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The Effect of Nano-Scale Surface Roughness on Copper Cold Spray Inactivation of Influenza A Virus

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Abstract

Copper has been identified as an antimicrobial material, with over 450 copper alloys approved by the EPA [8]. Previous work with conventional and nanomaterial copper cold spray surfaces has demonstrated efficacy in percent reduction of both MRSA and Influenza A Virus, with nanomaterial copper having better results for virus efficacy than conventional copper [1]. The main mechanism of copper contact killing is believed to be Cu ion release[12]. The surface roughness of the conventional and nanomaterial copper cold spray surfaces was examined in order to further explain the mechanisms that caused the observed difference in Influenza A virus efficacy testing. Results showed that both the percentage of grain boundaries and surface roughness may be contributing factors in copper's kill-mechanism at the nano-scale. The surface roughness below 0.1um2 and above 1000um2 was much greater for nanomaterial copper surfaces than conventional copper, however the opposite was true between these values. It was determined that there appeared to be a localized relationship at the nano-scale between increased surface roughness and percent Cu ion diffusion, similar to the relationship of ion diffusion to the percentage of grain boundaries[1]. However, further research is needed to uncouple the individual contribution of surface roughness and grain boundaries to copper kill rate.

Keywords: Surface Roughness, Relative Area, Cold Spray, Nanomaterial Copper

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Background

The Center for Disease Control (CDC) states that the number of deaths per year due to Influenza and Pneumonia related illnesses has reached up to 55,227 in select years [7]. Additionally, MRSA causes approximately 14,000 deaths and 250,000 hospitalizations per year [5]. Self- cleaning surfaces, such as copper, are being used in hospitals to prevent the spread of infectious diseases across medical equipment and work surfaces [20, 14]. It is believed that the contact killing mechanism for microbes is copper ion diffusion, indicating that with increases in Cu concentration there is an increased kill rate [12]. Cold spray is an additive manufacturing method used to coat surfaces with Cu and other metals. Cold spray propels metal powder particles at a high velocity (600-1000 m/s) and relatively low temperature (100-500°C) at a substrate to form low porosity and low oxide coatings. The high hardness and density of the coating from the cold spray process results in an increased kill rate as compared to other additive methods using lower-velocity processes [9].

This is a follow-on study to the paper "Effectiveness of Nanomaterial Copper Cold Spray Surfaces on Inactivation of Influenza A Virus" [19]. This paper explores the relationship between work hardening, grain boundaries and ion diffusion. Where, the velocity of the cold spray process coupled with the unique grain structure of the nanomaterial Cu allowes for increased kill rate of Influenza A Virus. Cold sprayed nanomaterial Cu coating was compared to cold sprayed conventional Cu coating, which contained larger particles with fewer grain boundaries. The results from testing are shown in Figure 1. [19]

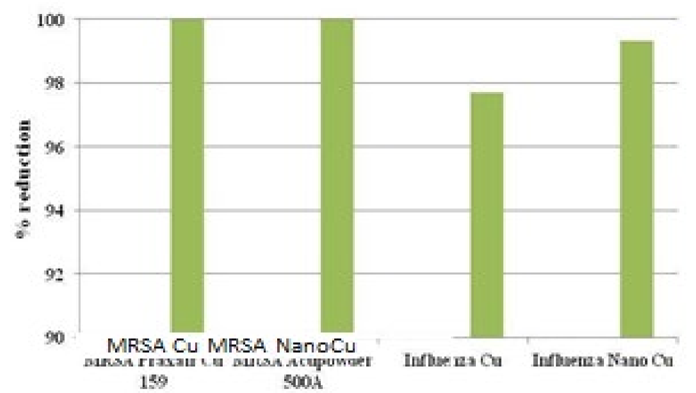

Figure 1: Percent reduction of MRSA and influenza A virus after 2 hour exposure to various copper deposits.

In the above graph, it is shown that copper surfaces have a higher kill rate for MRSA than for Influenza A. It is hypothesized that surface roughness at the MRSA (0.5- 1.0 μm) and Influenza A (80-120 nm) scales are a leading factor in percent reduction [17, 6]. A mismatch in size between the surface roughness and the scale of the microbe leaves large surface pockets available which increases the likelihood of microbe congregation to form reservoirs of thriving bio organisms [21]. Additionally, the mismatch in roughness can contribute to the ability of microbes to adhere to a surface [13]. Roughness may cause destabilization of cell membranes and viral capsids through both increased toxic Cu ion transfer and puncture of the cell wall [11].

Topography also results in greater contact area and may significantly influence the transfer or dissolution of ions from the material [22]. The roughness of the cold sprayed nanomaterial copper on the nanoscale could be providing a greater advantage for microbe reduction than that of conventional copper. Similarly, the relationship between surface roughness and conventional copper on the micron scale could provide a greater advantage than that of nanomaterial copper at that level. In order to measure at both the micro- and nanoscales, this paper uses 3D confocal microscopy and atomic force microscopy, respectively.

Current research on contact killing of bacteria on copper shows that the suppression of bacteria-metal contact prevents killing [13]. Less work has been done quantitatively for topology, with most surface assessments relying on conventional roughness parameters such as Ra, which lack spatial specificity and fine scale sensitivity [4]. Microbes of different sizes interact with a surface at different length-scales, thus it is important to characterize the surface topography with a scale sensitive technique. This paper focuses on multiscale analysis, using the fractal tiling method developed by Brown et al which simulates the effect of different observational scales on surface area [2]. Unlike many multiscale methods, this technique has a clear physical interpretation, and surface area has a direct effect on a wide range of surface phenomena[4].

Through multiscale analysis, area scale, complexity, and volume scale plots can be formed. Area scale is a fractal tiling method where the surface is tessellated with triangles of the same size in order to determine the observable area at the scale of those tiles. Large-scale features affect relative area values at finer scales. Large-scale features are still contributing to the total area regardless of the observable scale. Complexity removes the influence of large-scale features at the scale of observation, whereas relative area is dependent on all the features that are larger than it. Complexity is the first derivative of the area scale plot, which shows the roughness at a particular scale. Volume scale analysis looks at how volume varies at different scales of observation or the fraction of space that can be filled.[2]

The aim of this research is to provide further analysis of surface metrology on the micro- and nanoscales to better understand the reason why consolidated nanomaterial Cu performs better than conventional Cu in percent reduction of Influenza A Virus and to relate findings to why the coating did not perform as well as in percent reduction of MRSA. Characterizing the factors affecting Cu antimicrobial efficiency will help in better understanding the kill mechanism and in future optimization of the Cu coatings for healthcare applications.

Test Procedure

Cold Spray

Cold spray samples from the prior paper, "Effectiveness of Nanomaterial Copper Cold Spray Surfaces on Inactivation of Influenza A Virus" [19], were used. The pure Cu cold spray coatings on aluminum substrates are approximately 0.05 mm thick. The nano-agglomerate copper (Eltron) and conventional copper (Praxair Cu-159) powders are produced through spray drying and gas atomization, respectively. Due to the low mass of the nanoparticles, they are bound into agglomerates using conventional Cu as the binder and spray dried [16, 18]. For more information on the materials and cold spray process parameters see reference [19].

Visual and Microstructural Analysis

The interior of the coatings was studied through microscopy to illustrate the difference in grain size and number of grain boundaries between the nano and conventional copper coatings. The conventional Cu coating was cross sectioned in a Helios NanoLab G3 UC DualBeam scanning electron microscope/focused ion beam (SEM/FIB) and imaged with the ion beam. A thin film of the nano Cu coating was prepared in the same SEM/FIB and imaged in an FEI Talos F200X scanning/transmission electron microscope (STEM).

Measurement Analysis

The grain sizes of the conventional and nanomaterial Cu cold spray deposits were measured in an Olympus Stream Image Analysis Software. More than 50 grains were measured per category. Grain outliers were removed with Dixon's Q-test, and the range and average are reported for each category.

Surface Roughness Measurement

Topographic measurements at micrometer length scales were taken with an Olympus Lext OLS4100 scanning laser confocal microscope. Imaging was performed with the 50x objective providing magnifications at 1080x.

To investigate the topography at nanometer length scales, measurements were taken with a Nanosurf Naio AFM operating in contact mode. The cantilevers used were MikroMasch HQ:CSC17 cantilevers and the image resolution was 1024x1024.

Measurement Analysis

The confocal measurements were filtered to remove spurious points using the outlier filter in software MountainsMap by Digital Surf. The points removed comprised <0.01% of the total surface. The AFM images were not filtered. Area-scale fractal analysis was performed with Sfrax by Surfract with the Four Corners Full Overlap tiling algorithm. Sfrax was used to analyze the surface data for volume scale where the volume-ratio was used with the ratio being one [3].

Results and Discussion

Microstructure

The cross-sectional FIB image of the conventional Cu coating cold sprayed with gas-atomized particles is shown in Figure 2. The lens-shaped deformed particles are evident, as well as highly plastically deformed particle boundaries. A large particle with several larger grains is found in this cross section. A higher magnification image of the largest grains and a particle-particle interface is shown in Figure 3. This region at the particle-particle interface, where the most plastic deformation occurred, contains bands that formed during the cold spray process with smaller grains than in the interior of the particles [15].

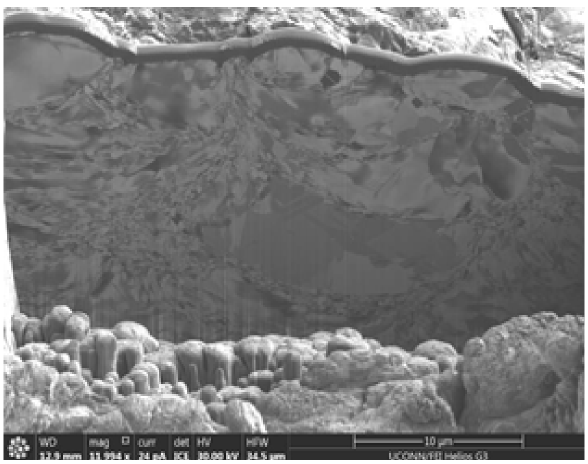

Figure 2: Cross-sectional focused ion beam image of the conventional copper coating

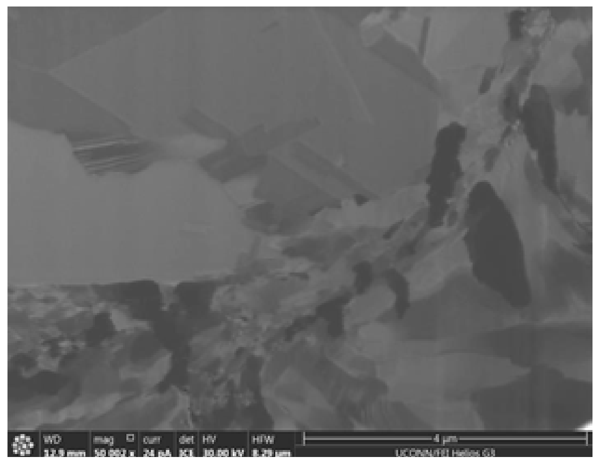

Figure 3: Higher magnification cross-sectional focused ion beam image of the conventional copper coating

A STEM image of the nanomaterial Cu coating cold sprayed with spray dried nanoparticle agglomerates is shown in Figure 4. The different contrasting stripes are an artifact from sample preparation. In order to understand the different regions of this sample, it is important to know the material inputs. During fabrication, micron-sized particles are formed by spray-drying nano particles that were aglomerated with a pure copper binder [18]. As a result, this sample includes regions of conventional copper with micron-sized grains, as well as areas of nanostructured copper. The lighter regions that are swirled with the micron-sized grains contain the nanostructure. A higher magnification image of the nanostructure copper is shown in Figure 5.

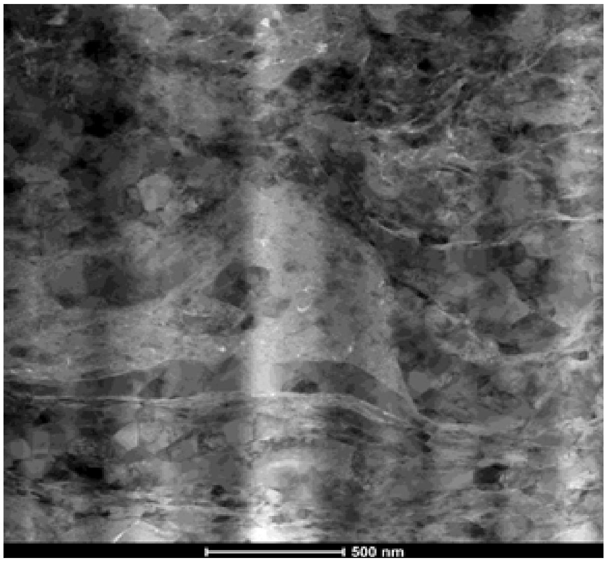

Figure 4: STEM image of nano copper coating

The nano-sized grains of the nanomaterial Cu are shown clearly in Figure 5. The particle in the middle that is surrounded by a hole in the sample that formed during sample preparation is hypothesized to be one of the agglomerated particles that contains nanoparticles. It is known that an increase in grain boundaries and dislocations increases ion diffusion, which is believed to be the main mechanism for contact killing of microbes [12]. The nano- grains in the nanomaterial Cu allow for increased kill rate of the Influenza A virus.

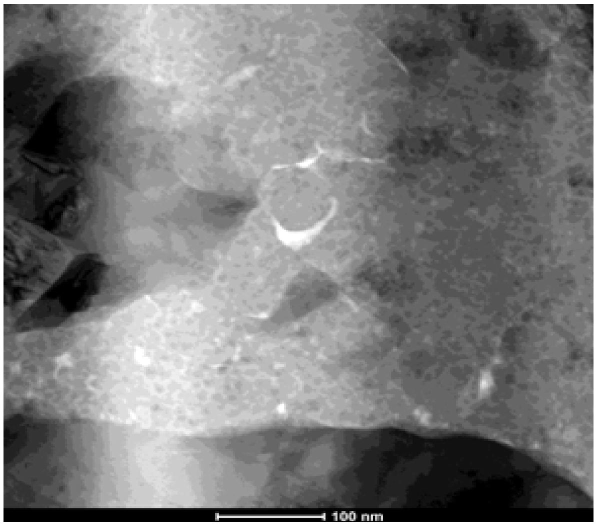

Figure 5: STEM image of the nano copper coating showing the nanostructured particles

Grain size measurements were made of the nanomaterial and conventional Cu and a summary of the results is reported in Table 1. For the cold sprayed conventional Cu, the average size of the majority of the grains was 0.2 +/-0.2 microns, while the largest of the grains in the sample were 2.2 +/-1.5 microns with the largest being almost 5 microns in diameter. The cold sprayed nanomaterial Cu contained nano-sized grains as well as micron-sized grains, measuring an average 10.6 +/-2 nm and 0.4 +/-0.1 microns, respectively. The size of the nano-grains is the clear difference between the samples and must be one of the factors affecting the higher virus kill rate in the nanomaterial Cu.

	Conventional Coating		Nanostructured Coating	
	Conv grains (µm)	Large grains (µm)	Nano grains (nm)	Conv grains (µm)
Min	0.1	1.0	7.3	0.2
Max	1.0	4.9	15.1	0.7
Ave.	0.2	2.2	10.6	0.4
σ	0.2	1.5	2.0	0.1

Table 1: Grain size measurements

Surface Roughness

The results of the area-scale analyses are displayed in Figures 6, 7, and 8. Area scale analysis looks at how area varies at different scales of observation [2]. In this case, the below figures detail changes in surface roughness on the micro- to nanoscales representative of the size scales for MRSA (0.5-1um) and Influenza A virus (80-120nm) [17, 6]. The cold sprayed conventional and nanomaterial copper surfaces are shown at the 50X objective in Figures 6. Less of a change in slope is seen between the large-scale measurements taken with the 3D confocal microscope as compared to the smaller-scale measurements taken with AFM.

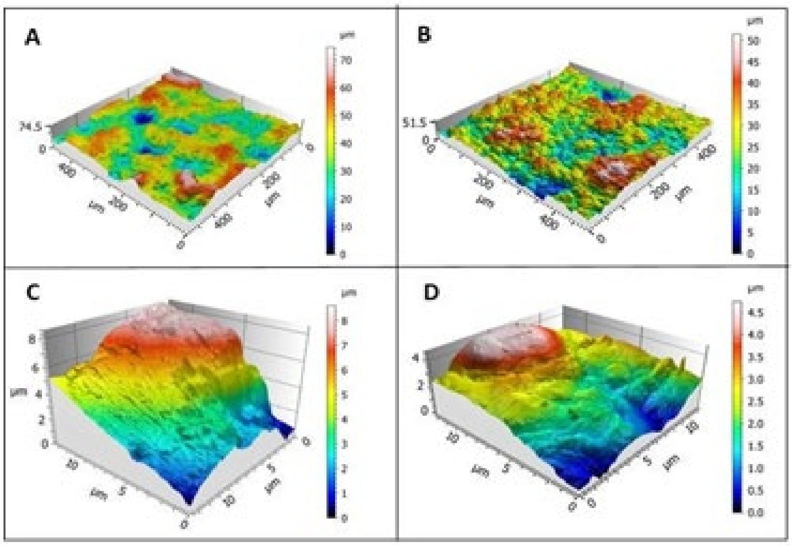

Figure 6: 3D confocal Images at 50X A) conventional Cu B) nanomaterial Cu. AFM images at 15x15 um^2 C) conventional Cu D) nanomaterial Cu.

Visual inspection of the 3D confocal measurements between the conventional and nanomaterial Cu shows what appears to be greater roughness in the latter sample. The relative area and complexity plots confirm the visual assessment made above. Plots based off the 3D confocal measurements are shown in Figure 7. The yellow line in both the relative area and complexity plots indicates the scale at which MRSA is present, 0.5-1um [6]. The green line in the plots is an indicator for 3D confocal optical resolution limit.

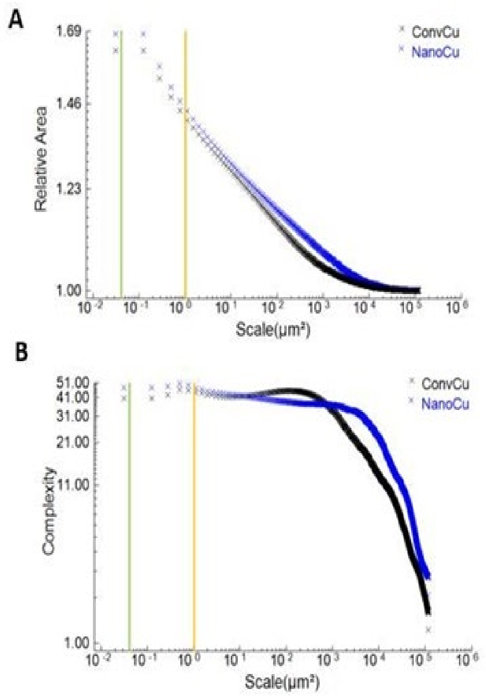

Figure 7: From 3D Confocal data- A) Relative Area Plot at 50X, and B) Complexity Plot at 50X

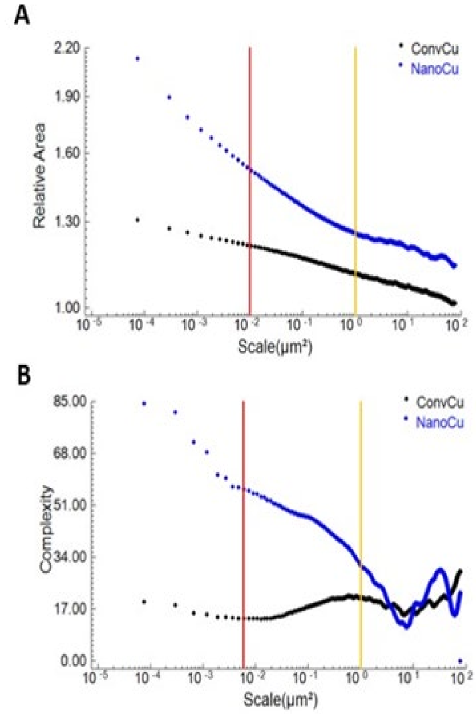

Figure 8: From AFM data- A) Relative Area Plot at 50X, and B) Complexity Plot at 50X

Quantification of the relative surface area of the 3D confocal measurements in Figure 7A shows that above 1000um2, nanomaterial Cu surface area is greater than that of conventional Cu. Again, below 10um2, nanomaterial Cu has the greater surface area. The complexity plot in Figure 7B allows for a clearer image of the transition in surface roughness at 10um2, as complexity removes the influence of large-scale features at the scale of observation.

Despite nanomaterial copper's greater surface roughness, it does not seem to have an impact on the kill rate of MRSA at the 2-hour mark, as both the nanomaterial and conventional surfaces show 99% reduction of MRSA at that timepoint [19].

The relative area and complexity plots based on AFM measurements are shown in Figure 8. The yellow line in both the relative area and complexity plots indicates the scale at which MRSA is present, 0.5-1um [17]. And the red line indicates the scale at which Influenza A Virus is present, 80-120nm [6]. Note that while the green line indicating 3D confocal optical resolution limit is not present, the value falls between the red and yellow lines

The relative area plot of the AFM data shows an increase in nanomaterial Cu surface roughness with decreasing scale. The complexity diagram in Figure 8B shows a relationship between decreasing scale and increasing difference in surface roughness between the nanomaterial and conventional Cu, where nanomaterial Cu continues to increase in surface area and conventional Cu stagnates.

Greater surface roughness may impact antimicrobial effectiveness of cold sprayed nanomaterial Cu against Influenza A Virus, as the nanomaterial Cu surface shows greater perecent reduction of the virus over a 2-hour period compared to conventional copper [19].

Volume scale analysis looks at how volume varies at different scales of observation [3]. Volume scale comparison at the micro- and nanoscale is presented in Figure 9. The y-axis of the graphs is a measurement of fraction volume filled, which shows at a given scale how much of the surface is filled in. Nanomaterial Cu has much greater fraction volume filled at both the nano-scale in Figure 9B and micron-scale in Figure 9A than conventional Cu. Viewing the volume scale graphs at the MRSA scale, 0.5-1um, and Influenza A Virus scale, 80-120nm, the same observation holds true. This indicates that the nanomaterial Cu has greater pockets and reservoir formation than conventional Cu. While the exact diameter of the pockets/reservoirs is not able to be determined from the volume scale data provided, the starting size can be estimated at ~102 um2 as seen from the confocal measurement in Figure 9A.

Fraction volume filled is a good determination of the amount of volume held by the sample in relation to itself, but it does normalize the effect of minimum and maximum points on the surface, where nanomaterial Cu has a much wider range than that of conventional Cu, as previously shown in surface roughness Figures 7 and 8. While fraction volume filled shows the structural difference between the two samples, it does not allow for a direct comparison between the two; total volume measurement is needed for that.

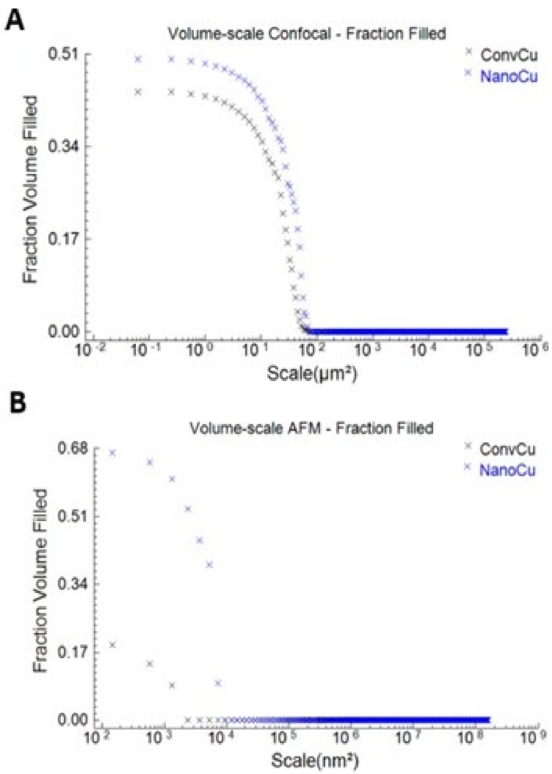

Figure 9: Volume scale A) Confocal B) AFM

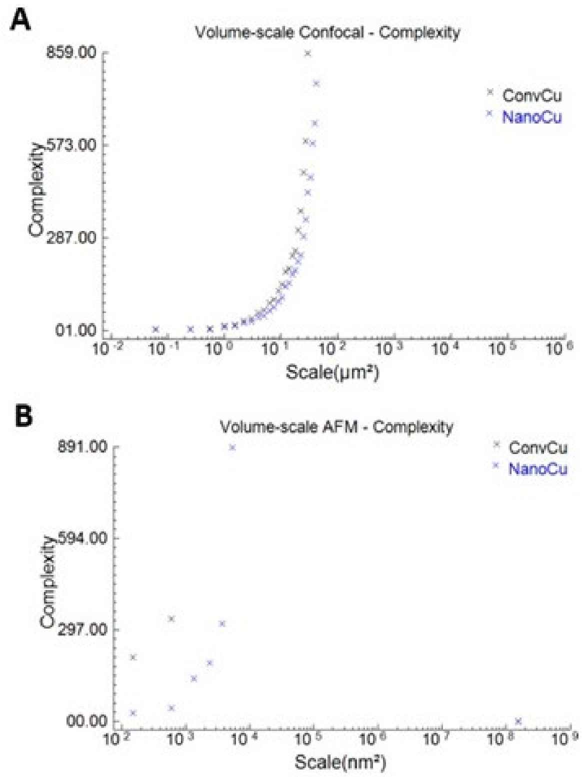

Figure 10: Volume scale Complexity A) Confocal, B) AFM

Volume scale complexity indicates how quickly the surface jumps from small to large pockets/holes. High complexity values indicate a short gradient between small and larger holes, indicative of a bi-modal distribution with very small and very large holes but minimal medium-sized ones. Whereas low complexity values indicate longer, more gradual or gausian-like gradients with a wider range of sizes between the small and large holes. Cold sprayed conventional Cu has slightly higher complexity values than nanomaterial Cu showing a quicker change in pore size. The nanomaterial has a more gradual change in pore size, most likely due to the powder containing both nanoparticles and conventional Cu binder.

Conclusion

Two factors affecting copper kill mechanisms include the percentage of grain boundaries and surface area. An increase in grain boundaries allows for increased Cu ion diffusion [10]. Surface roughness is proportional to surface area [22]. Depending on the powder particles used in the cold spray process, increased surface area could be an indicator of an increased number of grain boundaries. However, greater surface roughness can also mean more area for reservoir development [13].

As seen in the micrographs and measurement of grains, cold sprayed nanomaterial Cu has much smaller grains than cold sprayed conventional Cu. This supports the efficacy testing data showing nanomaterial copper with greater percent reduction of Influenza A Virus than conventional copper. However, both the nanomaterial and conventionalcopper surfaces appear to have the same percent reduction on MRSA after 2 hours.

Surface roughness may also contribute to copper kill- rate at the nanoscale, as seen in the 50X relative area scale plot in Figure 8. The surface roughness of nanomaterial Cu is much greater than conventional Cu at the scale of Influenza A and supports the efficacy testing that showed greater percent reduction of Influenza A by nanomaterial Cu than conventional Cu. The surface roughness of nanomaterial and conventional Cu is much closer for the MRSA scale, with nanomaterial Cu having slightly greater values. Surface roughness does not seem to affect the efficacy testing of MRSA as the percent reduction results at 2 hours are the same for both materials [19].

Volume scale data supports the area scale results with cold sprayed nanomaterial Cu having much greater fraction volume filled than conventional Cu at both the micro- and nanoscales. Interestingly, complexity values show steeper gradient for conventional Cu then nanomaterial Cu, indicating a greater pore size variation for the latter, most likely due to the differences in powder production.

At larger scales (0.1 um2 and above) surface roughness seems to have less of an effect on kill-rate than at the nanoscale. Follow-on research is needed to determine to what extent the variables of percent grain boundaries and surface roughness are coupled. Performing regression testing to determine optimal material surface roughness for fastest kill-rate may provide further insight [2]. Additional testing is also needed to determine if the kill-rate between the two surfaces varies prior to the 2-hour exposure time. And quantification of the percent of grain boundaries on both the conventional and nanomaterial copper surfaces at the micro- and nanoscale is needed to confirm that proportionality between surface roughness and percent grain boundaries.

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