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ANTIBACTERIAL MECHANISM TRANSITION IN BRUSH-PLATED Cu-Ag HIERARCHICAL COATINGS

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Abstract

Hierarchical Cu-Ag composite coatings were fabricated by brush plating on AISI 316L stainless steel, integrating a sub-micron columnar copper matrix (750 nm-1 μm), homogeneously distributed nanoscale Ag particles (20-30 nm, ~38 wt.%), and surface-localized Ag microparticles (3-5 μm, 3-5 wt.%). SEM, EDS, and XRD confirmed the coexistence of Cu and Ag phases without detectable intermetallic formation. Unlike pure copper coatings-which exhibited diffusion-mediated antibacterial activity with inhibition zones of 20-24 mm against Staphylococcus aureus and Escherichia coli-the Cu-Ag composites showed no measurable zone of inhibition (<0.5 mm), while optical microscopy revealed no obvious bacterial colonization at the coating-agar interface. This difference in antibacterial response is interpreted in terms of the hierarchical architecture: the columnar Cu matrix increases the effective surface area available for bacterial-surface interactions, nanoscale Ag particles provide localized Ag-rich sites that may contribute to membrane perturbation and oxidative stress, and surface Ag microparticles physically cover approximately 65-75% of the Cu matrix, reducing the directly exposed Cu area and limiting outward Cu-ion transport. These results demonstrate that brush plating can be used to tailor the dominant antibacterial response through hierarchical microstructural design, providing a potential strategy for functional biomedical surfaces where reduced ion diffusion is desirable.

Keywords: brush plating; Cu-Ag composite; hierarchical microstructure; surface-confined antibacterial response; biomedical coatings

INTRODUCTION

Bacterial colonization of metallic surfaces remains a persistent challenge in medical devices and hospital infrastructure, where hygiene and reliability are critical [1,2]. Functional antibacterial coatings address this issue by limiting microbial growth directly at the material-environment interface.

Copper and silver are among the most extensively studied antimicrobial metals. Copper exerts rapid biocidal effects primarily through ion release (Cu^{+}/Cu^{2+}), which can disrupt cell membranes and interfere with intracellular processes [3-5]. Silver remains effective at substantially lower concentrations and can interact with bacterial membranes and cellular components, while silver nanoparticles have also been associated with reactive oxygen species (ROS) generation [6-8]. Combining both metals in a single coating provides an opportunity to exploit potentially complementary antibacterial effects [9-11].

Metallic antibacterial coatings can operate through different, partially overlapping pathways. Diffusion-mediated activity involves the release of metal ions into the surrounding medium, producing an antibacterial concentration field beyond the material surface. Surface-confined activity, in contrast, involves direct physical and chemical interactions between bacterial cells and the material interface [3,12]. Although

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ion release can provide effective antibacterial action, excessive metal-ion leaching may raise concerns regarding cytotoxicity in biomedical applications [13,14]. Consequently, controlling the balance between ion-mediated and surface-localized antibacterial effects is an important materials-design consideration.

Surface architecture can play a significant role in this balance. Hierarchically organized metallic surfaces can increase the number and complexity of local interaction sites available to bacterial cells, potentially enhancing surface-associated antibacterial effects. At the same time, selective coverage of a reactive metallic matrix may modify the area available for direct contact and influence the transport of ions from the underlying material.

Brush plating - a form of localized, dynamic electrodeposition - offers a flexible approach for producing such engineered surface structures. The process maintains a thin, continuously refreshed electrolyte film between a moving anode and the workpiece. High mass-transfer rates and strong hydrodynamic conditions can influence nucleation, growth kinetics, particle incorporation, and coating morphology [15–17]. Previous work on pure copper brush-plated layers demonstrated clear diffusion-mediated antibacterial activity, with inhibition zones of 20-24 mm [18].

The novelty of the present work lies in examining whether hierarchical Cu-Ag microstructural design can alter the dominant antibacterial response of brush-plated coatings. By combining a sub-micron columnar Cu matrix with nanoscale and micron-scale Ag particles in a single deposited structure, the study investigates the relationship between coating architecture and the experimentally observed transition from measurable diffusion-mediated inhibition to a predominantly surface-localized antibacterial response.

EXPOSITION

Experiment and characterization

Austenitic stainless steel AISI 316L coupons (20×60×3 mm) were mechanically polished to a mirror finish, ultrasonically cleaned, and air-dried. Coatings were applied using a portable SIFCO ASC POWER PACK (60 A/30 V) with a graphite anode wrapped in absorbent material. The anode traversed the substrate at 15 m/min, covering an effective area of 875 mm² per pass.

Deposition was carried out at 3-12 V, 30-45 °C, and a current density of 0.46 A/cm² for 255-262 s, targeting a nominal coating thickness of approximately 10 μm. Profilometry confirmed an average thickness of 9.8 ± 0.7 μm (n = 5).

Two coating types were prepared:

1. CuAC: pure copper deposited from an acidic electrolyte (COPPER ACID 2050, SIFCO ASC; pH 0.5).
2. CuAC+Ag: copper matrix co-deposited with bimodal silver particles.

Silver was introduced as a mixture of colloidal Ag nanoparticles (20-30 nm, 99.9%, 37-39 wt.%) and Ag microparticles (3-5 μm, 99.9%, 3-5 wt.%). The particles were ultrasonically dispersed immediately before use. Surface coverage by Ag microparticles was quantified using ImageJ and was estimated to be approximately 65-75% of the exposed Cu matrix.

Surface morphology was examined by SEM (HIROX SH-5500P) at 15 kV. Cross-sectional analysis was performed using FIB, including preparation of electron-transparent lamellae with an approximate thickness of 100 nm for TEM analysis. Elemental distribution was evaluated by EDS (QUANTAX 100 Advanced, Bruker), using five independent measurements per sample. Phase composition was determined by XRD (TDM 10) with Cu-Kα radiation over the 2θ range of 20-90°.

Antibacterial performance was evaluated using a combination of zone of inhibition (ZOI) measurements and direct surface microscopy. Testing was conducted in an ISO/IEC 17025-accredited laboratory against Staphylococcus aureus ATCC 29213 and Escherichia coli ATCC 25922.

Samples were autoclaved and placed on Müller-Hinton agar inoculated with bacterial suspensions adjusted to OD_{600} = 0.5. After

incubation for 24 h at 37°C, inhibition zones were measured (n = 5). The coating surfaces were subsequently inspected by optical microscopy to assess bacterial colonization at the coating–agar interface.

Hierarchical microstructure and composition

High-resolution SEM imaging revealed a distinct hierarchical architecture in the CuAC+Ag coating (Fig. 1). The surface morphology is characterized by a sub-micron columnar Cu matrix, with column diameters ranging from approximately 750 nm to 1 μm. The FIB cross-sectional view confirms that the columnar morphology extends through the coating thickness and is not restricted to the outermost surface.

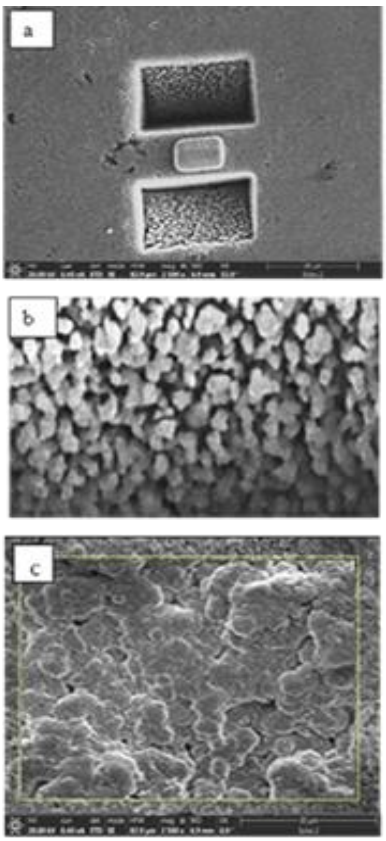


Fig. 1. *Hierarchical surface and cross-sectional microstructure of the brush-plated Cu-Ag composite coating: (a-b) SEM surface morphology at different magnifications; (c) FIB cross-sectional view revealing the sub-micron columnar Cu matrix*

The persistence of this architecture during preparation of the electron-transparent TEM lamella further supports its structural character. The observation of the columnar morphology after thinning to approximately 100 nm indicates that the observed structure is not merely a superficial imaging effect or a feature restricted to the outermost surface.

EDS elemental mapping revealed a continuous Cu-rich matrix with Ag distributed throughout the observed region. Nanoscale Ag features were observed within the Cu-rich matrix, while larger Ag features were preferentially associated with surface topographic features. The resulting structure therefore combines three characteristic length scales: a sub-micron columnar Cu matrix, nanoscale Ag inclusions, and micron-scale Ag particles concentrated toward the outer surface.

The phase composition of the coatings was further examined by XRD (Fig. 2). Both coatings exhibited diffraction peaks characteristic of metallic fcc Cu, while the CuAC+Ag composite additionally showed peaks corresponding to fcc Ag. No additional diffraction peaks or detectable peak shifts attributable to intermetallic phase formation were observed.

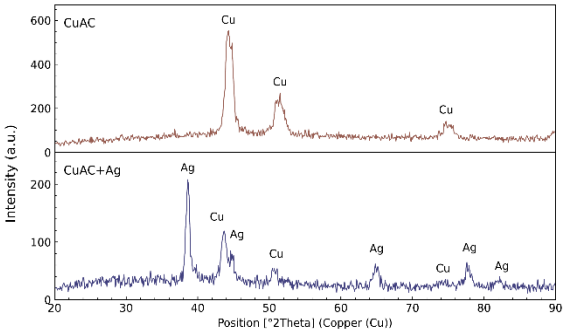


Fig. 2. *XRD patterns of brush-plated CuAC and CuAC+Ag coatings showing the coexistence of fcc Cu and fcc Ag phases without detectable intermetallic phases.*

These results indicate that the deposited Cu-Ag coating consists of separate Cu and Ag phases within the detection limits of the XRD analysis.

EDS analysis further confirmed the distinct compositional characteristics of the two coatings. The pure CuAC coating consisted almost entirely of copper, with 99.8 ± 0.1 wt.% Cu and 0.2 wt.% O. The CuAC+Ag composite contained 58.3 ± 1.2 wt.% Cu, 40.1 ± 1.0 wt.% Ag, and 1.6 wt.% O.

The EDS maps showed a Cu-rich background corresponding to the matrix,

while Ag was distributed throughout the analyzed region. The compositional results are consistent with the hierarchical morphology observed by SEM and FIB, in which Ag occurs both as finely distributed nanoscale features and as larger particles associated with the outer surface.

Antibacterial activity and mechanism transition

ZOI testing revealed a pronounced difference between the antibacterial responses of the two coating systems (Fig. 3). Pure CuAC coatings produced measurable inhibition zones of 24.3±1.2 mm for S. aureus and 22.8±0.9 mm for E. coli. These results are consistent with a diffusion-mediated antibacterial contribution associated with the release of copper ions into the surrounding agar, in agreement with the behavior previously reported for brush-plated copper coatings [18].

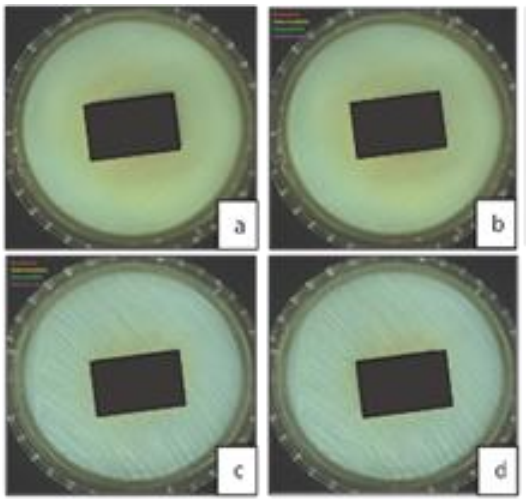


Fig. 3. *Antibacterial response of brush-plated CuAC and CuAC+Ag coatings after 24 h incubation at 37 °C: ZOI measurements against S. aureus ATCC 29213 and E. coli ATCC 25922 and representative optical microscopy observations of the coating-agar interface.*

In contrast, the CuAC+Ag composite produced no measurable inhibition zone (<0.5 mm) for either bacterial strain. Despite the absence of a measurable ZOI, optical microscopy did not reveal obvious bacterial colonization at the coating–agar interface.

The combination of these observations indicates a substantial difference in the spatial extent of the antibacterial response. The pure CuAC coating produced an antibacterial effect extending into the surrounding agar, whereas the CuAC+Ag coating showed no detectable inhibition field beyond the immediate surface while also showing no obvious bacterial colonization in the examined interface region.

The absence of a measurable ZOI in the CuAC+Ag composite, together with the absence of obvious bacterial colonization at the coating-agar interface, suggests a transition from predominantly diffusion-mediated activity toward a predominantly surface-confined antibacterial response (Fig. 4). This interpretation is consistent with the hierarchical organization of the coating.

First, the sub-micron columnar Cu matrix provides a highly developed surface morphology that increases the effective area available for bacterial-surface interactions. The columnar architecture therefore provides a large number of local surface features where bacteria may interact directly with the coating.

Second, the nanoscale Ag particles (20–30 nm) distributed within the Cu matrix provide localized Ag-rich sites. Their proximity to the bacterial–surface interface may contribute to membrane perturbation and localized oxidative effects during direct interaction. However, the present experiments do not directly quantify ROS generation or bacterial viability and therefore do not establish these processes independently. Third, the larger Ag particles (3-5 μm) concentrated toward the outer surface provide substantial physical coverage of the underlying Cu matrix, estimated at approximately 65-75%. This surface coverage reduces the directly exposed Cu area and may limit the outward

transport of Cu ions from the underlying matrix into the surrounding environment.

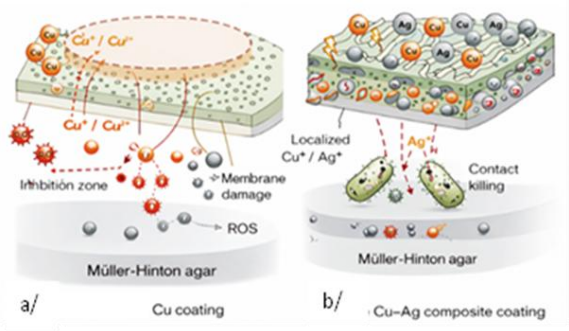


Fig. 4. *Schematic representation of the proposed transition in antibacterial response: (a) diffusion-mediated activity of the CuAC coating through Cu-ion release; (b) predominantly surface-confined response of the hierarchical Cu-Ag coating;*

The proposed interpretation is therefore based on the combined relationship between coating architecture and antibacterial observations: the CuAC coating exhibits a measurable inhibition field extending into the agar, whereas the hierarchical CuAC+Ag coating exhibits no measurable inhibition field while maintaining an interface without obvious bacterial colonization.

The proposed mechanism should be considered an interpretation of the combined structural and antibacterial observations rather than a direct measurement of individual bacterial killing pathways. In particular, the present study does not directly quantify bacterial viability, intracellular ROS generation, or local Cu/Ag ion concentrations. These parameters will be addressed in future work.

Note on galvanic effects:

Galvanic coupling between Cu (E° = +0.34 V) and Ag (E° = +0.80 V) would thermodynamically favor accelerated copper dissolution rather than suppression of copper dissolution. Therefore, the absence of a measurable diffusion zone cannot be attributed to electrochemical passivation of Cu by Ag. Within the framework of the present observations, the more plausible explanation is the physical surface coverage of the Cu-rich matrix by Ag microparticles and the resulting restriction of the directly exposed Cu surface, together with local retention and interaction of released species at the interface [13,14].

Limitation of the antibacterial assessment:

The present assessment distinguishes diffusion-mediated from predominantly surface-confined behavior through the combined use of ZOI measurements and direct surface microscopy. However, the study does not directly quantify bacterial viability by live/dead staining, colony-forming-unit counting, or biofilm assays. Similarly, ROS generation and local metal-ion concentrations were not directly measured. Consequently, the proposed surface-confined mechanism is mechanistically consistent with the observed structural and antibacterial behavior, but individual molecular and cellular pathways cannot yet be considered experimentally proven. Future work will therefore focus on quantitative viability measurements, biofilm characterization, ROS evaluation, and analysis of local Cu and Ag ion release.

CONCLUSION

Hierarchical Cu-Ag composite coatings were successfully deposited on AISI 316L stainless steel by brush plating. The resulting architecture integrates a sub-micron columnar Cu matrix, distributed nanoscale Ag particles, and surface-concentrated Ag microparticles within a single deposited structure.

The antibacterial tests demonstrated a pronounced difference between the two coating systems. Pure CuAC coatings produced measurable inhibition zones of approximately 20-24 mm against both S. aureus and E. coli, consistent with a diffusion-mediated antibacterial contribution. In contrast, the CuAC+Ag composites produced no measurable ZOI (<0.5 mm), while optical microscopy revealed no obvious bacterial colonization at the coating–agar interface.

The observed behavior is interpreted as a transition toward a predominantly surface-confined antibacterial response associated

with the hierarchical microstructure. The sub-micron columnar Cu matrix increases the surface area available for bacterial interaction, nanoscale Ag particles provide localized Ag-rich sites, and surface Ag microparticles cover approximately 65–75% of the exposed Cu matrix, reducing the directly exposed Cu area and potentially limiting outward Cu-ion transport.

The proposed mechanism remains to be validated by direct measurements of bacterial viability, ROS generation, biofilm formation, and local Cu/Ag ion release. Nevertheless, the present results demonstrate that brush plating provides a practical route for engineering the spatial character of antibacterial activity through hierarchical control of metallic phases and surface architecture. This approach may be relevant to the development of functional biomedical surfaces where localized antibacterial activity and reduced ion diffusion are desirable.

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