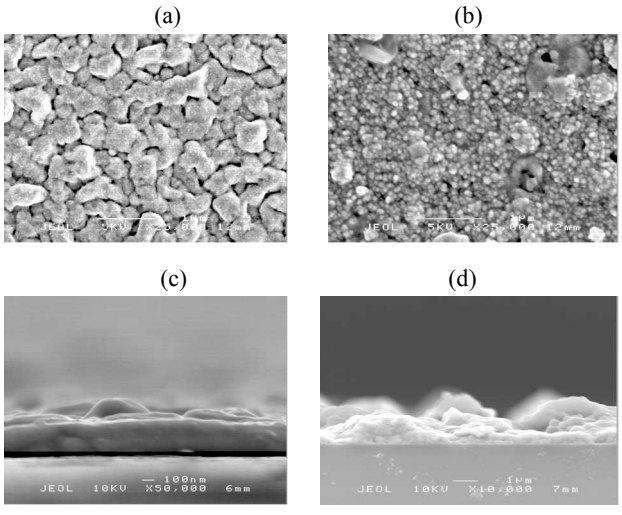

Figure 2. SEM micrograph of (a) Cu film grown via AACVD of $[Cu(NO_3)_2.3H_2O]$ in methanol at 350 °C and cooled under N_2 and (b) a Cu_2O film deposited from $[Cu(NO_3)_2.3H_2O]$ in methanol at 350 °C and cooled in air. Side-on SEM of (c) the Cu film and (d) the Cu_2O film.

All films were uniformly deposited on the glass substrate and all were well adhered to the substrate. The films remained attached after the use of Scotch™ tape, with only damage observed after scratching with a scalpel. The stability of the Cu and Cu_2O thin films were tested using different solvents. Both films were left unchanged and adhered to the substrate when wiped using water and acetone. However, when wiped using nitric acid the films visibly looked damaged and begin to remove from the substrate.

The morphology of the film was investigated using scanning electron microscopy (SEM). There are many different ways a film can grow on the substrate during the AACVD process and the microstructure can have a major impact on the properties of the film.[27] The SEM images of the copper films deposited onto microscope slides are shown in Figure 2a. The microstructure consists of large particles ranging from 300 – 600 nm. This is consistent with a Volmer Weber or Island Growth model.[37] This type of growth occurs when atoms experience stronger bonding interaction between themselves than with the substrate. Copper oxide films deposited onto microscope slides appear rougher with different particle sizes ranging from 100 – 600 nm, as shown in Figure 2b.

The film thickness of the Cu and Cu_2O films were measured using images taken from cross-sectional SEM (Figure 2c and d). The copper and copper oxide films produced were relatively thick. The copper films range from 200 – 300 nm whereas the copper oxide film is much thicker with a larger range of 0.4 –

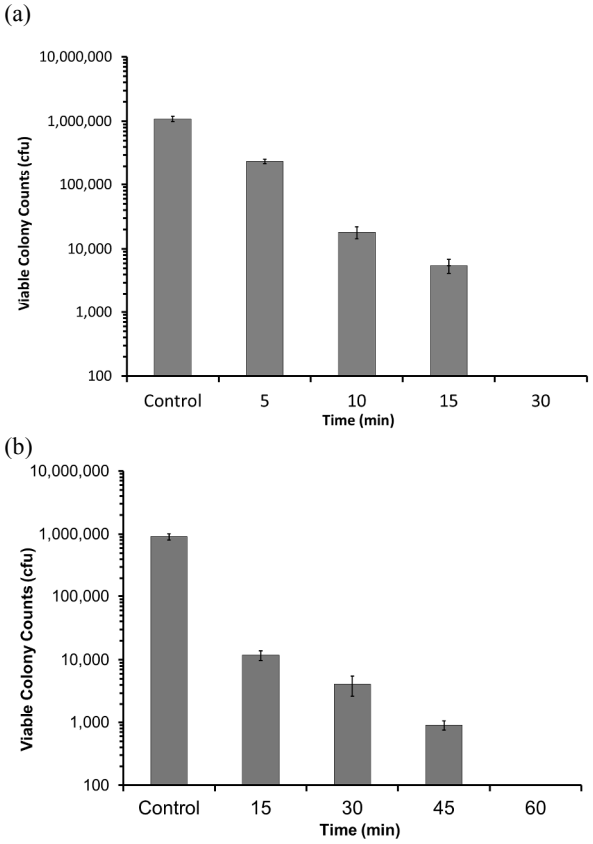

Figure 3. Viable counts of bacteria after incubation on Cu thin films tested against (a) *E. coli* and (b) *S. aureus*

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1μm. Both films have a structure composed of particles ranging in sizes which can be related to the microstructure of the films.

To further characterise the Cu_2O films, the optical band gap of the film was calculated using a Tauc plot.[38] The band gap for the Cu_2O films was 2.25 eV, corresponding well with literature values of 2 – 2.2 eV, reported previously.[39]

Antimicrobial activity

The antimicrobial properties of the copper and copper oxide films, deposited from $[Cu(NO_2)_3.3H_2O]$ via AACVD, against *E. coli* and *S. aureus* were studied. Samples were cut in 1x1 cm sample sections and were covered with 25 μL of bacterial cell suspension containing approximately 10^6 cfu. Through enumeration and plating of the bacterial suspensions and subsequent overnight incubation (37 ^oC), the resultant viable counts of bacteria for each sample section was determined.

Mann Whitney U Test were performed on the results of the antimicrobial testing, which showed that all the reduction in bacteria cell viability were highly significant (P = 0.001) for all exposure times.

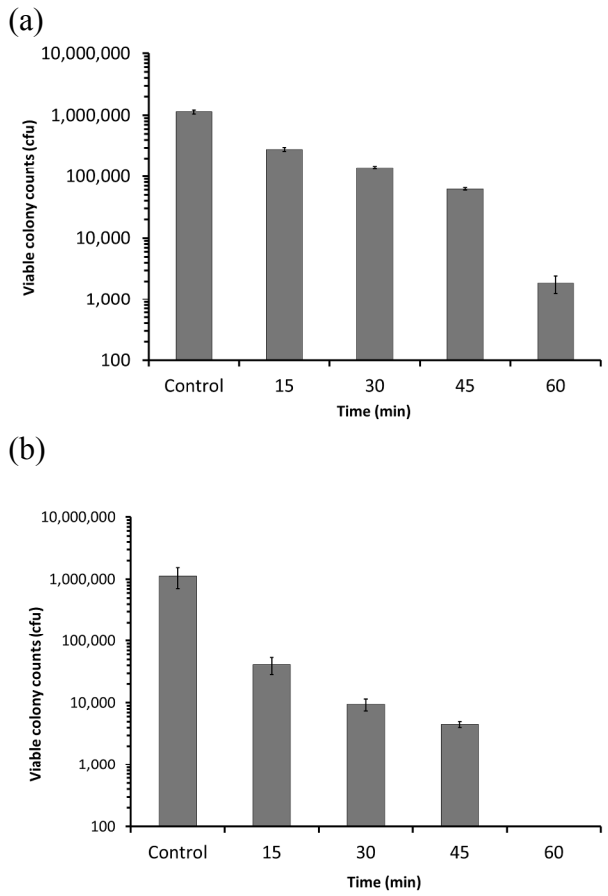

Figure 4. Viable counts of bacteria after incubation on Cu_2O thin films tested against (a) *E. coli* and (b) *S. aureus*

The results for the copper films, shown in Figure 3, demonstrate that the films have antimicrobial activity against *E. coli* and *S. aureus*. For *E. coli*, a 2.1-log_{10} reduction in viable bacteria was observed after 15 minutes and 4-log_{10} reduction was obtained after 30 minutes, as shown in Figure 3(a). In the case of *S. aureus*, a 2.7-log_{10} reduction in viable bacteria was achieved after 30 minutes and 4-log_{10} reduction was observed after 1 hour (Figure 3(b)). For both bacteria, the copper films produced highly significant reduction of the bacterial cells when compared to the glass controls. Moreover, these copper films deposited via AACVD showed similar levels of bacterial activity to previous reports describing the antimicrobial activity of copper coupons.[40,41] Figure 4 shows the antimicrobial activity of copper oxide against *E. coli* and *S. aureus*. The copper oxide samples tested against *S. aureus* gave a 2.7-log_{10} reduction in viable bacteria after 45 minutes and a 4.7-log_{10} reduction after 1 hour. In the case of *E. coli* the copper oxide films were less active giving a 1.2-log_{10} reduction in viable count after 45 minutes and a 2.7-log_{10} reduction after 1 hour.

In the work described herein the antimicrobial activity of copper was compared to copper(I) oxide in order to see if the oxidation would increase the antimicrobial effectiveness. Both samples were tested against *E. coli* and *S. aureus*. The results show that the two types of film have different antimicrobial efficacies against *E. coli* (gram negative) and *S. aureus* (gram positive). From the results presented, *E. coli* had lower bacterial cell counts when exposed to copper than to copper oxide, whereas with *S. aureus* similar levels of bacterial activity were observed with copper and copper(I) oxide films. The toxicity of the samples can be attributed to the release of copper ions under wet conditions. Studies have shown that copper ions play an important role in toxicity of copper containing materials.[42] Some research has shown that particular strains of bacteria are more vulnerable to different copper ions.[41] Previous reports have shown that the antimicrobial efficacy of Cu_2O approaches that of copper which is thought to be due to the fact that localised corrosion could lead to the release of Cu(I) ions.[30] Hence oxide formation on copper objects should not greatly impair the antimicrobial efficiency. The mechanism for copper materials toxicity are not fully understood currently but the results presented herein demonstrate the high antimicrobial activity of both copper and copper(I) oxide films deposited via CVD.

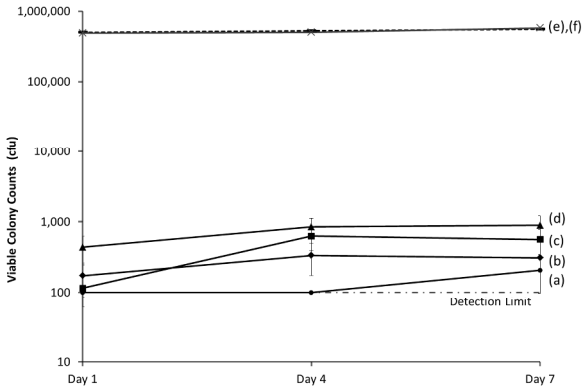

Figure 5. Viable counts of bacteria after incubation on Cu and Cu_2O thin films tested three times over 7 days. (a) Cu and (b) Cu_2O were tested against *S. aureus* (1 h incubation). (c) Cu and (d) Cu_2O against *E. coli* (30 min or 1 h incubation respectively). The glass control tested against (e) *E. coli* (---) and (f) *S. aureus* (-x-) (1 h incubation).

The repeatability of the Cu and Cu_2O thin films were investigated over a period of 7 days. The time exposure was chosen as the

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longest exposure times investigated that had previously shown the highest bacterial activity. These results showed that the reduction in viable bacteria counts were maintained over the 7 day period and the antimicrobial activity of the films was maintained when they were repeatedly used. However, after the initial testing on day 1 the reduction of E. coli and S. aureus was effected but the reduction rate was highly significant, as shown in Figure 5. HAI bacteria, such as *E. coli* and *S. aureus,* can potentially survive on touch surfaces for days even though cleaning procedures are used by healthcare workers'. The Cu and Cu_2O thin films, however, have demonstrated the ability to maintain the reduction in bacterial cell viability over a 7 day period.

4. Conclusions

The antimicrobial efficacy and repeatability of copper and copper(I) oxide thin films deposited via AACVD using $[Cu(NO_3)_2.3H_2O]$ in methanol at 350 ˚C either cooled under nitrogen or air were investigated. This thin film technique is a simple and industrially scalable process. An advantage of AACVD is the ability to deposit on a wide range of substrate including the commonly used materials on touch surfaces in hospitals, such as stainless steel. We examined the antimicrobial activity of copper and copper(I) oxide films against *E. coli* and *S. aureus*. The antimicrobial activity of both copper and copper(I) oxide showed a highly significant reduction in viable bacterial counts. The results indicate that antimicrobial effectiveness is not reduced when copper is oxidised. The results have also shown that after repeated cleaning and testing over 7 days the activity of the thin films is still highly significant. Further studies would involve quantifying the release of copper ions from the films, as well as investigating the impact of morphology on antimicrobial activity.

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Notes and references

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† Electronic Supplementary Information (ESI) available: [details of any supplementary information available should be included here]. See DOI: 10.1039/b000000x/

‡ Footnotes should appear here. These might include comments relevant to but not central to the matter under discussion, limited experimental and spectral data, and crystallographic data.

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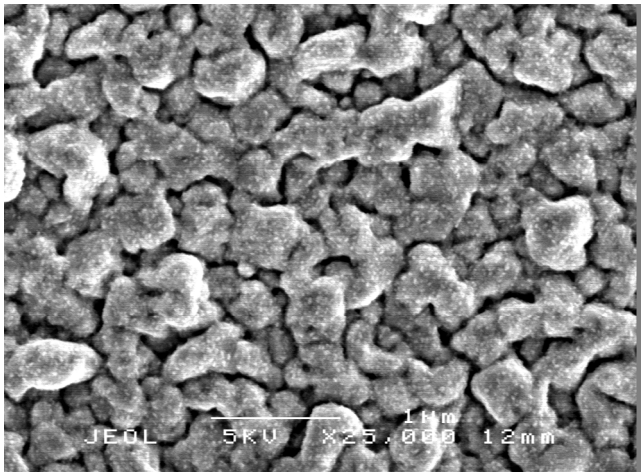
Graphical Abstract:



High antimicrobial efficacy of Cu and Cu_2O films, deposited via aerosol-assisted chemical vapour deposition, was observed against *E. coli* and *S. aureus*.

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