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Antimicrobial effects and dissolution properties of silver copper mixed layers

G. Gotzmann[a,*,1], C. Jorsch[a,1], C. Wetzel[a], H.W.R. Funk[b]

[a] Fraunhofer Institute for Organic Electronics, Electron Beam and Plasma Technology FEP, Winterbergstraße 28, 01277 Dresden, Germany
[b] Faculty of Medicine Carl Gustav Carus, Institute for Anatomy, Technical University of Dresden, Dresden, Germany

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ABSTRACT

Within the last years, research in the areas of hygiene and healthcare has focused on the resurgence of antimicrobial coatings to prevent infections and control their spreading.

We used a physical vapor deposition technology to deposit coatings of either silver or copper individually or as mixtures on flexible polymeric substrates. Five combinations of silver and copper coatings were produced with a thickness of 50 nm. The respective amounts of silver and copper on the coated surfaces were determined using scanning electron microscopy and energy dispersive X-ray spectroscopy (SEM/EDX). The antimicrobial characteristics of silver and copper against *Escherichia coli* K12 (*E. coli*) were investigated, focusing on the possible synergistic effects of the two metals.

The deposited coatings showed homogeneous mixtures of silver and copper clusters. The mixtures of silver and copper as well as pure silver showed antimicrobial properties. In contrast, pure copper did not exhibit antimicrobial effects.

The dissolution behavior of copper and silver was determined by incubation of mixed layers in physiological media (synthetic sweat). Copper coatings proofed to be fully dissolvable whereas silver showed lower solubility. With increasing copper content in the mixed coatings, the dissolution of silver increased as well. The observed dissolution behavior was correlated to the results of the antimicrobial activity of the investigated coatings.

1. Introduction

With the emergence and increase in the number of antibiotic resistant microbes and the continuing emphasis on healthcare costs, research has focused on the development of alternative, effective antimicrobial reagents. This basic idea has also led to the resurgence in the use of silver based antiseptics as well as novel antibiotics based on metallo-pharmaceuticals, which are a promising class of compounds to overcome highly resistant pathogen strains [1–3]. Silver is a well-known antimicrobial material and besides coatings with pure silver or silver alloys, also silver nanoparticles and their incorporation in various materials are under investigation [4,5]. As an example, silver as antimicrobial coating for urinary catheters is FDA approved [4] and even textiles can be coated using novel deposition techniques [6]. The antimicrobial activity of the metal ions is believed to be driven by several mechanisms leading to diminished membrane integrity, protein dysfunction or inhibition and oxidative stress [4,7,8]. As a consequence, the affected microorganism becomes inactivated.

The antibacterial effects of silver salts have been noted since antiquity [9]. The in 1967 first synthesized silver sulphadiazine, used as wound dressing, is still commercial available as water soluble cream to dress burned skin [10]. Silver is currently used to control bacterial growth in a variety of situations, including dental work, catheterization, and the treatment of burn wounds [4,7,9,11,12]. Moreover, it is well known that free silver ions are highly toxic to a wide variety of organisms including bacteria. The detailed mechanism has not yet been fully elucidated [12,13], but several possibilities have been suggested [8,13–17]. Furthermore, it has been discovered that some bacteria can acquire silver resistance [18,19]. In addition to silver, copper has also been a focus of research because of its antimicrobial properties [20].

The sensitivity to an antimicrobial agent like silver or copper depends on the targeted organism. For example, *Bacillus subtilis* is more sensitive to copper, whereas *E. coli* and *Staphylococcus aureus* are more sensitive to silver [9]. It has also been reported that mixtures of silver and copper showed synergistic antimicrobial effects [17].

Currently, research focuses strongly on the use of silver in the form of nanoparticles [4,5,21–23]. Though fabrication of antimicrobial nanoparticles becomes more and more eco-friendly [24,25], many

* Corresponding author.
E-mail address: gaby.gotzmann@fep.fraunhofer.de (G. Gotzmann).
[1] Authors contributed equally.

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Table 1
Targeted layer compositions and their abbreviations (first column). Elemental composition of the layers determined by SEM/EDX (third and fourth column).

Sample	Aimed amount of Ag and Cu	Content of silver [wt%]	Content of copper [wt%]
Ag^{PURE}	100% Ag	100	–
Ag^{HIGH}/Cu^{LOW}	75% Ag/25% Cu	78	22
Ag^{MEDIUM}/Cu^{MEDIUM}	50% Ag/50% Cu	53	47
Ag^{LOW}/Cu^{HIGH}	25% Ag/75% Cu	30	70
Cu^{PURE}	100% Cu	–	100

investigation report about problems with aggregation phenomena when using nanoparticles for antimicrobial surface preparation [5,26,27] and also cytotoxic effects of nanoparticles in contact with mammalian cells are discussed diversely [23–25]. Therefore, we preferred using a coating technology to deposit the antimicrobial elements silver and copper on a medical surface.

Surface coating is a widely used process to change and adapt surface characteristics for biological applications [28]. Currently, many studies exist regarding the use of coating processes to apply metal-based antibacterial layers to medical surfaces [6,29–32]. Among others, physical vapor deposition (PVD) has often been used to deposit such antimicrobial coatings [33–36].

2. Materials and methods

2.1. Deposition of silver copper mixed coatings

Polyurethane (PUR) as described in [37] was used as a substrate and was coated with combinations of either silver and/or copper according to the designation in Table 1. According to Table 1, five different coating compositions were prepared: pure silver and copper (Ag^{PURE} and Cu^{PURE} respectively), mixtures of 75% silver and 25% copper (Ag^{HIGH}/Cu^{LOW}), 25% silver and 75% copper (Ag^{LOW}/Cu^{HIGH}) and a 1:1 mixture of 50% silver and 50%copper (Ag^{MEDIUM}/Cu^{MEDIUM}). The coating deposition was performed in the vertical inline sputter plant “ILA 900” at the Fraunhofer Institute for Organic Electronics, Electron Beam and Plasma Technology (FEP) in Dresden, Germany.

The substrate was prepared inline using intense sputter etching with argon gas to pre-clean the substrate surface; the rated power was adjusted to 100 W at a process pressure of 0.3 Pa. Layers of silver and copper as well as silver copper mixtures were prepared using the DC magnetron sputtering technology. For the deposition process, argon was used as process gas at a pressure of 0.3 Pa and a rated power of 1000 W. The deposition conditions were kept constant for all coatings. For the deposition of mixed layers, the silver and copper targets were used in parallel, pulsing them ten times one after another with a high frequency. The final coating thickness was adjusted to 50 nm by adapting the velocity of the substrate holder according to the previously determined dynamic deposition rates that were $33\ nm * m * min^{-1}$ and $25\ nm * m * min^{-1}$ for silver and copper, respectively. The samples were coated on one side, leaving the backside uncoated. By placing the PUR samples on a large glass surface, it was possible to coat the temperature sensitive material without influencing its properties as the glass led to a fast heat deduction.

For the biological investigation and the investigation of the dissolution behavior, the samples were cut into pieces of $3 \times 3\ cm^2$ or $1 \times 1\ cm^2$, respectively. Sample sterilization was done using two-sided low energy electron-beam irradiation with a dose of 25 kGy [38].

2.2. Elemental analysis

The actual elemental composition of the mixed layers was verified using scanning electron microscopy (SEM). By detecting backscattered electrons (BSE), information about the elemental distribution on the surfaces was gained. Energy dispersive X-ray analysis (EDX) was then used to determine the amount of silver and copper in the mixtures (in wt%). For SEM/EDX analysis, a field emission scanning electron microscope (SU8000 from Hitachi High-Technologies Corporation; Tokyo, Japan) was used with the imaging detector positioned over the objective lens. Secondary electrons (SE) and BSE were detected. The EDX analysis was performed using an Apollo XV (EDAX) device. The excitation voltage was 20 keV, which guaranteed an excitation depth of roughly ten micrometers. Since the coatings had a thickness of approximately 50 nm, it was possible to determine the elemental composition of the entire coating by this method. The coating substrate itself (PUR) does not influence the measurement, as it does not consist of either silver or copper components.

2.3. Microbiological evaluation

Antimicrobial effectiveness was investigated against *Escherichia coli* K12 (DSMZ 498). *E. coli* was cultured in standard nutrient broth (standard I nutrient broth, Carl Roth GmbH + Co. KG, Karlsruhe, Germany; 15 g/L peptone, 3 g/L yeast extract, 6 g/L NaCl, 1 g/L glucose, pH 7.5 ± 0.2) at 37 °C and 100 rpm shaking. The coated samples (9 cm^2) were incubated in an Erlenmeyer flask with 15 mL of *E. coli* suspension. For the experiments, the bacterial starting density in the nutrient broth was adjusted to 10^3 CFU/mL using a Neubauer counting chamber. Growth curves were obtained by determining the absorption of the nutrient broth at 600 nm once an hour by transferring 100 µL aliquots into a 96-well microtiter plate and subsequent measurement with a Tecan multi well plate reader. Sterile nutrient broth without bacteria but also incubated with the samples was used as a blank. An *E. coli* suspension without sample incubation was used as reference for the measurement. To compare the growth curves in the exponential growth phase, a defined value of absorption (0.3) was used as checkpoint. The different samples were compared concerning the incubation time, for which their absorption was below this value. The absorption of the samples was recorded over a test period of 100 h. If a sample did not reach the value over the whole time of the experiment, the sample was declared as sterile, meaning no vital bacteria were present. To confirm sterility, an aliquot of these samples was plated afterwards on standard agar plates (standard I nutrient agar from Carl Roth GmbH + Co. KG, Karlsruhe, Germany; 15 g/L peptone, 3 g/L yeast extract, 6 g/L NaCl, 1 g/L glucose, 12 g/L agar, pH 7.5 ± 0.2), incubated for two days at 37 °C and observed for any colony formation.

2.4. Dissolution of the mixed coatings

In contrast to the microbiological investigations and for analytical reasons, smaller sample sizes and smaller liquid amounts were used for the investigation of the dissolution behavior. Coated samples of 1 cm^2 were incubated in 7.5 mL synthetic sweat with a pH of 5.5 (according to DIN EN ISO 105-E04:2013-08 [39]) at 37 °C. After 24 h of incubation, the samples were removed and the medium was used for analysis of the dissolved silver and copper contents by ICP-MS.

2.5. ICP-MS analysis

To determine the dissolution behavior of the silver copper mixed layers, inductively coupled plasma mass spectrometry (ICP-MS, Perkin Elmer, ELAN 5000) was used. For sample preparation, 100 µL of nitric acid suprapur were added to 7.5 mL of the media, which was obtained as described above before ICP-MS analysis. Certified reference materials for copper and silver (1000 mg/mL in 2% nitric acid, Carl Roth GmbH + Co. KG, Karlsruhe, Germany) were used as a standard for the evaluation of the results.