The Potential Use of Nanostructured Surfaces and Metallic Nanoparticles to Combat the Spread of Infectious Diseases

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Emmelia Ashton is a junior studying Metallurgical and Materials Engineering and has been involved in research since her first year. She works as a member of Dr. Terry Lowe's Transdisciplinary Nanostructured Materials Research Team. During this time, she was part of the FIRST and SURF programs, and works to research medical device metals, sustainable magnets, and investigates material properties for industry sponsors. In the future, she plans to attend graduate school and earn a PhD in the field so she can further pursue research related to nanoscale biomaterials.

ABSTRACT

In recent years, serious health problems have been caused by the increasing resistance of pathogens to traditional antibiotics [1]. The development of novel, effective, and improved antimicrobial agents is necessary to prevent the spread of illness and pathogens. Copper possesses natural antimicrobial properties. However, its use as an antibacterial agent is not common due to the low rate of antimicrobial activity compared to traditional antimicrobial agents. Recent advances in the understanding of naturally biocidal surfaces have contributed to the development of nanopatterning technology that can be used to increase the antimicrobial effect of metallic substrates. The surface channelization of copper powder and mesh was performed to determine whether the natural biocidal properties of copper could be enhanced using nano-structuring techniques to create nanoscale channels across the surface. An in vitro trial of the nano-channelized copper powder against the Pseudomonas virus, Phi6, demonstrated an increase in the antimicrobial potential. These results suggest etching can be used to influence the geometry of surface features and improve the antimicrobial properties of naturally biocidal materials. The increased biocidal effect of the copper substrate could be implemented in numerous applications to help combat the growing issue of bacterial resistance and limit the spread of infectious disease.

“[Emmelia’s work] represents part of a thrust within the Transdisciplinary Nanostructured Materials Research Team (TNMRT) to develop biocidal alloys and surfaces. The project was spawned by the “Mines Pandemic Challenge” in 2020 initiated by Vice President of Research and Technology Transfer, Stefanie Tompkins. [...] Emmelia’s exemplary laboratory skills and microscopy are part of a team effort to optimize copper to incorporate into hospital air filtration systems, water filters, and high-touch surfaces to protect people from disease.”

— Dr. Terry Lowe

Introduction

In recent years, serious health problems have been caused by the increasing resistance of pathogens to traditional antibiotics [1]. Bacterial resistance occurs when modifications enhance the ability of a microorganism to resist inactivation by antimicrobial agents, typically in response to any inappropriate use of antibiotic agents [2],[3]. The increasing bacterial resistance to antibiotics is of concern as the use of traditional antibiotics can become ineffective in treatment of bacterial infections and illness. Due to the genetic variability and high rate of bacterial mutations, the development of novel, effective, and improved antimicrobial agents is important to prevent the spread of illness and pathogens.

Metal-based nanoparticles (NPs) have been explored as a promising solution to address the increasing resistance of bacteria to traditional antibiotics as they are known to possess non-specific microbial toxicity properties [4]. As metal-based NPs do not exhibit selectivity, they act as unbiased antimicrobial agents that inactivate any microbe in contact with the surface. The particle size of NPs is known to be a significant factor in antimicrobial effectiveness [2]. NPs and topographical features with high aspect ratios have demonstrated properties that could allow for their use as a non-specific antimicrobial agent.

In the natural world, there are many examples of natural bactericidal surfaces that employ nanoscale topographical features as a mechanism for cell death. The wings of the cicada Psdaltoda claripennis have been found to possess uniform and regularly spaced nanopillars that allow for natural bactericidal activity [5],[6]. This mechanism has been studied and successfully used to replicate nanostructured surfaces that possess bactericidal properties through the prevention and reduction of bacterial adhesion and surface contamination. As a result of these findings, many biomimetic nanoscale modifications to metallic substrates have been induced to increase the toxicity of engineered surfaces for specific applications.

Biomimetic nano-protrusions can inhibit microbial adhesion, an essential step in the bacterial colonization on a surface and limit the growth of bacterial colonies. These nanostructured protrusions have been engineered on titanium to inhibit the growth of biofilms on medical implants [5]. The exact bactericidal mechanism by which surfaces exhibit microbial toxicity is unknown. However, several mechanisms have been proposed based on experimental observations. Many of the theories emphasize the importance of surface interaction with microbes and highlight the importance of surface geometry and topographical features.

"Copper is an affordable metallic substrate that demonstrates the potential for use in the creation of antimicrobial surfaces."

The nanopatterning of metallic surfaces (creation of nano-protrusions and nanochannels) has been conducted using liquid etching. This method has been proven effective in increasing the biocidal activity of the material substrates [7]. Etching can be used to induce surface changes in any polycrystalline material and can create nanoscale channels (nanochannels) throughout the material. The method of etching could be used to modify the geometry of surface asperities, theoretically enhancing the antimicrobial properties of metal surfaces.

Copper is an affordable metallic substrate that demonstrates the potential for use in the creation of antimicrobial surfaces. The creation of copper NPs is also advantageous due to their robustness, stability, affordability, and ease of synthesis using various techniques [4]. Copper possesses natural antimicrobial properties and exhibits efficiency in contact killing of microbes. It is currently the most used metal in the coating and creation of antimicrobial surfaces, the global market of which is estimated to be valued at USD 20.71 billion by 2028 [8]. The antimicrobial effect of copper surfaces is generally attributed to the mechanism of membrane depolarization [9],[8]. Due to a combination of damage by copper ions and reactive oxidative species, material surfaces can exhibit toxicity to bacteria and microbes. The combination leads to lipid peroxidation and the resulting reduction of membrane integrity, leading to protein and DNA damage, and eventually resulting in cell death.

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While copper has natural antimicrobial properties, its use as an antibacterial agent is not common due to the slow rate of antimicrobial activity compared to traditional antimicrobial agents. To allow for the widespread use of copper as a non-specific antimicrobial agent, the rate of the antimicrobial activity must be enhanced and accelerated. The recent advances in the understanding of natural biocidal surfaces and the resulting technologies can be implemented in conjunction with copper, a naturally biocidal material, to engineer an affordable biocidal surface. If the surface of a copper substrate can be engineered to possess nanoscale topographical features, then it may have a more substantial biocidal effect and the technology could be introduced to help combat the growing issue of bacterial resistance and limit the spread of infectious disease.

Experimental Methods

To determine whether induced nanopatterning via channelization of the copper substrate could enhance the antimicrobial properties of the material surface, several copper samples were prepared. This experiment examines mainly induced channelized surfaces; however, the antimicrobial testing results for copper substrate with nano-protrusions were also measured to compare the antibacterial properties of each nanopatterning technique.

The surface asperities for the nano-channelized samples were induced using an aqueous hydrochloric acid (HCl) solution containing iron chloride ($FeCl_3$). This etchant has been used in literature to etch nanoscale features on copper surfaces at room temperature [10]. Within the etchant solution, the $FeCl_3$ dissociates into Fe^{3+} and Cl^- ions acting as the etchant and the coordinating ligand, respectively. The dissolution of the etched copper particles is assisted by the HCl present within the etchant solution.

The etchant was applied to the surfaces of copper substrates (both mesh and powder) to induce asperities and channelize the surface. Each copper substrate was then characterized, and micrographs were obtained. High-resolution scanning electron microscopy (SEM) images of the copper surfaces were obtained using a FESEM (JEOL-7000F) at 15kV accelerating current with magnifications of 2000x for the etched copper powder and 10,000x for the etched copper mesh. The obtained micrographs were used to compare and examine the surface asperities and nanoscale topographical features induced by the etching procedure.

Measurement of the effectiveness of the surface at inactivating microbes was tested using an enveloped bacteriophage, Pseudomonas virus Phi6, in vitro. The Phi6 is a nonpathogenic bacteriophage and possesses no risk of infection to humans. It has a lipid membrane around its nucleocapsid, allowing for its use as a nonpathogenic surrogate for more hazardous enveloped RNA viruses such as SARS-CoV-2, influenza, and Ebola [11], [12]. This is beneficial when testing the antimicrobial properties of the engineered surfaces, as it can be used to determine whether the biocidal surfaces can be used to limit the spread of pathogens and enveloped viruses that currently pose a serious health risk, while using a biosafe model.

To test the antimicrobial properties of the engineered copper powder, a series of timed experiments were carried out to measure the degree of neutralization of the Phi6 virus. A titer of 20mL of the virus was added for 60 seconds in different wells of a cell culture plate with five different loadings of each powder type (etched/channelized copper, nano-protrusion copper, as-received copper, and stainless steel) ranging from 0.1 to 0.5 milligrams [13]. The virus-containing solution was removed and plated to determine the log fraction of surviving Phi6 virus. All materials were tested against the Phi6 bacteriophage with the same procedure and conditions as the nano-channelized copper powder.

Results and Discussion

The nano-channelized copper powder was etched to create nanoscale surface asperities to determine whether it would enhance its natural antimicrobial properties. After undergoing etching, the channelized copper powder was imaged and the resulting micrograph is shown in Figure 1.

The SEM micrograph shows a group of etched copper powder particles that have started to develop nanoscale surface features and become channelized, confirming the effectiveness of the etchant at inducing nanoscale surface features. Since the copper used is a polycrystalline material, creation of nanochannels and surface asperities occurs as the etchant attacks the particle surface at varying rates

The antimicrobial activity was measured to compare the antimicrobial properties of nanopatterned copper powder (both nanochannels and nano-protrusions) to the naturally bactericidal, as-received copper powder. The decay curve constructed from the data obtained in the experimental trials is shown in Figure 2.

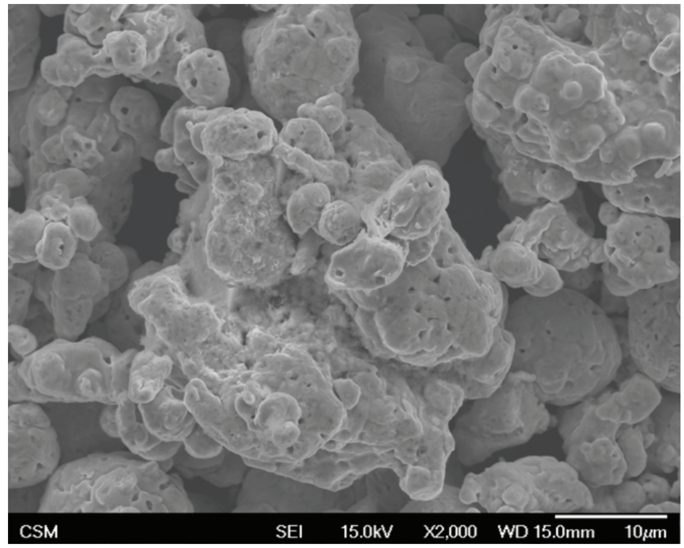

Figure 1 *Scanning Electron Microscopy (SEM) image of etched copper powder particles at a magnification of 2,000x, showing the effects of selective chemical etching on the surface topography of the sample.*

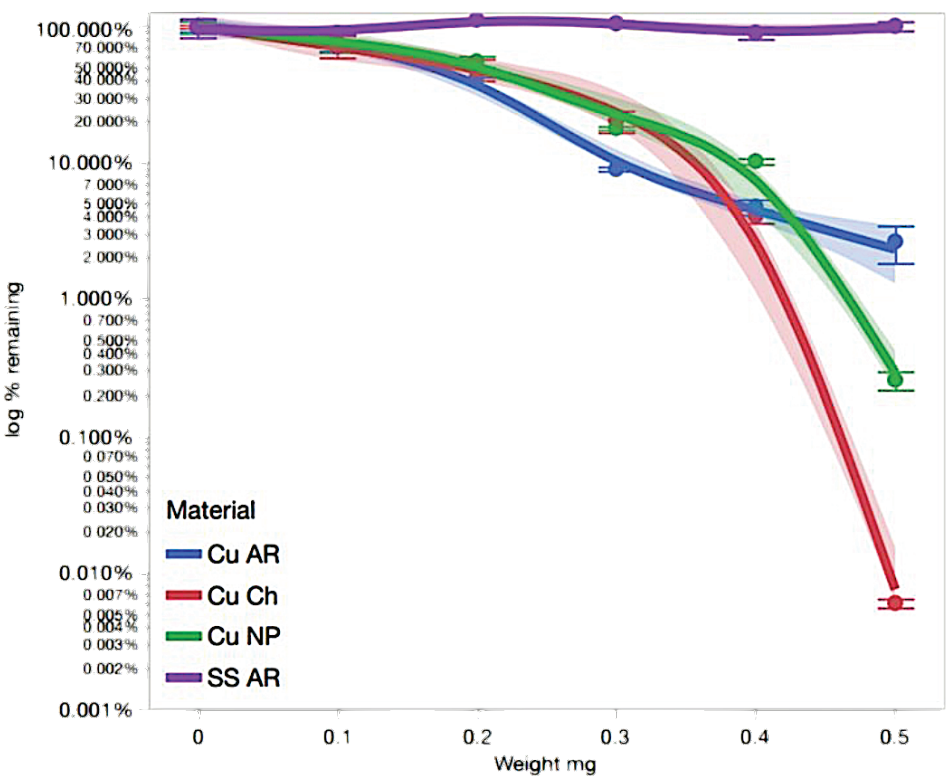

Figure 2 *From Reiss, R. A., unpublished data, used with permission (2022). Decay curves for antimicrobial testing of nanostructured copper powder. Five loadings of each powder ranging from 0.1 to 0.5 milligrams were tested and the log percentage of Phi6 bacteriophage was measured for a time interval of 60 seconds. Cu AR, blue, is the as-received copper powder (no nano-structuring was performed). Cu Ch, red, is the channelized copper powder that underwent etching processes. Cu NP, green, is the copper particles with nano-protrusions. SS AR, purple, is as- received stainless steel powder used to compare the inherent antimicrobial effects of the as-received copper powder. The graph shows the standard deviation of each curve throughout the testing process.*

As shown in the decay curve, the nanostructured channelized copper powder was shown to decrease the viral load of Phi6 faster than any other powder type. The nano-channelized copper was experimentally found to deactivate 99.999% of Phi6 virus in under 60 seconds. This is a significant increase compared to the as-received copper powder, indicating that the nanostructured asperities enhance the natural biocidal properties of copper. This result demonstrates the potential use of nanostructured copper as an antimicrobial agent. In addition to the use of powders as biocidal surfaces and antimicrobial agents, there is potential use of other copper substrates such as mesh. To further understand the surface asperities induced by the etching procedure, etched copper mesh was characterized, and the resulting micrograph is shown in Figure 3.

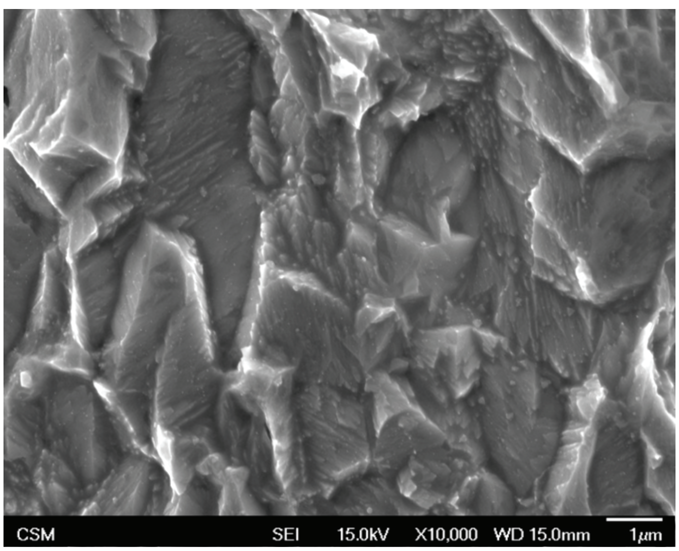

Figure 3 *Scanning Electron Microscopy (SEM) image of etched copper mesh at a magnification of 10,000x, showing the effects of selective chemical etching on the surface topography. The lighter sections of the angular surface asperities display the charging that occurs on the high aspect ratio asperities.*

The SEM micrograph for the etched mesh indicates the etching process was effective in creating surface asperities. Additionally, an uneven charge distribution is shown to accumulate along the channelized surface, where the sharper asperities appear lighter because of their increased charge concentration. This uneven charge distribution can also be observed, to a lesser extent, in the etched copper powder micrograph.

Copper is a highly conductive material with an electron configuration of [Ar]3d104s1 and a highly reactive valence electron, allowing each copper atom to act as an electron donor during conduction [14]. In conductive materials, charge is known to concentrate on angular surface features like nanoscale ridges and channels [15]. The conductive properties of copper likely contribute to the observed accumulation of charge around the angular surface features. This non-uniform charge accumulation around nanopatterned features could potentially explain the antimicrobial properties of copper NPs and the etched surfaces.

The uneven charge distribution and accumulation of electrostatic charge could result in a disruptive charge transfer between the charged metallic substrate asperities and the microbe. This could lead to the inactivation of the microbe, contributing to the mechanism by which the observed antibacterial properties occur. To further examine this potential mechanism, an in-depth study of the asperities is necessary.

Since the electrostatic charge concentration is dependent on the geometry of the surface features [15], non-uniform charge distributions can be created through nano-structuring processes such as etching. The natural biocidal properties of copper can therefore be improved through modifications to the nanostructure. The enhancement of the non-specific antimicrobial properties of copper could be a potential solution to the ongoing bacterial resistance crisis, allowing for the implementation of numerous technologies that can limit the spread of infectious diseases.

Conclusions and Future Work

In conclusion, nano-structuring can be used to enhance the natural biocidal properties of copper. The surface channelization of the copper substrate was shown to drastically increase the antimicrobial potential of the material. The enhanced rate of antimicrobial properties because of the created surface asperities could allow for the widespread use of copper as a non-specific antimicrobial agent. Although the roughness of the induced surface asperities could explain the antimicrobial properties mechanically, the observed mechanism of antimicrobial properties is likely affected by the electrical properties of the material and the geometry of the induced asperities. The charge transfer between the non-uniform charge distribution of the surface could explain the observed inactivation of microbes, though further research is necessary to confirm these observations.

Further characterization of the induced surface asperities is necessary to further understand the observed antimicrobial properties and the mechanism by which they function. The quantification of surface asperity geometry could be used to compare the antimicrobial effectiveness of various induced nanostructures. To further characterize the asperities, the radius of curvature could be measured, and the results of this characterization could be used to compare the antimicrobial effectiveness of varying degrees of surface channelization and to determine the optimal size of surface features.

This technology could revolutionize the antimicrobial agent and antibacterial surface markets. There are numerous possibilities for the implementation of nanostructured antimicrobial copper substrates to prevent the spread of diseases. Applications, including antimicrobial surfaces and coatings, integration into air filtration systems, and integration into clothing or other fabrics, could be used to limit the spread of pathogenic viruses and bacteria. Copper is an affordable material that can be efficiently enhanced to exhibit highly antimicrobial properties, which will allow for the potential development of highly accessible antimicrobial technologies.

References

[1] S. M. Dizaj, F. Lotfipour, M. Barzegar-Jalali, M. H. Zarrintan, and K. Adibkia, "Antimicrobial activity of the metals and metal oxide nanoparticles," Mater. Sci. Eng. C, vol. 44, pp. 278–284, 2014, doi: https://doi.org/10.1016/j.msec.2014.08.031.

[2] A. Khezerlou, M. Alizadeh-Sani, M. Azizi-Lalabadi, and A. Ehsani, "Nanoparticles and their antimicrobial properties against pathogens including bacteria, fungi, parasites and viruses," Microb. Pathog., vol. 123, pp. 505–526, 2018, doi: https://doi.org/10.1016/j.micpath.2018.08.008.

[3] S. Modi et al., "Nanostructured Antibiotics and Their Emerging Medicinal Applications: An Overview of Nanoantibiotics," Antibiotics, vol. 11, no. 6, 2022, doi: 10.3390/antibiotics11060708.

[4] E. Sánchez-López et al., "Metal-Based Nanoparticles as Antimicrobial Agents: An Overview," Nanomaterials, vol. 10, no. 2, 2020, doi: 10.3390/nano10020292.

[5] M. I. Ishak, X. Liu, J. Jenkins, A. H. Nobbs, and B. Su, "Protruding Nanostructured Surfaces for Antimicrobial and Osteogenic Titanium Implants," Coatings, vol. 10, no. 8, 2020, doi: 10.3390/coatings10080756.

[6] E. P. Ivanova et al., "Bactericidal activity of black silicon," Nat. Commun., vol. 4, no. 1, p. 2838, Nov. 2013, doi: 10.1038/ncomms3838.

[7] F. Xue, J. Liu, L. Guo, L. Zhang, and Q. Li, "Theoretical study on the bactericidal nature of nanopatterned surfaces," J. Theor. Biol., vol. 385, pp. 1–7, 2015, doi: https://doi.org/10.1016/j.jtbi.2015.08.011.

[8] M. Birkett, L. Dover, C. Cherian Lukose, A. Wasy Zia, M. M. Tambuwala, and Á. Serrano-Aroca, "Recent Advances in Metal-Based Antimicrobial Coatings for High-Touch Surfaces," Int. J. Mol. Sci., vol. 23, no. 3, 2022, doi: 10.3390/ijms23031162.

[9] D. Mitra, E.-T. Kang, and K. G. Neoh, "Antimicrobial Copper-Based Materials and Coatings: Potential Multifaceted Biomedical Applications," ACS Appl. Mater. Interfaces, vol. 12, no. 19, pp. 21159–21182, May 2020, doi: 10.1021/acsami.9b17815.

[10] T.-S. Choi and D. W. Hess, "Chemical Etching and Patterning of Copper, Silver, and Gold Films at Low Temperatures," ECS J. Solid State Sci. Technol., vol. 4, no. 1, p. N3084, Oct. 2014, doi: 10.1149/2.0111501jss.

[11] R. A. Reiss, O. Makhnin, and T. C. Lowe, "Rapid Method to Quantify the Antiviral Potential of Porous and Nonporous Material Using the Enveloped Bacteriophage Phi6," Environ. Sci. Technol., vol. 56, no. 12, pp. 8350–8362, Jun. 2022, doi: 10.1021/acs.est.1c07716.

[12] Á. Serrano-Aroca, "Antiviral Characterization of Advanced Materials: Use of Bacteriophage Phi 6 as Surrogate of Enveloped Viruses Such as SARS-CoV-2," Int. J. Mol. Sci., vol. 23, no. 10, 2022, doi: 10.3390/ijms23105335.

[13] R. A. Reiss, "Unpublished Data, used with permission," 2022.

[14] K. Chung et al., "Non-oxidized bare copper nanoparticles with surface excess electrons in air," Nat.Nanotechnol., vol. 17, no. 3, pp. 285–291, Mar. 2022, doi: 10.1038/s41565-021-01070-4.

[15] F. M. Gilles, F. M. Boubeta, O. Azzaroni, I. Szleifer, and M. Tagliazucchi, "Modulation of Polyelectrolyte Adsorption on Nanoparticles and Nanochannels by Surface Curvature," J. Phys. Chem. C, vol. 122, no. 12, pp. 6669–6677, Mar. 2018,