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Laser cladding of stainless steel with a copper–silver alloy to generate surfaces of high antimicrobial activity

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ABSTRACT

Copper and silver are used as antimicrobial agents in the healthcare sector in an effort to curb infections caused by bacteria resistant to multiple antibiotics. While the bactericidal potential of copper and silver alone are well documented, not much is known about the antimicrobial properties of copper–silver alloys. This study focuses on the antibacterial activity and material aspects of a copper–silver model alloy with 10 wt% Ag. The alloy was generated as a coating with controlled intermixing of copper and silver on stainless steel by a laser cladding process. The microstructure of the clad was found to be two-phased and in thermal equilibrium with minor Cu_2O inclusions. Ion release and killing of *Escherichia coli* under wet conditions were assessed with the alloy, pure silver, pure copper and stainless steel. It was found that the copper–silver alloy, compared to the pure elements, exhibited enhanced killing of *E. coli*, which correlated with an up to 28-fold increased release of copper ions. The results show that laser cladding with copper and silver allows the generation of surfaces with enhanced antimicrobial properties. The process is particularly attractive since it can be applied to existing surfaces.

1. Introduction

With more than 4 million infected people and over 37,000 deaths per year in Europe alone [1], healthcare-associated infections pose a major problem to the healthcare system. In this context, copper and silver receive growing attention as intrinsically antimicrobial materials to combat multi-resistant germs like the "superbug" MRSA (methicillin resistant *Staphylococcus aureus*) [2–6]. Silver, due to its high cost, is often applied in the form of nanoparticles [7,8]. Copper and copper alloys, on the other hand, are also used for massive, metallic components which exhibit so-called contact killing of bacteria [9,10]. Although both elements have been recognized for their beneficial properties for approximately 4500 years [11,12], the mechanism of their antimicrobial action and the correlating material properties are still not fully understood. Recent studies account for multiple toxic effects toward bacteria, fungi and even viruses, which seem to be mainly based on the dissolved ions of these elements [13–15]. In the following section, killing mechanisms of ionic copper and silver are compared, and the current state of design for metallic, antimicrobial materials is discussed.

Due to the similar electron configurations of copper and silver (Cu: [Ar] 3d10 4s1; Ag: [Kr] 4d10 5s1), the two elements behave as mimetics in biological systems and some of the suggested killing mechanisms for bacteria are believed to work similarly for the two metals [8,16–18]. Silver mainly forms single charged ions in solution (Ag^+), whereas copper may form Cu^+ and Cu^{2+}. Copper ions, in contrast to silver ions, can thus induce redox cycling and thereby act as a catalyst in the generation of free radicals (e.g. Fenton reaction) which are also able to severely damage bacteria [19]. Copper is an essential trace element for most, if not all organisms. For humans, the latest dietary reference intakes for copper range from 0.5 g d^{-1} for infants to 1.3 mg d^{-1} for lactating women, with 0.9 mg d^{-1} recommended for healthy adults [20]. The biological function of copper contrasts with silver, which has no recognized biological role.

Depending on the strain, bacteria exhibit different sensitivities to copper and silver ions [21–24] and several studies account not only for an additive but also a synergistic effect of these ions

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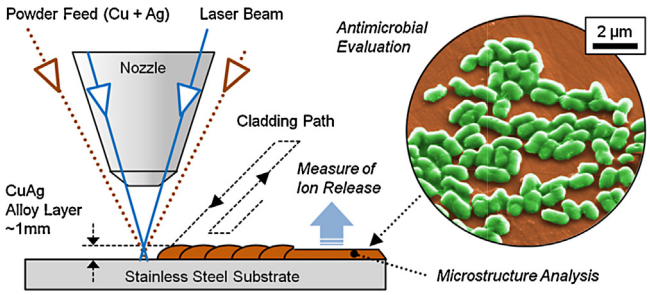

Fig. 1. Schematic of the experimental design. A 9:1 weight ratio copper–silver powder mixture was used to generate a homogeneous CuAg alloy layer on stainless steel following a meander-like path. The colored SEM image (right) shows dried *E. coli* bacteria (strain W3110) on the polished CuAg alloy during a killing assay. (For interpretation of the references to color in this figure legend, the reader is referred to the web version of this article.)

against microbes [14,23,25,26]. Lin et al. quantitatively described the synergism between copper and silver ions against *Legionella pneumophila* with a fractional parameter related to the minimum inhibitory concentration for each metal ion alone [27]. They suggested the synergy to be based on copper ions primarily permeabilizing the cell wall and thereby facilitating the interaction of silver ions with proteins inside the cells.

Due to these complementary and even synergistic antimicrobial modes of action, the combination of metallic copper and silver seems to be a beneficial approach to achieve highly functionalized antimicrobial materials. Indeed, Jing et al. showed that an additional silver coating on porous copper increased the antibacterial properties toward *E. coli* [28]. The role of metallic ions however was not investigated in this study. Hahn et al. combined copper, silver and/or gold nanoparticles embedded in a silicon matrix to achieve a controlled release of antimicrobially active metal ions [29]. Although they could electrochemically tune ion release by these materials, no killing tests with bacteria were performed.

As the aforementioned studies relate the antimicrobial effect mainly to dissolved ions, the design of metallic, antimicrobial materials requires a special focus on corrosion properties. In line with this, it has been shown that the antimicrobial performance of metallic copper and silver changes under different environmental conditions. The studies of Michels et al. and Warnes et al. suggest that silver is generally more effective in humid environments whereas copper appears to exhibit an increased killing rate under dry conditions [30,31]. This may explain why silver is considered as an antimicrobial coating agent for titanium implants which are continuously exposed to a liquid environment [32]. Gibbard et al. alleged that silver oxide, which can be assumed to be the predominant material compound for silver nanoparticles in solution, is more antimicrobially active than pure silver [18]. Recent studies on copper oxides indicated Cu_2O to be nearly as antimicrobial as pure copper, whereas CuO, which is the predominant copper oxide formed in humid conditions, shows a lower killing behavior [33].

To correlate copper–silver materials design with ion release and bactericidal efficiency, alloyed copper and silver were compared to the pure elements under wet killing conditions. The different steps of the experiment are summarized in Fig. 1.

For metallic surface coating, a laser cladding procedure (LC) was employed. This technique allows additive surface manufacturing as well as re-alloying of metallic surfaces with additional components on the millimeter scale [34,35]. Copper and silver in particular have already been successfully used for LC experiments in different combinations with other materials and substrates [36–38]. The microstructural and chemical properties of the generated LC alloys were characterized in detail by X-ray diffraction, scanning electron microscopy and energy dispersive X-ray spectroscopy and were found to be two-phased. Bacterial killing by the alloy was determined by wet plating procedures with *E. coli*. An enhanced killing rate was found, which correlated with increased release of copper ions. Overall, these findings suggest that co-acting antimicrobial mechanisms of copper and silver as a metallic alloy allow the design of improved antimicrobial materials for the healthcare sector.

2. Materials and methods

2.1. Materials and sample preparation

Rolled metal sheets of 99.99% pure copper, 99.99% pure silver and of stainless steel (AISI 304: X5CrNi18-10) were used as references and as steel substrate for the copper–silver clads. The copper and silver powders used for the LC of alloy layers were 99.9% and 99.92% pure with grain dimensions of less than 44 μm (<325 mesh). All metallic samples were polished finishing with oxide particle solution of 0.05 μm particle size. To remove residues from the polishing process, samples were successively sonicated in ethanol, acetone, and cyclohexane for 15 min each and stored in a protective nitrogen atmosphere immediately.

2.2. Laser cladding procedure

For a schematic drawing of the LC process, see Fig. 1. Copper and silver powders were mixed at a weight ratio of 9:1 (1 h) using a bead mill. A 500 W continuous diode laser (λ = 800–980 nm, LDM 500-20, Laserline) and a powder feeder (TWIN-10-C, Sulzer Metco) were used for the LC experiments. The laser head was controlled by a three-axis CNC system (Bosch-Rexroth). Helium was employed as protection gas.

2.3. Materials characterization

Phase analysis was conducted on an X-ray diffraction (XRD) system (PANalytical X'Pert MPD) using Cu-Kα radiation in Brag Brentano configuration. Energy dispersive X-ray spectroscopy (EDX) and scanning electron microscopy (SEM) were performed on a dual beam microscope (FEI Strata DB 235) at 5 and 20 kV using an Si-Li EDX detector (EDAX) and secondary electron contrast. Topographical surface analysis was done by White Light Interferometry (New View 7300, Zygo LOT) at 360 nm lateral and <0.1 nm vertical resolution.

2.4. Measurement of contact killing by wet plating

Contact killing by wet plating was assessed essentially as described by Molteni et al. [15]. Briefly, *E. coli* W3110 was grown aerobically overnight in Luria Broth (10 mL, LB) at 37 °C in a shaking water bath at 250 rpm. An aliquot was removed to determine the cell titer of the culture. For contact killing assays, 20 μL of cells were applied to the polished metallic samples as a drop. Following incubation for various times in a water-saturated atmosphere, 15 μL were withdrawn and serially diluted in PBS, followed by spreading on LB agar plates. After incubation (37 °C for 24 h), colony forming units (cfu) were determined. Prior to contact killing experiments, all coupons were dipped in 2-propanol, dried on sterile tissue paper and stored in sterile Petri dishes until use.

2.5. Ion release analysis

Ion release experiments were performed in a closed setup on a sample surface area of 3.14 cm^2 with PBS (20 mL). Liquid samples (1 mL) were taken at different times and resuspended in 1.2 wt% nitric acid (5 mL, analytical grade). Every 10 min and before taking samples, the solution was stirred. The inductively