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Original Article

Laboratory-based study of novel antimicrobial cold spray coatings to combat surface microbial contamination

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Abstract

Objective: To investigate the touch-contact antimicrobial efficacy of novel cold spray surface coatings composed of copper and silver metals, regard to their rate of microbial elimination.

Design: Antimicrobial time-kill assay.

Setting: Laboratory-based study.

Methods: An adapted time-kill assay was conducted to characterize the antimicrobial efficacy of the developed coatings. A simulated touch-contact pathogenic exposure to Gram-positive *Staphylococcus aureus* (ATCC 25923), Gram-negative *Pseudomonas aeruginosa* (ATCC 27853), and the yeast *Candida albicans* (ATCC 10231), as well as corresponding resistant strains of gentamicin-methicillin–resistant *S. aureus* (ATCC 33592), azlocillin-carbenicillin–resistant *P. aeruginosa* (DSM 46316), and a fluconazole-resistant *C. albicans* strain was undertaken. Linear regression modeling was used to deduce microbial reduction rates.

Results: A >7 log reduction in microbial colony forming units was achieved within minutes on surfaces with cold spray coatings compared to a single log bacterial reduction on copper metal sheets within a 3 hour contact period. Copper-coated 3-dimensional (3D) printed acrylonitrile butadiene styrene (ABS) achieved complete microbial elimination against all tested pathogens within a 15 minute exposure period. Similarly, a copper-on-copper coating achieved microbial elimination within 10 minutes and within 5 minutes with the addition of silver powder as a 5 wt % coating constituent.

Conclusions: In response to the global need for alternative solutions for infection control and prevention, these effective antimicrobial surface coatings were proposed. A longitudinal study is the next step toward technology integration.

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The persistence of hospital acquired infections and the increasing rates of antibiotic resistance pose a serious threat to healthcare services, including staff, patients, and visitors.[1,2] Surface contamination is a major contributing factor; with many infections, including resistant microbial strains, are transmissible via contact sites.[3,4] Common hospital surfaces, including stainless steel and plastic polymers, can harbor microorganisms for extended periods. Methicillin-resistant *Staphylococcus aureus* (MRSA), for example, has been reported to survive on these surfaces for >6 months.[1] Multiple surfaces provide an intermediary for infection transmission, via recurrent contact: bed rails, door handles, medical equipment, and even portable electronic devices (eg, tablets, smartphones, and smartwatches).

Although hospitals offer mitigation strategies for both practitioners and patients, they are often ineffective. Hand hygiene protocols, for example, are at the forefront of awareness programs, encouraging the use of sanitizer stations within hospitals. The World Health Organization reported that compliance with hand hygiene protocols for healthcare providers was at 38.7% as of 2009.[5] Since then, this compliance has not significantly improved. Recently, the Centers for Disease Control and Prevention reported that hospital staff clean their hands less than half the times they should.[6] Together with hand hygiene practices, surface-cleaning protocols have been the predominant solution for infection control. However, many hospital surfaces fail to comply with regulated contamination standards, and noncompliance ranges between 50% and 90%.[7,8]

It is well established that the metals of copper and silver possess innate antimicrobial properties.[7,9] Copper, particularly, has been used within the hospital environment as a favorable antimicrobial surface.[10–12] The current burden of nosocomial infections and a renewed interest in alternative approaches to infection control has encouraged a reintroduction of these metals.

A number of studies have considered various surface-coating techniques, from blended copper and silver laser cladding,[13] to copper-wire arc spraying of stainless steel substrates,[14] to copper magnetron sputtered coatings on copper substrates.[7] Another

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comparative study investigated the relative antimicrobial efficacy of copper-coated aluminum using three spray techniques: plasma, wire arc, and cold spray.[15]

Two emerging trends from previous studies have prompted a new and alternative design approach related to the substrate materials and the observed efficacy timelines. These studies utilized metal substrates of stainless steel, aluminum, and copper. However, a large proportion of high contact hospital surfaces are plastic. Additionally, an authentic time course was considered for test protocols, considering an observed 5 minute contact window, related to the observed average elapsed time between contact with hospital surfaces by patients, visitors, or hospital staff.[7] Previous efficacy timelines including the aforementioned studies,[7,13–15] and even many test standards,[16] consider biocidal efficacy from a 1 hour contact time onward (24 hours for the last three listed standard test methods), thus inadvertently neglecting more realistic contact time effects that occur within minutes and not hours.[7]

An international patent application, filed on behalf of the inventors Lucas, Botef, and van Vuuren,[17] details the method of applying an antimicrobial surface coating to a substrate using the additive manufacturing techniques of cold spray and 3D printing as well as the contact killing properties of copper and silver metals, for the reduction of micro-organisms on surfaces. This current study presents the results of an adapted time-kill assay as a means of evaluating the touch-contact antimicrobial efficacy of novel cold-spray surface coatings over incremental microbial contact time periods.

Methods

Cold spray coatings

A custom built cold spray unit from Centerline Supersonic Spray Technology (SST; LaSalle, Ontario) was used to produce coatings based on the novel multi-step and multi-material additive manufacturing approach developed by Lucas, Botef, and van Vuuren.[17] The cold spray coatings were deposited onto a copper sheet and 3D printed acrylonitrile butadiene styrene (ABS) substrates of dimension 12 mm × 12 mm. High purity copper powder (>99.7% Cu) and silver powder (>99.99% Ag) were obtained from SST Centerline and Sigma-Aldrich (St Louis, MO), respectively, and the ABS substrates were printed using a Stratasys uPrint SE 3D printer (Stratasys, Rehovot, Israel).

Antimicrobial susceptibility testing

A dry contact time-to-microbial elimination (time-kill) assay was devised to simulate the touch-contact activity on surfaces. The test procedure was adapted from the US Environmental Protection Agency test protocol.[16] A critical variation in the test methodology used in our study was the introduction of additional sampling periods and the extension to include fungal contamination. The selection of test organisms was based on the highly prevalent ESKAPE pathogens.[18] The following pathogens from the American Type Culture Collection (ATCC) were selected: *Staphylococcus aureus* (ATCC 25923), *Pseudomonas aeruginosa* (ATCC 27853), and *Candida albicans* (ATCC 10231). Furthermore, we also investigated antimicrobial efficacy against the following resistant strains: gentamicin-methicillin–resistant *S. aureus* (ATCC 33592), azlocillin-carbenicillin–resistant *P. aeruginosa* strain from the German collection of microorganisms and cell cultures at the Leibniz Institute (DSM 46316), and a fluconazole-resistant *C. albicans* strain from the University of the Witwatersrand, Faculty of Health Sciences, Department of Clinical Microbiology and Infectious Diseases (*C. albicans* 4122).

Test samples were cleaned with an anti-pill cloth to remove any visible contaminants (eg, residual spray powder) and were then disinfected via immersion in 70% alcohol. The samples were then air dried under aseptic conditions in a laminar airflow unit under an ultraviolet light. For each test period, a culture suspension, with a microbial concentration of ~3.75×10^7 colony-forming units (CFU) of inoculated Tryptone Soya broth (CFU/mL) was spread onto a test sample surface. The sample was then aseptically transferred to a neutralizing saline solution after a predetermined contact period (ie, 0.5, 5, 10, 15, 20, 60, and 180 minutes). Two consecutive serial dilutions, with a dilution factor of 1:100 each, were sufficient to achieve an effective colony count. At each dilution level, an aliquot of this solution was plated onto Tryptone Soya agar plates. Samples were then incubated at 37°C for 16–24 hours for bacterial quantification and for 48 hours for the yeast. Colony counts were performed using Open CFU, an open-source colony-counting software, and were validated by manual counts. The final quantified data are presented as the geometric mean CFU/mL at each period and are plotted to represent effective time-kill rates.

Statistical methods

Linear regression modelling was used to evaluate the dry contact test results. The relative microbial reduction rate (m_{ar}), given by Equation 1 and with units of $\log_{10}$ (CFU/mL) per minute, was defined as the relative microbial reduction rate of a test coating (m_{ts}) compared to the response of a stainless steel control (m_{ss}). The relative microbial reduction rate (m) represents the gradient of a linear line-of-best-fit in the standard form $y = mx + c$, with the sampling time as the x abscissa data points, the logged contamination concentration of $\log_{10}$ (CFU/mL) as the y ordinate data points and the vertical axis intercept c representing the initial inoculate concentration.

$$m_{ar} = m_{ts} - m_{ss} \qquad \text{Eq.1}$$

The larger the antimicrobial rate, the faster microbial reduction takes place. The suitability of a regression model was confirmed based on an evaluation of the coefficient of determination (R^2) as a measure of data variability, which represents the square of the Pearson correlation (Equation 2),[19] where x and y are the relevant coordinates for the graphed data points, i is the counter and n the number of data points in the analysis. The coefficient of determination, as a measure of data variability, has a value between 0 and 1 and may therefore be indicative of the percentage of the data that supports a linear trend relationship.

$$R_{xy} = \frac{\sum_{i=1}^{n} y_u(x_i - \overline{x})}{\left[\sum_{i=1}^{n} y_i(y_i - \overline{y})^2 \sum_{i=1}^{n} (x_i - \overline{x})^2\right]^{0.5}} \qquad \text{Eq.2}$$

In addition to the microbial reduction rate (m_{ar}), the highest average percentage reduction was included as a deterministic factor for antimicrobial efficacy.

Results

The antimicrobial efficacy of the copper containing coatings was compared to that of high purity copper and a stainless steel sheet, tested under simulated dry, touch-contact conditions. Figure 1 illustrates the antimicrobial efficacy rates of these test samples

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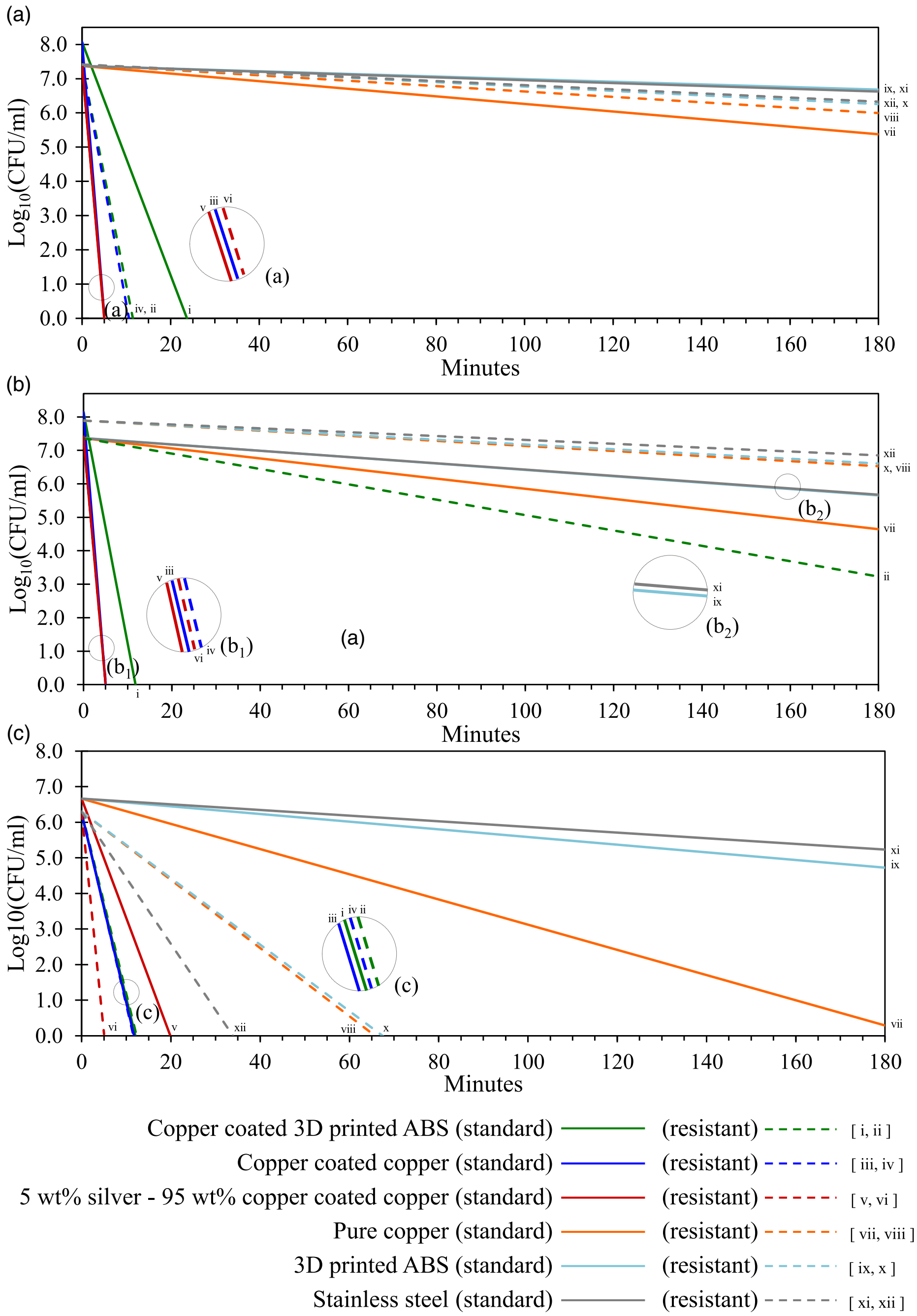

Fig. 1. Touch-contact antimicrobial efficacy of copper and silver metallized coatings against (A) *Staphylococcus aureus* (reference) and gentamicin-methicillin resistant *S. aureus* (resistant), (B) reference and azlocillin-carbenicillin-resistant *Pseudomonas aeruginosa*, and (C) reference and fluconazole-resistant *Candida albicans*.

against the three pathogens (Fig. 1A, *S. aureus*; Fig. 1B, *P. aeruginosa*; Fig. 1C, *C. albicans*) and their associated resistant strains. The copper-sheet control effectively validates the enhanced antimicrobial efficacy demonstrated by the cold spray coatings. Copper metal, a known antimicrobial agent, exhibited a considerably lower efficacy rate as a solid metal sheet than as a cold spray coating, regardless of the substrate material and test pathogen. In particular, the copper control achieved, on average, just >90% reduction on reference pathogenic strains within a contact time of two hours and 20 minutes. Against the resistant strains, copper metal was ineffective as a contact-killing surface; it exhibited no attributable antimicrobial activity. This activity stands in contrast to the cold spray coatings, all of which achieved >7 log microbial reduction within minutes.

The microbial reduction observed for stainless steel and uncoated 3D printed ABS over the three hour test period (Fig. 1), though not indicative of active antimicrobial activity, represents the effects of system noise. These uncontrollable system variations include plating stress, environmental conditions, and potential inoculate volume loss during sample loading, which influence test conditions and subsequent microbial growth. These noise effects are clearly differentiated from the biocidal activity evident with the cold spray coatings.

The effects of system noise are not unique to this study. A study investigating wire-arc sprayed copper coatings on stainless steel, reported a 60% microbial load reduction over a six hour contact period for stainless steel control samples.[14] This finding may be

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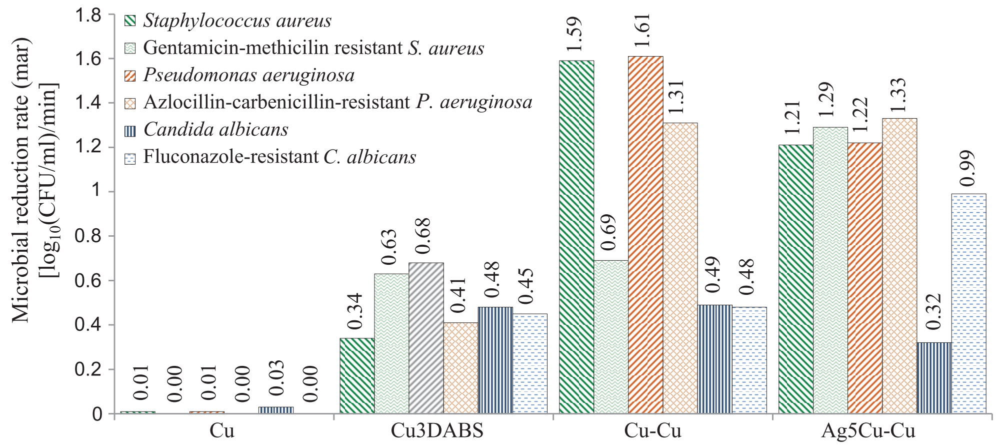

Fig. 2. Microbial reduction rates for copper cold spray coatings and copper metal control against both reference and resistant *Staphylococcus aureus*, *Pseudomonas aeruginosa*, and *Candida albicans*. Cu refers to the copper metal sheet control; Cu3DABS refers to the copper coated 3D printed ABS; Cu-Cu refers to copper coated copper samples; and Ag5Cu-Cu refers to a copper and 5 wt% silver additive powder blended coating on a copper substrate.

attributed to plating stress and other system noise effects, along with genuine environmental conditions that limit microbial survival. When investigating antimicrobial efficacy of surfaces, all factors other than the innate material effects should be isolated.

As a way of accounting for the effects of noise while demonstrating the microbial reducing activity attributed to the antimicrobial activity of the test samples, the relative microbial reduction rate (m_{ar}) was used. The rate of microbial reduction from coated samples defined antimicrobial activity relative to the system noise effects accounted for by the pseudo-efficacy of stainless steel. Rapid microbial elimination, represented by a steep slope from the regression lines in Figure 1, suggests decreasing risk of subsequent pathogenic transmission from such surfaces. For example, a copper coating on a 3D printed ABS substrate against reference pathogens, exhibited rapid microbial elimination within 25 minutes of exposure to *S. aureus* (Fig. 1A) and within 15 minutes of exposure to *P. aeruginosa* and *C. albicans* (Fig. 1B and 1C, respectively).

Figure 2 illustrates the antimicrobial efficacy, defined by the relative microbial reduction rate (m_{ar}), of the cold spray coatings and the copper metal control. Again, the limited efficacy of the copper metal control (Cu) contrasts with the high rates of microbial elimination from the coatings. The efficacy of the cold spray coatings remained fairly consistent against both reference and resistant strains. Contrasting activities were observed for copper-coated-copper against *S. aureus* and a 5 wt% silver–95 wt% copper-coated-copper against *C. albicans*. Copper-coated-copper against the multidrug-resistant *S. aureus* showed a marked reduction in antimicrobial activity, and the addition of a silver additive was three times more efficient against the clinically resistant *C. albicans*.

The evidence indicates that the resistance to antibacterial and antifungal drugs does not significantly affect the performance of cold spray coatings. On average, copper cold spray coatings on 3D printed ABS achieved complete microbial elimination within 15 minutes against both reference and resistant microbial strains. Similarly, copper-coated-copper materials exhibited complete microbial elimination within 10 minutes.

The ideal microbial elimination time proposed for high contact surfaces was 5 minutes and was attained by 5 wt% silver–95 wt% copper cold spray coated copper test material (Fig. 1). The corresponding microbial reduction rate of 1.2 $\log_{10}$ (CFU/mL) per minute (Fig. 2) represents a ~93.7% reduction in viable CFU for every minute the micro-organisms spends in contact with this material. Such activity in a dry contact environment demonstrates the ability of such a coating to effectively prevent surface contamination.

Discussion

Under dry touch-contact conditions, the cold spray coatings performed as effective self-sanitizing surface coatings against both reference and resistant pathogenic strains. The activity of these cold spray coatings was compared to a study investigating the antimicrobial activity of plasma sprayed, wire arc sprayed, and cold sprayed copper coatings on aluminum.[15] In the study, after a two hour exposure to *S. aureus*, the remaining viable bacterial presence was >10%, >5% and <0.001% for each respective coating type. This experiment clearly demonstrates the advanced antimicrobial efficacy of cold spray coatings. The additional sampling periods used in this current study demonstrated effective antimicrobial efficacy over a more realistic time course. By demonstrating complete microbial elimination within 15 minutes for the polymer metallized coatings and within 10 minutes for cold spray coatings on copper, this experiment has proven the capability of these coatings to provide a realistic and positive impact on high contact surfaces. Further studies involving characterized coating durability and coating wear resistance are needed.[20]

Cold spray, as a low temperature, solid-state deposition technique, uniquely allows coating of thermally sensitive and chemically dissimilar materials, Furthermore, direct fabrication is possible, making this an attractive additive manufacturing approach.[21] Coupling this technology with polymer 3D printing led to the development of novel and functionally versatile antimicrobial surface coatings.

An increase in the diffusion of ions is believed to be a key factor affording the heightened effective antimicrobial activity demonstrated by the cold-spray coatings. It is widely accepted that the generation and diffusion of metal ions plays a fundamental role in the antimicrobial mechanism of action of such metals.[1,7,22] The increased rate of microbial elimination when comparing coatings on copper substrates to those on polymer substrates may be a consequence of improved ion diffusivity. In a study investigating the cold spray of copper onto aluminum substrates,[15] the cold spray process for such metal-on-metal depositions resulted in impact hardening of the coatings and enhanced diffusion of cupric copper ions, which is needed for microbial destruction. However,

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while this trend of greater efficacy from coated metal substrates held for tests against *S. aureus* and *P. aeruginosa* strains, it did not hold for the *C. albicans* strains. The mechanism of action may, in this case, differ from that suggested previously. In a clinical environment, this finding translates to a wider application potential for suitable coating sites. A considerable reduction in the rate of antimicrobial elimination was observed for a copper coating when tested against the multidrug-resistant *S. aureus* strain. The consequence was an observed contact killing elimination time of 10 minutes, which is double that achieved against the reference strain. The clinical relevance of this finding lies in the potential to mitigate infection transmission and possibly the associated rate at which surface cleaning should occur for high risk sites.

The addition of a silver additive (as a 5% weight constituent of the final powder blend) was investigated in the current study as a means to further enhance the antimicrobial efficacy of these coatings. Against the test pathogens, this was not the case. The inclusion of a 5 wt% silver powder constituent did not, to any appreciable degree, improve the antimicrobial efficacy of the cold spray coatings. A previous study commented on an observed collaborative interaction between copper and silver in terms of their independent antimicrobial mechanisms of action, whereby copper initiates cell wall breaching, granting access to silver ions, which proceed to impair cellular membrane function and interfere with DNA replication.[22,23] Explanations for the lack of enhanced activity from the silver containing coatings may relate to the relative quantity of silver included in the powder blend or to the operating conditions of the coatings in these tests. By design, these tests were performed under simulated dry touch-contact conditions. It has been suggested that silver has a tendency to perform better in a wet, diffusive environment.[13] With disc diffusion assays, silver was observed to be an effective additive, particularly against Gram-positive pathogens.[23]

A caveat, based on these findings and in agreement with a previous study that investigated the synergistic antimicrobial effects of various silver–transition-metal combinations,[9] relates to necessary precautions taken when considering metallic combinations for antimicrobial applications. Metal combinations may, under different environmental operating conditions, perform with additive effects, synergistic effects or, in certain circumstances, antagonistic effects. An example of this can be seen in the antimicrobial response of a copper-on-copper coating with a silver additive against the fluconazole-resistant *C. albicans* and the respective reference ATCC strain. Careful consideration of operating environments and intended antimicrobial behavior should therefore be made. If no additional activity is achieved through the use of an additional antimicrobial constituent, as was the case in this study against reference pathogens, that addition (silver for the standard coatings) becomes economically inadvisable.

Returning to the directive of a realistic time course for the effective antimicrobial activity of contact killing surface coatings, it is clear that the developed coatings (both polymer metallized depositions and metal-on-metal coatings) have proven to be effective contenders, outperforming the activity reported in previous studies. Additionally, the achievement of antimicrobially active, contact killing polymer metallized coatings via multi-step and multi material additive manufacturing has validated this novel technology.

Nosocomial infections have been a persistent and prevailing problem within hospitals, exacerbated by resistant microbial strains. Touch-contact surfaces provide an intermediary for infection transmission and were therefore targeted for solution development. Hand hygiene and surface cleaning practices, while effective in theory, have proven ineffective in successfully mitigating the prevalence of hospital acquired pathogens. To address this problem, we propose a supplementary means of infection control and prevention, specifically with regard to surface contamination as a means of reducing the CFU survival levels on contaminated surfaces.

Validation of effective antimicrobial surface coatings developed through the integrated additive manufacturing techniques of cold spray and polymer 3D printing was achieved. We have demonstrated enhanced antimicrobial efficacy, characterized by rapid reduction and effective elimination of a range of reference and resistant microorganisms, in a dry touch-contact environment. Both polymer metallized depositions and metal-on-metal surface coatings were confirmed as contact killing surfaces. Contact elimination from cold spray coatings was demonstrated by all coatings against both reference and resistant microbial strains within a contact window of 5 to 25 minutes. Furthermore, a copper coating with a 5 wt% silver additive has confirmed compliance within a five minute exposure window, achieving complete microbial elimination within this time.

In the pursuit of commercialization, a longitudinal clinical trial should be devised to evaluate the durability and sustained efficacy of these coatings under real-world test conditions. According to Professor Christopher Berndt, the director of the Australian Research Council's Surface Engineering for Advanced Materials (SEAM) Center, speaking at the inaugural International Thermal Spray Conference and Exposition (ITSC) virtual preview conference: Coatings for Antivirus, Bacteria, and Fungus Applications (June 2020), a key advantage of thermal spray technologies for antimicrobial coatings relates specifically to the longevity of such coatings compared to other painted or thin film coating technologies. Cold spray, a subset of thermal spray technologies, should therefore express these desirable properties for both the metal-on-metal and polymer-metallized surface coatings.

Considering the current novel coronavirus pandemic and the associated possible increase of bacterial resistance due to the overuse of sanitizers, coupled with the lack of biocidal protection afforded by common hospital surfaces; this surface coating technology may offer a supplementary solution to current infection control and prevention strategies. Further clinical studies are thus recommended to confirm efficacy.

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Conflicts of interest. All authors report no conflicts of interest relevant to this article.

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