

# Effectiveness of Nanomaterial Copper Cold Spray Surfaces on Inactivation of Influenza A Virus

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## Abstract

Bacterial and viral contamination of touch surfaces allows for transmission of pathogens leading to increased risk of infection. Previous work has demonstrated the antimicrobial properties of copper for contact-killing of microbes for use in hospitals. Less research exists on copper as an antiviral surface and on the effects of nanomaterial copper surfaces in the contact-killing of viruses. Nano agglomerate and conventional copper powder feedstock is used in the cold spray process to form copper coatings on aluminum substrates. The nano and conventional copper surfaces formed are tested for antiviral contact-killing of influenza A virus. After a two hour exposure to the surfaces, the surviving influenza A virus was assayed and the results compared. The differences in the powder feedstock used to produce the test surfaces were examined in order to explain the mechanism that caused the observed differences in influenza A virus killing efficiency. Results showed that the nano copper surface was antiviral, but less effective than a study on antimicrobial killing of MRSA on copper surfaces. The nano copper surface was more effective at percent reduction of influenza A virus than that of conventional copper. It was determined that the work hardening caused by the cold spray process in combination with the high number of grain boundaries results in a copper microstructure that enhances ionic diffusion. Copper ion diffusion is the principle mechanism for microbial and viral destruction on copper surfaces. Testing determined significant microbiologic differences between nano- and conventional Cu surfaces and demonstrates the importance of nano copper surfaces as an antiviral agent. The nano agglomerate powder shows superior antiviral effectiveness to that of conventional Cu due to an increase in grain boundaries at the nano level. Further research is needed to determine the effects of nano and conventional copper surface roughness on the contact-killing rate of viruses versus microbes on both a micro and nano-scale.

**Keywords:** Cold spray; Influenza A virus; Nanomaterial; Copper; Antiviral

## Background

The epidemiology of nosocomial pathogens is a cause of concern in hospitals due to the possibility of increased risk of infection [1,2]. Influenza A, a viral pathogen, is one of the leading causes of infection and mortality of young children and the elderly (>65) [3]. During the 2012 and 2013 influenza seasons, the percentage of deaths attributed to pneumonia and influenza in the United States peaked at 9.9%, exceeding the epidemic threshold for 13 consecutive weeks [4].

It is indicated by the Center for Disease Control and Prevention (CDC), that the three main mechanisms of influenza virus transmission are the spread of infectious droplets through: airborne, person to person, or surface contact [5]. Research shows, influenza that is distributed by airborne nuclei can contaminate objects in the environment, leading to easy transfer at the hand-surface contact interface [6-8]. The spread of bacteria and viruses via hospital surfaces, including medical equipment, work stations, and patient rooms is well documented [2,6,9,10]. The addition of self-disinfecting surfaces in conjunction with required cleaning practices further prevents the spread of infectious diseases [11].

Research exists supporting the role of copper surfaces as an antimicrobial material [12-15]. A correlation is shown between the increase in copper content and the increased contact-killing of *E. coli*, *Listeria monocytogenes*, and Methicillin-Resistant *Staphylococcus aureus* [12]. Two hypothesized mechanisms for contact killing of microbes is the interaction of copper ions with the cellular membrane causing an influx of ions in the intracellular matrix, and copper induced DNA fragmentation, both resulting in cell death [16]. Testing of *S. aureus* and *E. coli* on nano Cu-incorporated diamond-like carbon films shows post-incubation TEM images of cytoplasm leakage from cell

membranes and data from Transmission electron microscopy- Energy dispersive X-ray spectroscopy (TEM-EDS) indicating copper ion presence in the membranes of both microbes. This shows the primary contact-killing mechanism to be membrane deformation by copper ions [17].

Research also exists supporting the role of antimicrobial copper surfaces in the contact-killing of influenza A virus [18]. It is shown that Cu(II) ions added in solution to influenza A virus result in morphological abnormalities [19]. Similar studies with copper ion solutions show the increased sensitivity of enveloped viruses to Cu(II) ions as compared to non-enveloped viruses, suggesting that the virus's lipid membrane becomes overwhelmed by intracellular copper, similar to the microbial kill mechanism [20].

The Copper Development Association (CDA) currently lists more than 450 wrought and cast copper alloys under the unified numbering system (UNS) that are registered with the U.S. Environmental Protection Agency (EPA) as antimicrobial [21]. All EPA approved surfaces contain a copper content of at least 60%. EPA-approved laboratory studies show that copper kills 99.9% of disease-causing bacteria including, *Staphylococcus aureus*, *Enterobacter aerogenes*, *Escherichia coli*

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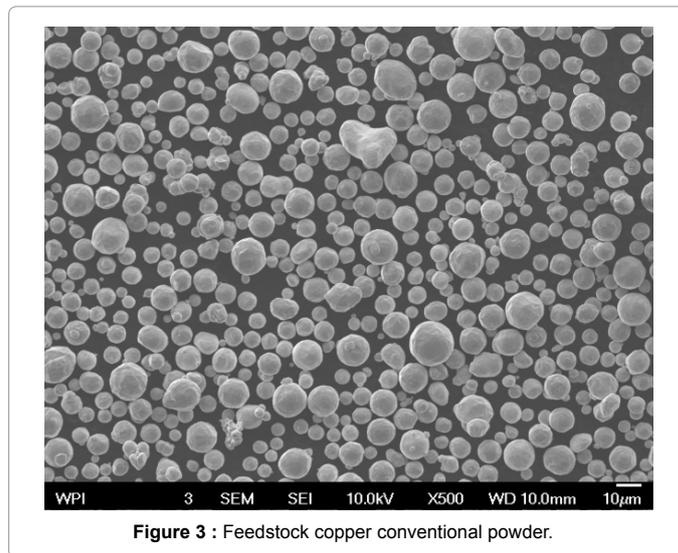
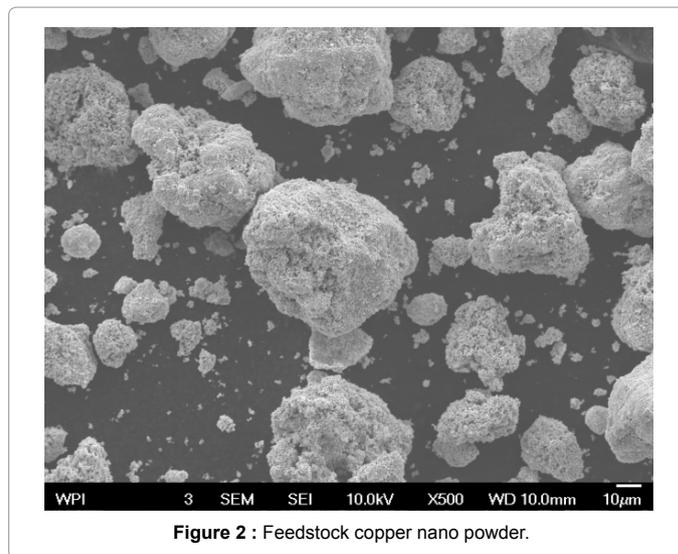
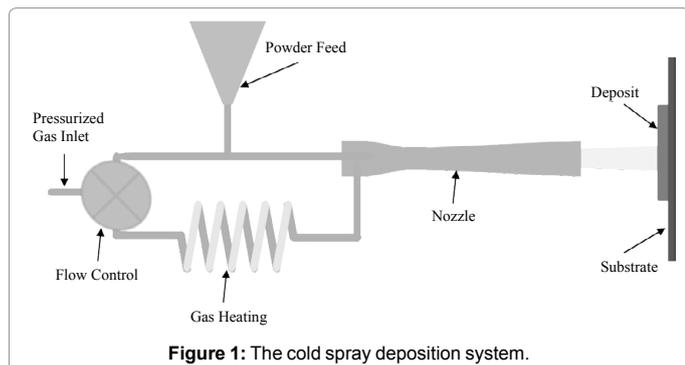
O157:H7, *Pseudomonas aeruginosa*, Vancomycin-resistant *Enterococcus faecalis* (VRE) and MRSA, within 2 hours of contact. All EPA registered materials can be used for frequently touched surfaces, such as hospitals, for control and inactivation of microbes [22]. While there are specific guidelines for testing the efficacy of antimicrobial surfaces against microbes, there are no EPA approved protocols for testing their efficacy against viruses.

Increasing the kill rate of influenza A virus is important, as the survival of infectious influenza on fomites can last up to 72 hours [6]. Research shows that processing of copper material does affect the ability of copper to inactivate microbes [23]. Copper surface coatings can be created using various metal spray techniques including plasma, arc, and cold spray processes. During the cold spray process, a heated carrier gas brings copper powder to supersonic velocities, causing them to plastically deform and adhere to a metal substrate, forming a thin solid copper coating. A schematic of the cold spray process is shown in Figure 1. Powder particles are fed into a converging-diverging nozzle filled with a stream of heated helium or nitrogen gas and then blasted at the substrate, forming a mechanical bond with the surface upon impact. In general, the cold spray process operates at a temperature between 100-500°C and at a velocity range of 600-1000 m/s, resulting in <1% porosity and <1% of oxides.

Champagne et al. [23] indicates that of the plasma, arc, and cold spray processes, the later has the higher velocity impact of copper particles, resulting in increased material hardness and higher kill rate of *Staphylococcus aureus*. The aim of this research is to increase copper material hardness through the use of nano copper cold spray particles, which will create a harder copper coating that will show a higher kill rate towards Influenza A Virus.

## Test Procedure

The cold spray surface coating technique was used to produce copper coatings approximately 0.05 mm thick on aluminum substrates. The nano and conventional copper powder feedstock used for cold sprays is shown in Figures 2 and 3. The particle size distribution for the nano copper (Eltron) and conventional copper (Praxair Cu-159) is an average of 25 microns and a range of -31/+5 microns, respectively [24,25]. To confirm powder particle size as reported by the powder producer, a particle size distribution analysis was performed on the Horiba LA-910 Laser Scattering Particle Size Distribution Analyzer, in a 10 g/L solution of Sodium Hexametaphosphate in DI Water. The nano and conventional powder are both pure copper. The conventional powder is produced by gas atomization. For the nano powder, nanosized particles were dispersed in a solvent with a pure copper binder precursor and spray dried under controlled atmosphere to form perfectly spheroidized micron sized particles (~ 20 um in diameter) by Eltron Research and



Development Inc. [24,26] Copper nanoparticles offer many benefits over convention copper powders in terms of strength, hardness, wear and reactivity but cannot be used in the cold spray process due to insufficient mass. During the cold spray process, particles less than 5 microns in diameter can have insufficient momentum to penetrate the “bow shock” region next to the substrate [27]. Eltron utilizes spray drying to achieve strong, nanoparticle agglomerates with tight control of particle shape, size, distribution, and porosity with an ultra-fine grained (UFG) inorganic metallic binder that can match the nanoparticles in composition and that have sufficient mass and momentum to be used as a feedstock for the cold spray process [26]. Cross sections of the nano and conventional copper coupons produced are shown in Figures 4 and 5. Both deposits formed dense coatings with low porosity. Unlike the conventional Cu coating, the nano-structured deposit has areas of nano-Cu agglomerates surrounded by conventional Cu, which serves as a binder, as indicated by the darker colored regions of Figure 4. The clear difference in microstructure suggests a change in biological activity from conventional to nano copper surfaces.

The copper coated coupons were inoculated with influenza A virus, held at room temperature for two hours, and then survivors were

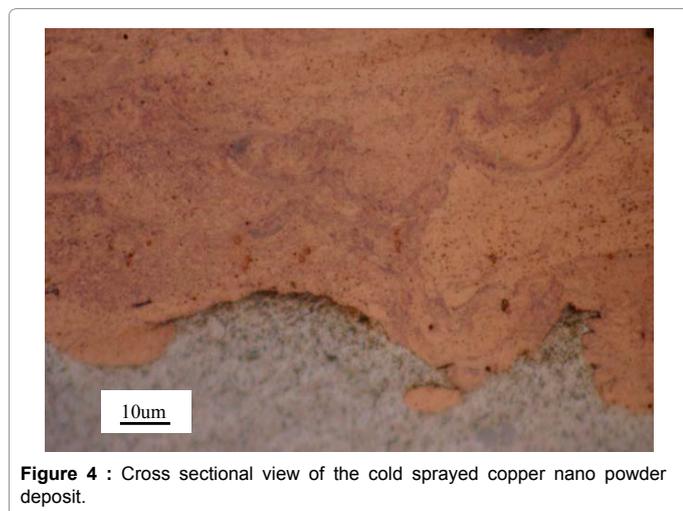


Figure 4 : Cross sectional view of the cold sprayed copper nano powder deposit.

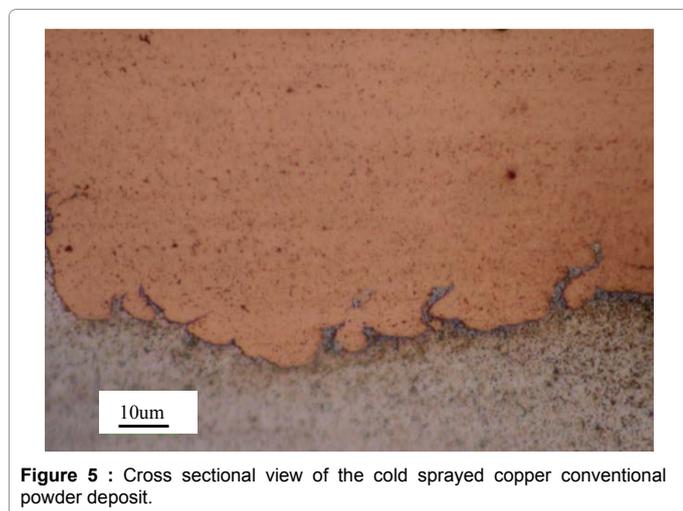


Figure 5 : Cross sectional view of the cold sprayed copper conventional powder deposit.

resuspended and cultured. There are no EPA approved test procedures for copper alloy surfaces as a sanitizer of viruses.

The EPA antimicrobial procedure, “Test Method for Efficacy of Copper Alloy Surfaces as a Sanitizer”, was used with some changes [27]. The full details of the modified procedure are given below.

### Carrier surfaces and preparation

**Carrier surfaces and preparation:** The surfaces of the nano and conventional copper coupons were the test carriers. The surfaces of the stainless steel squares were the control carriers. The carriers were sterilized with ethanol and allowed to dry prior to use in the test. Post sterilization, carriers were placed into Petri dishes.

**Inoculation of carriers:** A 100  $\mu$ L aliquot of influenza A virus, ATCC VR-544, strain Hong Kong was inoculated into the test and control carriers. The inoculum was spread within 1/8 inch of the carrier edge. The virus was then air-dried at room temperature (20°C) in a relative humidity of 23.5% until visibly dry (30 minutes). This temperature and humidity was selected to maximize virus survival while drying. Exposure time began at the 30 minute drying mark.

**Neutralization of subculture:** Following the two hour exposure time, a 1.00  $\mu$ L aliquot of neutralizing test medium was inoculated onto the carriers and the carriers were scraped with a sterile cell scraper. The

test medium is comprised of minimum essential medium (MEM) and 100  $\mu$ g/mL penicillin, 10  $\mu$ g/mL gentamicin, 2.5  $\mu$ g/mL amphotericin B, 2  $\mu$ g/mL TPCK-Trypsin, 0.2% bovine serum albumin (BSA), and 25 mM HEPES. Post scraping, 10-fold serial dilutions were performed in the test medium. An 100 $\mu$ L aliquot from each dilution was inoculated into the indicator cell cultures in quadruplicate. Indicator cell cultures were MDCK (canine kidney) cells.

**Incubation and observation:** The cell cultures were incubated at 36-38°C in 5-7% CO<sub>2</sub> for seven days. Following incubation, for each test the average TCID<sub>50</sub> was calculated. The average log and percent reduction in viral titer of each test substance were calculated using the corresponding average TCID<sub>50</sub> of the control carriers (stainless steel).

### Results and Discussion

The ability to increase the contact-killing rate of influenza A virus on copper coatings is suggested to relate to the increase in Cu ion diffusion through the virus’s lipid membrane [20]. Champagne et al. shows that diffusion of copper ions from conventional copper surfaces is increased by copper surface hardness, resulting in increased microbial destruction [23]. Surface hardness of copper coatings can be increased further by using nano copper powder as opposed to the conventional powder typically used, as detailed in the below research results.

The percent reduction of influenza A virus after two hours on the surface of nano and conventional copper cold spray surfaces is shown in Figure 6. Also included in Figure 6 is data provided by the Army Research Laboratories on testing of copper cold spray surfaces for percent reduction of MRSA [28]. The results show that nano copper and conventional copper have a 99.3% and 97.7% reduction in influenza A virus, respectively. The use of copper as an antimicrobial material shows >99.9% reduction of MRSA after 2 hours. Copper is antiviral, but less effective at contact-killing of viruses than microbes.

The contact-killing of viruses by nano copper surfaces is more efficient than that of conventional copper. This difference in antiviral effectiveness between nano and conventional copper requires an examination of how the powder feedstock for the cold spray deposition process affects the nature of a copper coating.

The cold spray process operates at temperatures between 100-500°C and velocities between 600-1000 m/s. Due to the unique cold spray process conditions, two specific properties can be attributed to the copper cold spray coating. The first, high velocity impact of the powder particles creates a low porosity coating. Generally, cold spray coatings result in <1% porosity and <1% of oxides. Second, by blasting the powder particles at a high velocity and relatively low temperature, work hardening is induced. Work hardening results in high material

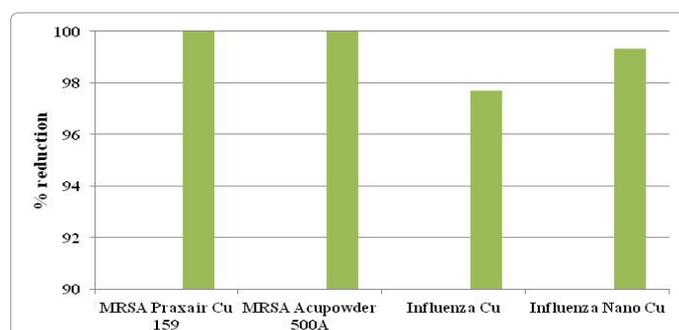


Figure 6: Percent reduction of MRSA (data taken from experiments [28]. and influenza A virus after 2 hour exposure to various copper deposits.

dislocation density cause by the plastic deformation of particles' as they hit the substrate and neighboring particles. Champagne et al. has shown that work hardening by cold spray increases dislocation density within copper [29]. By increasing the number of particles that collide with each other, the work hardening increases. This in turn will increase dislocation density and ultimately material hardness.

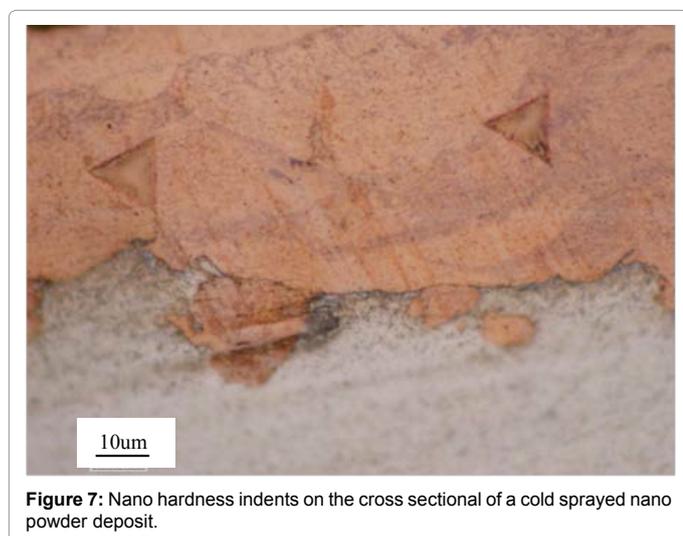
Particle size distribution analysis shows that the average particle size for nano and conventional copper powder was 37.8 microns and 10.4 microns, respectively. While the cold spray nano powder feedstock is larger, it contains many nano sized particles on the scale of 60-80 nm (Sky Spring Nanomaterials) [26]. Post agglomeration, the micron sized nano powder is heat treated using a proprietary process developed by Eltron to form micron sized particles with a nano grained structure [26]. The increase in nano grained boundaries in the nano powder cold spray feedstock allows for increased dislocation density post-processing. The relationship between dislocation density and diffusivity is  $D_p \propto \rho$  [30]. The diffusion of copper ions is increased with the hardness increase produced by nano grain boundaries, which enhances copper ion flow for viral destruction.

As illustrated in Figure 7, nano hardness testing was completed, with the resulting values for the nano copper and conventional copper surfaces as 2.44 and 1.45, respectively. The relationship between hardness and dislocation density is  $H_2 \propto \rho$  [31]. It follows that the nano copper surface has higher nano hardness, meaning a higher dislocation density from work hardening. Due to the reduced size of nano copper powder in comparison to the conventional powder, there are a greater number of particles present in the nano copper powder when sprayed. This means that there are a greater number of grain boundaries too. The more grain boundaries present upon impact with the substrate, the greater the work hardening resulting in higher dislocation density.

Dislocations at grain boundaries of metals increase ion diffusion, also known as pipe diffusion [32]. The increase in dislocation on the nano copper coating led to higher ion diffusivity reflected in a greater reduction in influenza A virus than conventional copper surfaces.

## Conclusion

The effectiveness of copper as antimicrobial for contact-killing on touch surfaces has been well documented [12-15]. Less research exists on the effectiveness of copper as an antiviral agent and on the effects



of nano grained copper surfaces on contact-killing rate of viruses. The significant differences between nano and conventional copper surfaces produced by cold spray technique, as shown here, demonstrate the importance of the copper powder processing and of the resulting deposition structure. The nano copper surface showed antiviral effectiveness caused by high dislocation density imparted to the sprayed particles by a combination of cold spray velocity and pre-processing heat treatment of nano particle agglomerates. The high dislocation density, measured by an increase in material hardness, allowed for higher copper ion diffusivity.

Cold spray technology is currently in use to produce metal copper coatings for hospital applications. Touch surfaces such as the copper coated hospital tray in Figure 8; show the current use of copper as an antimicrobial surface. Data collected on virus contact-killing properties of copper supports the potential future use of copper in hospitals and other industries for antiviral applications.

Both the nano and conventional copper for influenza A virus reduction have lower % reduction values than that of the copper surfaces used for contact-killing of MRSA. Surface roughness is documented to effect microbe retention [33]. Roughness of the copper surface could be a contributing factor to the higher contact-killing levels of influenza A virus by nano copper. Microbes, like MRSA, range in size from 0.5-1.0  $\mu\text{m}$  in diameter, whereas viruses like influenza A, are on a 80-120 nm scale [34,35]. The size difference may affect the ability of microbes and viruses to form reservoirs of survivors in microscopic pockets and crevices on copper surfaces. Further analysis of surface metrology on a micron and nano scale is needed to determine the effect of conventional and nano copper surface structures on the contact-killing of influenza A virus.

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