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Tuning the antimicrobial behaviour of $Cu_{85}Zr_{15}$ thin films in “wet” and “dry” conditions through structural modifications

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A B S T R A C T

The antimicrobial behaviour of $Cu_{85}Zr_{15}$ at.% thin films prepared by magnetron sputtering was studied in both wet and dry conditions. Small variations in key deposition processing parameters (pressure and substrate temperature) enabled the growth of thin films with similar nanostructures but different degrees of compactness, according to the Thornton's structural zone model. This model has proven its effectiveness in providing sensitive structural information to explain significant differences in antimicrobial behaviour of the CuZr thin films, even when processing conditions lie within the same structural zone. The antimicrobial behaviour has been studied for *E. coli* and *S. aureus* for up to 4 h of “dry” contact. Structures of lower compactness, grown at higher deposition pressure, are shown to provide higher antimicrobial activity for “dry” conditions than for “wet” conditions. For thin films of CuZr deposited at 0.5 Pa, the reduction percentage of bacteria is 99.47%, which is much higher than the results of 70–80% obtained for the films deposited at 0.1 and 0.3 Pa. Microscopy studies indicate that for 4 h of contact time, bacteria exhibit inner damage and even lysis, however, no morphological changes are detected because of the short timeframes used.

1. Introduction

Over the years, many authors have studied the antimicrobial behaviour of copper (Cu), Zinc (Zn) and silver (Ag) and their alloys because they can kill a broad spectrum of bacteria. The first papers about the biocidal properties of metallic thin films were focused on the addition of Ag, while those dealing with the antimicrobial properties of Cu were relatively scarce [1,2]. The antimicrobial behaviour of Ag-containing thin films [3] is attributed to the interaction of Ag ions with the thiol groups of bacteria proteins, affecting the replication of DNA, uncoupling the respiratory chain from oxidative phosphorylation or collapsing the proton motive force across the cytoplasmic membrane [4]. The antimicrobial properties of this element are highly temperature and humidity dependent, optimally at 35 °C and 95% RH, respectively [5] and are considered to be caused by silver oxide rather than pure silver [6–8].

However, in comparison, copper can kill bacteria across all levels of temperature and humidity [5] and its outstanding biocidal performance is associated to the liberation of Cu^{+1} and Cu^{+2} ions [9–11] as observed in alloy systems such as Cu-Zr-Ag [3] and Cu-Ti [12]. Most of the reported antimicrobial studies have been carried out on fully crystalline copper and copper based alloys, while the antimicrobial activity of these materials with amorphous/nanocrystalline structures obtained by rapid solidification techniques (i.e., magnetron sputtering) has been mostly overlooked. This is remarkable since these material structures are known to display very interesting properties such as higher strength, lower elastic modulus and superior corrosion resistance compared to their crystalline counterparts [13,14]. Some recent examples of the latter are Cu-Zr thin films that exhibit antimicrobial activity even for low Cu concentrations [15]. Even for copper concentrations as low as 30 at.%, such as in $(Zr_{55}Al_{10}Ni_5Cu_{30})_{100-x}Y_x$ (x = 0 or 1 at.%) alloys, the number of *S. aureus* was significantly reduced after 4 h of contact compared to the Ti-6Al-4 V control [16]. As could be expected, increasing the concentration of Cu in the alloy increases the killing efficiency (i.e., less time to kill bacteria) [17] of bacteria attached to the alloy.

There are several methods for the production of such nanostructured thin films, such as electroless plating [18], but physical vapour deposition (PVD) is perhaps the most widely used. Among the PVD processes, magnetron sputtering is especially relevant due to its ability to accurately control the microstructure and composition of the growing thin film [19,20] by fine tuning various key process parameters such as the substrate temperature and sputtering pressure. The microstructure of the thin film also depends on its chemical

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composition since the glass forming ability is composition-dependent. For example, co-sputtered binary Zr_xCu_{100-x} thin films [21] containing > 80 at.% Zr or Cu are crystalline while for intermediate compositions they exhibit a low order phase (i.e., amorphous) according to both experimental results and molecular dynamic simulations. Among all the amorphous thin films, the one with highest copper content reported until now is $Zr_{20}Cu_{80}$ at.% and there is no experimental information whether thin films of higher Cu concentrations (between 80 and 97 at.%) are amorphous or crystalline. Moreover, the antimicrobial activity of such alloys has not been studied despite their potential interest attributed to the high copper content.

There are several methods to quantify the antimicrobial activity of surfaces in the literature [22] that are similar to those observed in catheters or drainages, but these are "wet" test conditions, where the liquid phase in the broth media is always present. While for applications such as touch surfaces the conditions are always "dry". Consequently, some authors have begun to investigate this issue, encouraging the development of "dry" tests, where the media is completely evaporated [23–25]. However, the influence of air-drying on the morphology of *E. coli* and *S. aureus* is still not completely understood [26,27]. As previously mentioned, the antimicrobial activity may depend on the environmental conditions, and, it is of interest to explore the possibility of tuning the processing conditions of magnetron sputtering to control the antimicrobial performance. For this reason, this manuscript aims to shed light on the effect of varying the magnetron sputtering processing conditions of pressure and substrate temperature on the antimicrobial performance of Cu_{85}-Zr_{15} thin film evaluated when the liquid phase in the LB media is always present (considered as "wet" conditions), in contrast to samples where the media completely evaporated (denominated as "dry" tests).

2. Methods

2.1. Thin film fabrication

Thin films were prepared by the magnetron sputtering technique using a Teer Coatings UDP multi-cathode deposition plant. Substrates of 314 stainless steel (314SS) (5 × 5 × 1 mm thick) with a surface roughness, Ra < 0.5 μm and soda lime glass slides (25.4 × 76.2 × 1 mm thick) were used. Before deposition all substrates were thoroughly cleaned in 1:5 ratio of DECON90 solution to water for 60 s, rinsed in deionised water and dried with a nitrogen blast to clean off residues and particles before being loaded on a rotating carousel in the centre of the deposition chamber. The chamber was then evacuated to a base pressure of 5×10^{-4} Pa before pure argon was introduced at the required flow rate to control the working pressure. A rectangular copper (Cu) target (248 × 133 × 10 mm thick) and circular zirconium (Zr) target (100 mm dia. × 3 mm thick), both of 99.99% purity, were then sputtered cleaned at dc powers of 200 W and 160 W respectively for 10 min. Following cleaning, the cathode shutters were opened and the Cu and Zr targets were co-sputtered for 90 min onto the substrates mounted at a distance of 130 mm from the targets on the central carousel, which was rotated at a fixed speed of 5 rpm. These deposition conditions resulted in thin films of approximate chemical composition Cu/Zr 85/15 at.% and 1 μm in thickness. In total four different batches of Cu-Zr films were deposited; three at room temperature and varying working pressures of 0.1, 0.3 and 0.5 Pa and an additional batch at a substrate temperature of 403 K and working pressure of 0.3 Pa. Full details of the deposition conditions for these four batches are given in Table 1.

2.2. Structural and physical properties of thin films

The surface morphology and chemical composition of the Cu-Zr film samples sputtered on to 314SS substrates and borosilicate glass slides were analysed using a Tescan Mira 3, Scanning Electron Microscope (SEM) with 5–10 kV of acceleration voltage, coupled with an Oxford Instruments X-Max 150 Energy Dispersive X-ray (EDX) detector. The surface topography and roughness of the films was analysed and measured using a Digital instrument DimensionsTM 3100 atomic force microscope (AFM) system scanning in contact mode across a $3 \times 3\ \mu m^2$ sample area; data was visualised using Gwyddion software and processed in MATLAB software suite. The structural properties of the films were determined from X-ray diffraction (XRD) patterns collected using a Siemens D5000 diffractometer with Cu Kα radiation (λ = 1.54184 Å) at 40 kV and 40 mA, with a scanning speed of 0.01°/s in the 2θ range 10° to 90°.

Table 1
Cu-Zr thin film deposition conditions.

Batch ID	Base pressure (Pa)	Ar flowrate (sccm)	Working pressure (Pa)	Substrate temperature (K)	Cathode power (W)	
					Cu	Zr
1RT	5×10^{-4}	10	0.1	R_T	200	160
3RT	5×10^{-4}	30	0.3	R_T	200	160
5RT	5×10^{-4}	45	0.5	R_T	200	160
3HT	5×10^{-4}	30	0.3	403	200	160

Film thickness was measure using a Dekatak XTL stylus type profilemeter equipped with a 12.5 μm tip. A square edge step was created in the films grown on glass slides using a strip of 3 mm wide Kapton tape, which was removed after deposition.

Contact angle measurements were carried out using the sessile drop technique (Krüss drop size DSA30 analyser). A volume of 1 μL of deionised water was deposited at a rate of 30 μL/min on the surface of the samples, and the angle was immediately observed to prevent droplet shape change due to evaporation. Contact angle results are the average of five sessile drop tests (ten contact angle measurements).

2.3. Antimicrobial behaviour of thin films

The antibacterial activity of the thin films was assessed by following the reduction in colony forming units (CFU) recovered from the surface of the thin films over contact time using *E. coli K12* (Gram-negative) and *S. aureus* NCTC 6571 (Gram-positive) [28]. For the antimicrobial tests, bacteria were cultured in an orbital incubator (37 °C, 200 rpm), in 25 mL of LB (Luria Bertani) broth for 16 h. Culture yield was quantified by measuring optical density and bacteria were then diluted in sterile LB Broth to an optical density (OD_{600}) of 0.01. The diluted cultures were incubated at 37 °C until they reached an $OD_{600} \sim 0.3$; approx. 3×10^8 per mL. Thin films and control samples (bare 314SS) were immersed in pure ethanol and sonicated for 5 min to ensure a clean and disinfected surface. A quantity of 2 μL of the culture was dispensed directly onto the test surfaces. For the wet tests, these samples were placed inside a sterile petri dish containing tissue paper wetted with 1 mL of sterile LB Broth to prevent sample drying. For the dry tests, all samples were placed inside petri dishes, but no wetted tissue was added. To ensure complete drying, after inoculation, the petri dishes were placed 100 mm away from a Bunsen burner with the lid partially open. After the inoculum vehicle evaporated completely (around 8–9 min), all petri dishes were closed and left on a bench at room temperature. After the designated exposure time, the "wet" and "dry" samples were diluted in 198 μL of Tween 20 0.148 g/L (2 × CMC) and sonicated for 5 min. Finally, the recovered bacterial suspension was subjected to serial decimal dilution, spread onto LB agar plates and the resulting colonies were counted after 16 h of incubation at 37 °C. All tests were performed five times, with mean counts and standard deviation reported.