

CONTACT-MEDIATED ANTIBACTERIAL MECHANISM IN CU–AG COMPOSITE COATINGS DEPOSITED BY BRUSH PLATING

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Received on June 1, 2026

Presented by S. Hadjitodorov, Corresponding Member of BAS, on September 29, 2026.

Abstract

This study investigated the antibacterial mechanism of Cu–Ag composite coatings deposited by brushing on AISI 316L stainless steel. High Ag content coatings (~ 42 wt%) were fabricated using a SIFCO ASC brushing system from acidic electrolytes (pH 0.5) containing 1–5 μm Ag particles. SEM/EDS analysis revealed a uniform distribution of Ag in the Cu matrix without agglomeration, while XRD confirmed a phase separation between metallic Cu and Ag without detectable intermetallic compounds. Zone of inhibition (ZOI) assays against *Staphylococcus aureus* ATCC 29213 and *Escherichia coli* ATCC 25922 showed that, unlike pure Cu coatings that produced distinct zones of inhibition, the Cu–Ag composites did not exhibit a measurable ZOI (detection limit < 0.5 mm), but completely suppressed bacterial adhesion on the coating surface (Fig. 4). This shift from diffusion-mediated to predominantly contact-mediated antibacterial activity is due to the physical coating of the copper matrix surface by silver particles, which reduces the exposed copper area available for ion release, combined with the low solubility of silver compounds and the rapid complexation of the released Cu^{2+} ions with chloride ions in agar medium. Notably, galvanic coupling between Cu ($E^\circ = +0.34$ V) and Ag ($E^\circ = +0.80$ V vs. SHE) would thermodynamically accelerate Cu dissolution rather than suppress it; therefore, the observed reduction in ion diffusion is primarily explained by

This work was supported by the Bulgarian National Science Fund under the programme for financial support for fundamental research projects – 2022, Project “Investigation of the biocidal activity of selective plated copper-based coatings containing silver particles”, No. KP06-PH67-14.

<https://doi.org/10.7546/CRABS.2026.10.11>

the physical surface coating rather than by electrochemical effects. The brush coating allows for selective functionalization of critical areas of the implant without compromising osseointegration surfaces, offering a promising strategy for next-generation antimicrobial medical devices.

Key words: brush plating, copper–silver composites, contact-mediated killing, galvanic coupling, antibacterial coatings, stainless steel 316L

Introduction. Antibacterial resistance represents a critical global challenge in modern medicine. The World Health Organization estimates that infections caused by resistant microorganisms could result in up to 10 million deaths annually by 2050 [1, 2]. Medical device- and implant-associated infections, where bacterial adhesion and subsequent biofilm formation occur on surfaces, pose serious therapeutic challenges and often require surgical intervention [3, 4]. Copper and its alloys are well-known for their antibacterial properties, arising from both $\text{Cu}^+/\text{Cu}^{2+}$ ion release and direct interaction between the copper surface and bacterial cell membranes, leading to membrane disruption and microbial inactivation [5–7]. Numerous studies demonstrate the effectiveness of copper surfaces against a broad spectrum of Gram-positive and Gram-negative bacteria, including *S. aureus* and *E. coli* [8, 9]. Previous work established that brush-plated pure copper coatings exhibit clear antibacterial activity, with microstructure, phase composition, and surface topography significantly influencing ZOI size and overall efficacy [10].

Silver is another widely used antimicrobial agent with broad-spectrum activity [11, 12]. Its antibacterial action primarily involves localized Ag^+ release and contact-mediated effects, with limited diffusion in the surrounding medium due to the low solubility of silver compounds (e.g., AgCl , $K_{\text{sp}} = 1.8 \times 10^{-10}$) [13]. Combining copper and silver in composite coatings offers a promising approach to achieve complementary effects and sustained antibacterial protection [14–16]. Despite extensive studies on Cu–Ag systems, two gaps remain: (i) the antibacterial mechanism in brush-plated coatings has not been systematically explored, despite the potential influence of localized deposition and dynamic electrolyte renewal on ion release kinetics; and (ii) the possibility of a shift from diffusion-mediated to predominantly contact-mediated mechanisms induced by high Ag content has not been demonstrated in brush-plated systems. Previous reports on Cu–Ag coatings typically show mixed mechanisms with measurable but reduced ZOI (10–18 mm) at low Ag contents (< 10 at.%) [16, 17]. This work addresses these gaps by elucidating the relationship between microstructure and antibacterial mechanism in brush-plated Cu–Ag coatings with high Ag content (~ 42 mass%). We demonstrate that physical coverage of the Cu matrix by Ag particles shifts the dominant antibacterial mechanism toward contact-mediated effects while maintaining complete suppression of bacterial adhesion. Furthermore, the study emphasizes the unique advantage of brush plating for selective functionalization of critical implant areas while preserving osseointegration surfaces.

Materials and methods. Substrate preparation and brush plating deposition.. Austenitic stainless steel AISI 316L samples ($20 \times 60 \times 3$ mm) were mechanically polished to a mirror finish using a sequence ending with 1 μm aluminum oxide abrasive, followed by ultrasonic cleaning in acetone (10 min), ethanol (10 min), and deionized water (5 min). Coatings were deposited using the brush plating technique (localized electrodeposition) with a SIFCO ASC portable plating system (model: SIFCO Process[®]), under the following conditions:

- Maximum voltage: 15 V DC
- Maximum current: 5 A DC
- Operating mode: direct current (DC).

A graphite anode ($40 \times 20 \times 20$ mm) wrapped in absorbent material (White Tuff Wrap) served as the electrolyte reservoir and ensured uniform contact with the substrate, which acted as the cathode. Deposition was carried out as follows:

1. The absorbent material was saturated with electrolyte for 20 min prior to deposition.
2. The anode was positioned 2–3 mm from the substrate and moved at 8–12 mm/s along a predefined trajectory to uniformly cover an area of 875 mm².
3. Deposition was performed at a current density of 2.5 A/dm² for 180–240 s, yielding coatings ~ 10 μm thick.
4. Electrolyte was continuously replenished by saturating the absorbent material, preventing local ion depletion.

Four coating types were fabricated:

- CuAC: pure Cu from acidic electrolyte (0.5 M CuSO₄, 0.5 M H₂SO₄, pH 1.5);
- CuALK: pure Cu from alkaline electrolyte (0.3 M CuSO₄, 1.0 M NaOH, pH 8.5);
- CuAC+Ag: Cu with embedded Ag particles in acidic electrolyte;
- CuALK+Ag: Cu with embedded Ag particles in alkaline electrolyte.

Silver particles (99.9%, 1–5 μm) were ultrasonically dispersed (20 kHz, 15 min) in the electrolyte immediately before deposition. During brush plating, particles were incorporated into the growing Cu matrix via mechanical entrapment and electrophoretic migration toward the cathode.

Coating thickness was verified by profilometry ($9.8 \pm 0.7 \mu\text{m}$, $n = 5$) and cross-sectional SEM.

This experimental setup allows direct correlation between coating microstructure, phase composition, and antibacterial performance, providing a foundation for interpreting the transition from diffusion-mediated to contact-mediated antibacterial mechanisms.

Microstructural and phase analysis. Coating morphology and microstructure were examined using SEM (Bruker VEGA 3) at 15 kV. Elemental composition and distribution of Cu and Ag were analyzed by EDS at five independent locations per sample.

Phase composition was determined by XRD (TDM-10 Tongda diffractometer) using Cu-K α radiation ($\lambda = 1.5406 \text{ \AA}$) over $2\theta = 20\text{--}90^\circ$ with a $0.02^\circ/\text{s}$ scanning rate. Phases were identified by comparison with the X'Pert HighScore Plus database.

Antibacterial activity evaluation. Antibacterial activity was evaluated via ZOI assays in an ISO/IEC 17025-accredited laboratory (AO Research Institute, Davos, Switzerland) using *S. aureus* ATCC 29213 and *E. coli* ATCC 25922. Samples ($20 \times 40 \text{ mm}$) were cleaned under sterile conditions with a cotton swab dipped in isopropanol, followed by rinsing with sterile MilliQ water and air dried. Then the discs were packed and sterilized by autoclaving at 120°C for 15 min.

Bacterial strains *S. aureus* ATCC 29213 and *E. coli* ATCC 25922 were used for the inhibition zone testing.

For the testing both strains were inoculated into flasks containing Müller-Hinton Broth (MHB; Oxoid CM0405 Lot.#134486) and incubated overnight at 37°C . The overnight culture was poured into a 50 ml falcon tube and centrifuged for 5 min at 1320 rcf. The supernatant was discarded, and the pellet was resuspended with 10 ml sterile Phosphate buffered saline (PBS) and again centrifuged for 5 min at 1320 rcf. Again, the supernatant was discarded, and the pellet resuspended in 30 ml sterile PBS.

The suspension was sonicated in an Ultrasonication bath for 3 min. Then the optical density OD 600 was measured and the OD600 was adjusted to 0.5.

One hundred microliters of this solution was pipetted onto Müller-Hinton Agar (MHA; Oxoid CM0337 Lot.#316421) plates. The solution was streaked out with a cotton swab in four directions. Then the discs were placed onto the agar plates and incubated for 24 h at 37°C .

Results and discussion. Surface morphology and microstructure. SEM images (Fig. 1, 2) show a homogeneous surface morphology for the composite copper coatings. CuAC+Ag shows fine grains ($0.8 \pm 0.2 \mu\text{m}$), while CuALK+Ag shows larger aggregates ($1.5 \pm 0.3 \mu\text{m}$). The Cu–Ag composites show well-dispersed Ag particles ($1\text{--}5 \mu\text{m}$), fully embedded in the copper matrix, without cracks or delamination. The alkaline environment improves the distribution and attachment

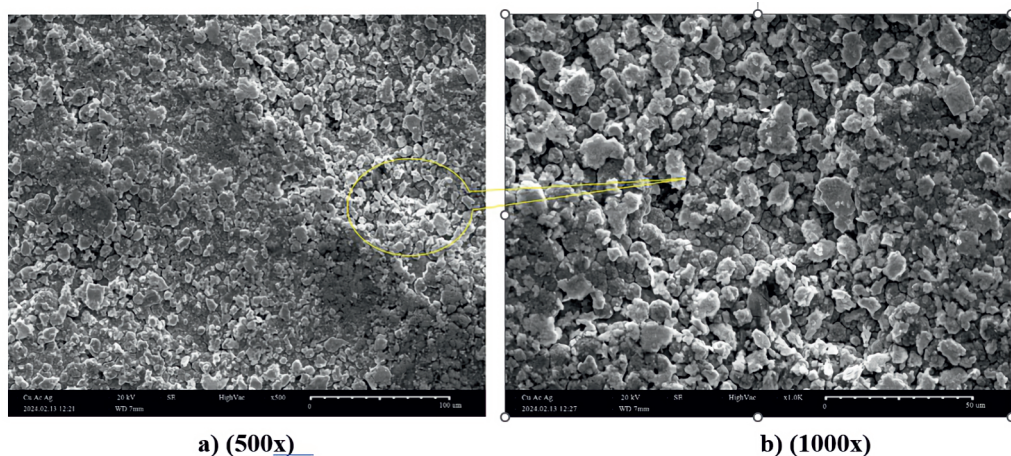


Fig. 1. Microstructure of selective copper-based coating CuAC+Ag containing silver particles with a size of 1–5 μm

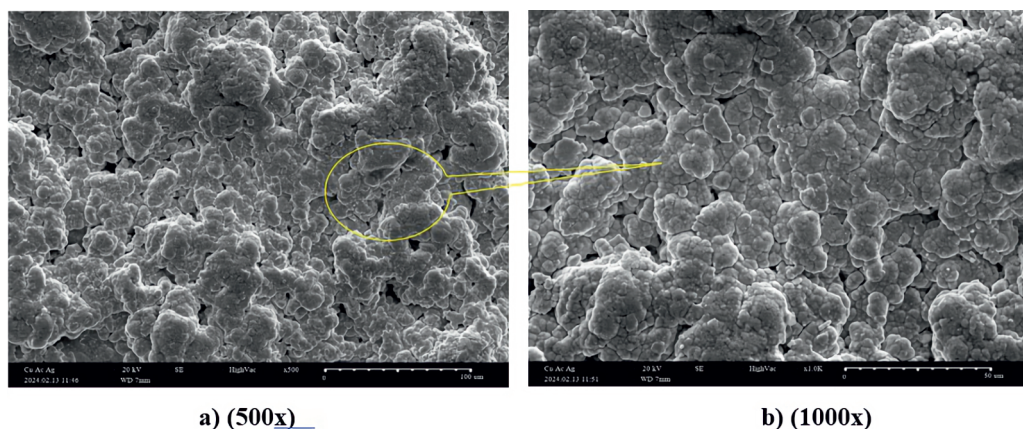


Fig. 2. Microstructure of selective copper-based coating CuALK+Ag containing silver particles with a size of 1–5 μm

of the particles. These differences probably arise from variations in deposition kinetics and mechanical embedding during brush application.

Elemental composition and distribution. EDS analysis confirms the presence of Cu and Ag as the main elements in composite coatings, without detectable impurities. Cu content in Cu–Ag coatings is 56–58 mass%, while Ag is 40–42 mass%. Elemental maps reveal uniform Cu distribution and localized but well-dispersed Ag particles, demonstrating effective embedding without agglomeration.

Phase composition. XRD patterns (Fig. 3) show fcc Cu peaks for pure coatings and additional fcc Ag peaks for composites. No peak shifts or intermetallic phases are observed, confirming phase separation and coating stability.

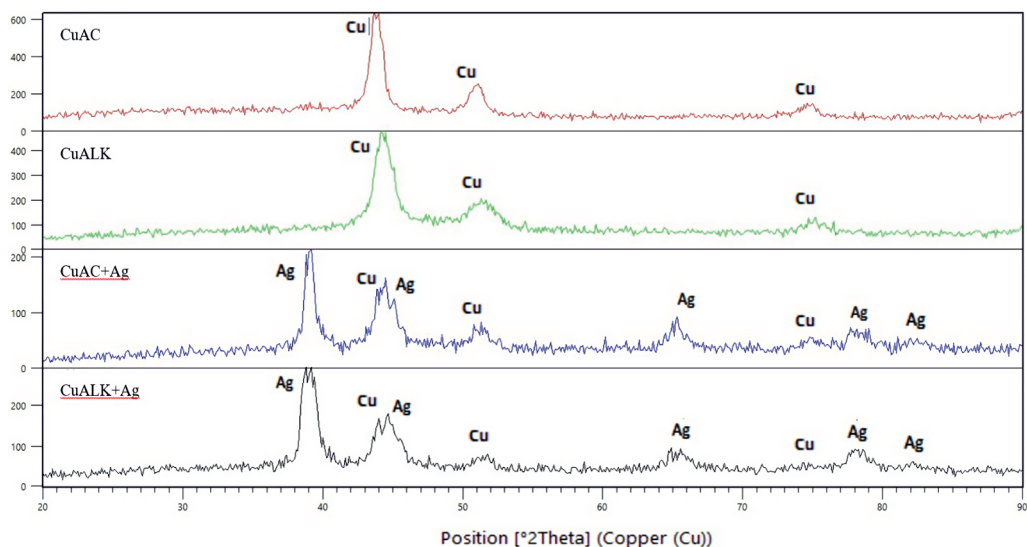
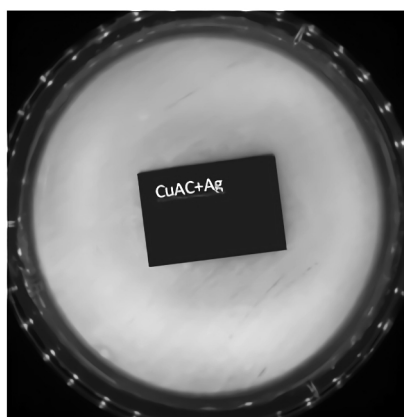


Fig. 3. XRD patterns of brush-plated coatings: (a) CuAC, (b) CuALK, (c) CuAC+Ag, (d) CuALK+Ag. Peaks indexed according to JCPDS: CuAC (96-410-5041), CuALK (96-431-3208), Ag (96-901-1609)

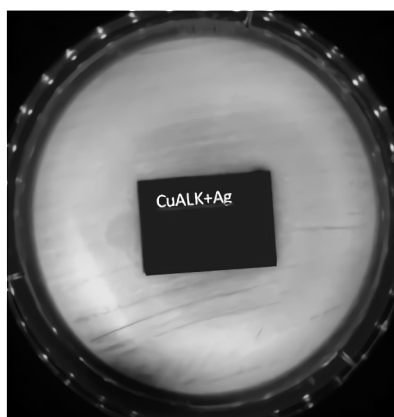
Antibacterial activity (ZOI analysis). Pure Cu coatings exhibit clear inhibition zones [10], indicating diffusion-mediated antibacterial activity. Cu–Ag composites show **no detectable inhibition zone** but completely suppress bacterial adhesion at the coating surface (Fig. 4), indicating a shift to contact-mediated killing.

Mechanism of antibacterial action. Pure copper coatings act by diffusion-mediated release of $\text{Cu}^+/\text{Cu}^{2+}$ ions, which migrate into the agar medium, forming macroscopic zones of inhibition [10]. The high solubility of copper compounds in chloride-containing media (e.g., Müller-Hinton agar) facilitates sustained release and diffusion of ions. In Cu–Ag composites, galvanic coupling between Cu ($E^\circ = +0.34$ V) and Ag ($E^\circ = +0.80$ V) limits diffusion of Cu ions, while Ag promotes localized contact-mediated killing. Phase separation provides stable, long-term antibacterial activity without over-release of ions. In Cu–Ag composites with high Ag content (~ 42 wt%), the dominant antibacterial mechanism shifts towards contact-mediated effects due to several interrelated factors:

1. Physical surface coverage: The high volume fraction of Ag particles significantly reduces the exposed Cu surface area available for ion release. Geometric modelling based on the particle size distribution (1–5 μm) and coating composition suggests that silver particles cover approximately 65–75% of the outer surface of the coating, which significantly limits the area through which copper ions can diffuse into the medium.

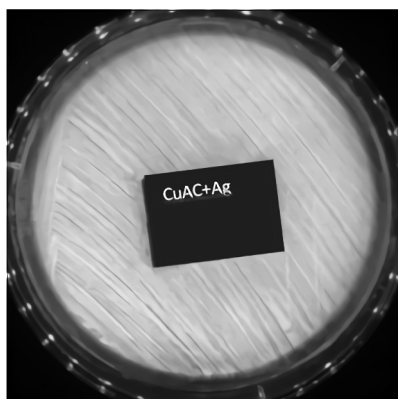


a)



b)

Bacterial strains *E. coli* ATCC 25922



c)



d)

Bacterial strains *S. aureus* ATCC 29213

Fig. 4. Antibacterial activity assessed by ZOI assay after 24 h at 37 °C: (a) CuAC+Ag, (b) CuALK+Ag, (c) CuAC+Ag, (d) CuALK+Ag against *E. coli* and *S. aureus*

2. Low solubility of silver compounds. This limits the release of silver⁺ and prevents the formation of a diffusion gradient in the agar medium.

This predominantly contact-mediated mechanism offers potential advantages for biomedical implants: it minimizes systemic ion release while maintaining effective surface protection against bacterial colonization, thereby potentially reducing the risks of cytotoxicity associated with elevated copper²⁺ concentrations in surrounding tissues.

Conclusion. Brush plating produces uniform Cu–Ag composite coatings (~ 10 μm thick) with well-embedded Ag particles (1–5 μm) at high concentra-

tion (~ 42 mass%) without agglomeration, as evidenced by SEM/EDS analysis (Fig. 1, 2). XRD analysis (Fig. 3) confirms phase separation between Cu and Ag with no detectable intermetallic compounds, consistent with the thermodynamic behaviour of the Cu–Ag system and the low processing temperature of brush plating.

Cu–Ag composites with high Ag content (~ 42 mass%) show no measurable inhibition zone but completely suppress bacterial adhesion at the coating surface, indicating a shift to predominantly contact-mediated antibacterial activity. This mechanistic shift is attributed to physical coverage of the Cu matrix by Ag particles (Fig. 1), which reduces the exposed Cu surface area and limits Cu^{2+} ion diffusion into the surrounding medium. Galvanic coupling between Cu and Ag would accelerate rather than suppress Cu dissolution; therefore, electrochemical effects do not explain the observed reduction in ion diffusion.

The predominantly contact-mediated mechanism offers potential advantages for biomedical implants by minimizing systemic ion release while maintaining effective surface protection against bacterial colonization. Brush plating enables selective functionalization of critical implant zones without compromising osseointegration surfaces, representing a practical advantage for clinical applications.

Acknowledgments. The authors acknowledge the AO Research Institute (Davos, Switzerland) for performing accredited antibacterial testing (ISO/IEC 17025).

REFERENCES

- [1] WHO (2014) Antimicrobial resistance: global report on surveillance, Geneva: World Health Organization.
- [2] O'NEILL J. (2016) Tackling drug-resistant infections globally: final report and recommendations, Review on Antimicrobial Resistance.
- [3] COSTERTON J. W., P. S. STEWART, E. P. GREENBERG (1999) Bacterial biofilms: a common cause of persistent infections, *Science*, **284**(5418), 1318–1322.
- [4] ARCIOLA C. R., D. CAMPOCCIA, P. SPEZIALE et al. (2012) Biofilm formation in *Staphylococcus* implant infections. A review of molecular mechanisms and implications for biofilm-resistant materials, *Biomaterials*, **33**(26), 5967–5982.
- [5] KAUR R., S. LIU (2016) Antibacterial surface design – Contact kill, *Prog. Surf. Sci.*, **91**(3), 136–153, <https://doi.org/10.1016/j.progsurf.2016.09.001>.
- [6] GRASS G., C. RENSING, M. SOLIOZ (2011) Metallic copper as an antimicrobial surface, *Appl. Environ. Microbiol.*, **77**(5), 1541–1547.
- [7] VINCENT M., P. HARTEMANN, M. ENGELS-DEUTSCH (2016) Antimicrobial applications of copper, *Int. J. Hyg. Environ. Health*, **219**(7 PtA), 585–591, <https://doi.org/10.1016/j.ijheh.2016.06.003>.
- [8] WARNES S. L., C. W. KEEVIL (2011) Mechanism of copper surface toxicity in vancomycin-resistant enterococci following wet or dry surface contact, *Appl. Environ. Microbiol.*, **77**(17), 6049–6059, <https://doi.org/10.1128/AEM.00597-11>.

- [9] SONG Q., S. Y. CHAN, Z. XIAO et al. (2024) Contact-killing antibacterial mechanisms of polycationic coatings: A review, *Prog. Org. Coat.*, **188**(12), 108214, <https://doi.org/10.1016/j.porgcoat.2024.108214>.
- [10] VESELINOV D., H. SKULEV, R. YANKOVA et al. (2023) Enhancing antibacterial performance: Structure, phase composition, and microroughness of selective plated copper coatings on medical-grade stainless steel 1.4021 and nickel alloy Ni200, *Appl. Sci.*, **13**(17), 9840, <https://doi.org/10.3390/app13179840>.
- [11] RAI M., A. YADAV, A. GADE (2009) Silver nanoparticles as a new generation of antimicrobials, *Biotechnol. Adv.*, **27**(1), 76–83.
- [12] CHERNOUSOVA S., M. EPPLE (2013) Silver as antibacterial agent: ion, nanoparticle and metal, *Angew. Chem. Int. Ed. Engl.*, **52**(6), 1636–1653.
- [13] HWANG I. S., J. H. HWANG, H. CHOI et al. (2012) Synergistic effects between silver nanoparticles and antibiotics and the mechanisms involved, *J. Med. Microbiol.*, **61**(Pt 12), 1719–1726, <https://doi.org/10.1099/jmm.0.047100-0>.
- [14] FAN X., L. YAHIA, E. SACHER (2021) Antimicrobial properties of the Ag, Cu nanoparticle system, *Biology*, **10**(2), 137, <https://doi.org/10.3390/biology10020137>.
- [15] SALAM A. A., R. SINGARAVELAN, P. VASANTHI et al. (2025) Electrochemical fabrication of Ag–Cu nano alloy and its characterization: an investigation, *J. Nanostruct. Chem.*, **5**(4), 383–392.
- [16] HAO Z., M. WANG, L. CHENG et al. (2024) Synergistic antibacterial mechanism of silver-copper bimetallic nanoparticles, *Front. Bioeng. Biotechnol.*, **11**, 1337543, <https://doi.org/10.3389/fbioe.2023.1337543>.
- [17] CIACOTICH N., R. U. DIN, J. J. SLOTH et al. (2018) An electroplated copper–silver alloy as antibacterial coating on stainless steel, *Surf. Coat. Technol.*, **345**, 96–104, <https://doi.org/10.1016/j.surfcoat.2018.04.007>.

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