Copper-Modified Mesoporous Silica Nanoparticles for Antimicrobial Applications

Elisa Recchia,[1] Amaia M. Goitandia,[2] Maialen Argaiz,[2] Miren Blanco,[2] Giorgia Grilli,[1] Alessandra Amoroso,[1] Nathalie Totaro,[1] Andrea Ciammaruconi,[1] Riccardo De Santis,[1,3] Fabiana Arduini,[4] Florigio Lista[1]*

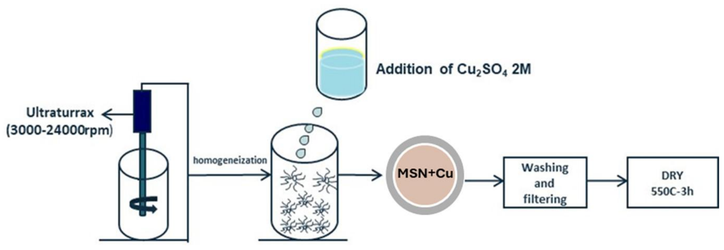
[1]*Defence Institute for Biomedical Sciences, 00184 Rome, Italy;*
[2]*Unidad de Química de superficies y Nanotecnología, Fundación Tekniker, Iñaki Goenaga 5, 20600 Eibar, Spain;*
[3]*Department of Public Health and Infectious Diseases, Sapienza University of Rome, Piazzale Aldo Moro, 5, 00185 Rome, Italy;*
[4]*Department of Chemical Science and Technologies, University of Rome "Tor Vergata", Via della Ricerca Scientifica 1, 00133 Rome, Italy.*

Introduction

Antimicrobial resistance (AMR) poses a serious threat to global health, leading to increased morbidity, mortality, and economic burden [1, 2]. Addressing this urgent issue requires innovative strategies to prevent bacterial spread, especially through contaminated surfaces. The overriding goal of Horizon Europe RELIANCE project is the development of advanced antimicrobial technologies by creating innovative coatings based on Mesoporous Silica Nanoparticles (MSNs) functionalized with metallic copper and essential oils (EO). These coatings are specifically designed to boost antimicrobial effectiveness beyond existing solutions [3]. The oxidation state of copper on the MSN surface was modulated through thermal treatments, allowing the evaluation of its influence on antimicrobial efficacy against two representative bacteria (*Escherichia coli* and *Staphylococcus aureus*), and viruses like influenza A (H1N1) pdm09, severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2), and MS2 bacteriophage (MS2). Two versions of copper-modified MSNs were prepared: Cu-MSN-1 (without calcination) and Cu-MSN-2 (calcined). The findings demonstrate that copper-modified MSNs exhibit significant biocidal activity against both bacterial and viral pathogens, offering a sustainable, antibiotic-free approach to infection prevention that bypasses the selective pressures driving antimicrobial resistance.

Syntesis and Characterization

Stober's optimized method: TEOS:EtOH:H_2O:NH_4OH 2M:CTAB - 1:20:100:0.16:0.1 + 20 mL mesitylene
average size = 104 nm, specific surface area = 900 m^2/g, pore size = 6 nm

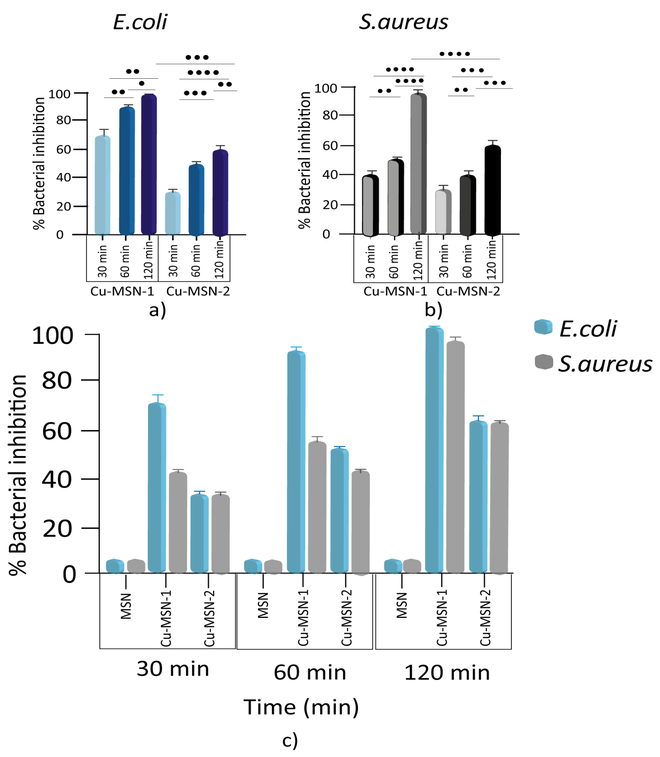


Synthesis mechanism for the incorporation of Cu

Cu loading: 1.3-1.5 wt%
Cu-MSN-1 (no calcination) vs Cu-MSN-2 (550°C calcined)

Antibacterial activity

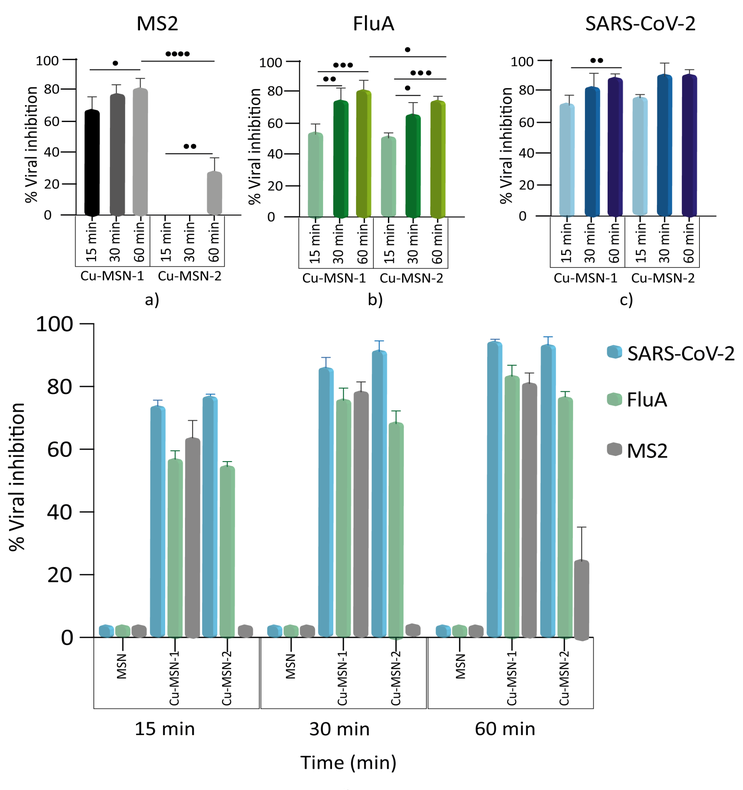
Comparative antibacterial evaluation using MIC, MBC, and time killing assays demonstrated that Cu-MSN-1 exhibited superior bactericidal activity against both *E. coli* and *S. aureus*. In fact, MIC values of 2.5 mg/mL were obtained for both Cu-MSNs preparations and both bacterial strains. However, an MBC value of 2.5 mg/mL was produced exclusively by Cu-MSN-1 against both bacteria. Moreover, after 120 minutes of incubation, Cu-MSN-1, at the concentration of 2.5 mg/ml, achieved complete bacterial eradication (100% killing) against *E. coli*, while Cu-MSN-2, at 2.5 mg/ml, demonstrated only 60.4% inhibition ($p = 0.0009$). Against *S. aureus*, Cu-MSN-1 showed 95.1% reduction compared to 60.5% for Cu-MSN-2 ($p < 0.0001$), confirming the superior broad-spectrum antimicrobial efficacy of the non-calcined formulation (Cu-MSN-1).



Bactericidal activity of Cu-MSN-1 and Cu-MSN-2 against *E. coli* (a) and *S. aureus* (b). Killing activity values were expressed as means ± the SD. Statistical significance was indicated as • for values with $p < 0.05$; •• for values with $p < 0.01$; ••• for values with $p < 0.001$; and •••• for values with $p < 0.0001$.
(c) Percentage of killing activity of MSN, Cu-MSN-1, and Cu-MSN-2 on *E. coli* and *S. aureus* over time (30, 60, and 120 min).

Virucidal activity

Cu-MSN-1 and Cu-MSN-2 were incubated with SARS-CoV-2, influenza A pH1N1 09, and MS2 bacteriophage at a final concentration of 0.25 mg/mL for 15, 30, and 60 minutes. Then, the virucidal activity was evaluated by plaque assay for SARS-CoV-2 and MS2, and TCID50 for influenza virus.
The results indicated an inactivation ratio, at 60 minutes, of 90% and 91.5% with Cu-MSN-1 and Cu-MSN-2, respectively, in SARS-CoV-2 ($p > 0.05$), whereas in influenza A it was around 80% with Cu-MSN-1 and 75% with Cu-MSN-2 ($p < 0.05$). Regarding MS2 bacteriophage, viral inhibition of Cu-MSN-1 reached 81.6%, whereas the activity of Cu-MSN-2 was about 25% ($p < 0.0001$). A significant increment of Cu-MSN-1 over time was observed for all the viruses, whereas for Cu-MSN-2, it was detected only for Influenza A and MS2 bacteriophage. In fact, for SARS-CoV-2, the degree of inhibition was high even at 15 minutes. The higher virucidal efficacy of Cu-MSN-1 compared to Cu-MSN-2 could be observed mainly in MS2 bacteriophage (p-value < 0.0001).



Virucidal effect of Cu-MSN-1 and Cu-MSN-2 against MS2 bacteriophage (a), influenza A virus (b), and SARS-CoV-2 (c). Virucidal titers were expressed as means ± the SD. Statistical significance was indicated as • for values with $p < 0.05$; •• for values with $p < 0.01$; ••• for values with $p < 0.001$; and •••• for values with $p < 0.0001$.
(d) Overview of the inhibitory effects of MSN, Cu-MSN-1, and Cu-MSN-2 on the three viruses at 15, 30, and 60 minutes.

Conclusions

The broad-spectrum antimicrobial performance of Cu-MSN, combined with their tunable physicochemical properties, supports their potential application in CBRNe-related contexts, including sustainable solutions against the global challenge of AMR, emerging viral pathogens, microbial coatings for high-touch surfaces, water treatment systems, and antiviral textiles for personal protective equipment.

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