Electronic Supplementary Information:

Tailored Ag-Cu-Mg multi-element nanoparticles for wide spectrum

antibacterial coating.

Giulio Benetti,^{a,b} Emanuele Cavaliere,^a Rosaria Brescia,^c Sebastian Salassi,^d Riccardo Ferrando,^d André Vantomme,^e Lucia Pallecchi,^f Simona Pollini,^g Selene Boncompagni,^g Beatrice Fortuni,^h Margriet J. Van Bael,^b Francesco Banfiⁱ and Luca Gavioli^{a,*}

- a. Interdisciplinary Laboratories for Advanced Materials Physics (i-LAMP) and Dipartimento di Matematica e Fisica, Università Cattolica del Sacro Cuore, Via Musei 41, 25121 Brescia, Italy.
- b. Laboratory of Solid State Physics and Magnetism, Department of Physics and Astronomy, KU Leuven, Celestijnenlaan 200D, 3001 Leuven, Belgium.
- c. Electron Microscopy Facility, Istituto Italiano di Tecnologia (IIT), Via Morego 30, 16163 Genova, Italy.
- d. Dipartimento di Fisica, Università di Genova, Via Dodecaneso 33, 16146 Genova, Italy.
- e. Institute for Nuclear and Radiation Physics, Department of Physics and Astronomy, KU Leuven, Celestijnenlaan 200D, 3001 Leuven, Belgium.
- f. Department of Medical Biotechnologies, University of Siena, Viale Bracci 1, 53100 Siena, Italy.
- g. Dipartimento di Medicina Sperimentale e Clinica, Università di Firenze, Largo Brambilla 1, 50134 Firenze, Italy.
- h. Molecular Imaging and Photonics, Department of Chemistry, KU Leuven, Celestijnenlaan 200F, 3001 Leuven, Belgium.
- i. FemtoNanoOptics group, Institut Lumière Matière, Université de Lyon, CNRS, Université Claude Bernard Lyon 1, 69622 Villeurbanne, France.
- * Corresponding author e-mail: luca.gavioli@unicatt.

1. SCBD Source Functioning

The deposition apparatus (Fig. S1) exploits pulses of high pressure inert gas (He@45bar) injected in the ablation chamber and a synchronized delayed discharge to generate a plasma spot at the cathode, i.e. the rod of the material to be deposited. Sputtered atoms start to condense inside the ablation chamber forming the NPs. The pressure gradient extracts the He and NP mixture through a nozzle and a set of focusing lenses into the first chamber (operating pressure 10⁻⁴ mbar). The central part of the beam is selected by a skimmer to increase the uniformity of the size of NPs that are directly reaching the substrate. Once deposited in the vacuum chamber, the samples are extracted to air and transferred either to the measurement apparatus or left exposed to the environment. The deposited coating thickness is estimated in real-time using a home-made quartz microbalance, calibrated through AFM.



Fig. S1 Drawing of the SCBD apparatus.

2. NP size analysis



Fig. S2 Histogram of the distribution of the circular diameter calculated from the area evaluated by ImageJ after application of a threshold over HAADF-STEM images of the two samples. [1] The corresponding average circular diameters are $1.8(\pm 0.8)$ nm for AgCuMg202060 and $3.0(\pm 1.7)$ nm for AgCuMg503020.

3. HR-TEM and EDX Details



Fig. S3 STEM- EDX spectra, normalized to the Cu K_{α} peak intensity (as visible in the bottom spectrum), for the AgCuMg202060 (red line) and AgCuMg503020 (black line) NPs samples, corresponding to the elemental maps in Fig. 1 in the main manuscript. The Au signal is due to the X-rays emitted by the TEM grid hit by backscattered electrons.



Fig. S4 HR-TEM analyses for the (a-d) AgCuMg202060 and (e-f) AgCuMg503020 NPs samples. In particular, while Ag NPs are found in both specimens (see a, e and their respective fast Fourier transforms, FFTs, b and f), in the Mg-richer one (AuCuMg202060) few nm-sized nanocrystals are found which are clearly ascribable to MgO (see panels c and corresponding FFT in d). In particular, the larger Ag NPs found in the sample AgCuMg503020 exhibit the typical multi-twinning often reported for metallic nanoparticles. Inorganic Crystal Structure Database (ICSD) reference structures are 44387 (Ag) and 98631 (MgO).

4. RBS Measurements

Fig. S5 shows the O, Mg, Cu and Ag signals of the RBS spectrum of the 85 nm thick AgCuMg203050 NP sample deposited on HOPG. The spectrum was obtained from the raw data after removing the minor (a few %) low-energy detector noise. Since this small contribution varies monotonically as a function of energy, it can be easily removed by a second order polynomial fit. For clarity, the low-energy carbon signal from the HOPG substrate is not shown. The relative elemental content obtained from the peak integration is O/Ag/Cu/Mg = 48.4/12.0/12.3/27.3%.



Fig. S5 Rutherford backscattering spectrum of the 85 nm thick film of AgCuMg503020 on HOPG. For the sake of clarity, only the relevant signals are shown. The arrows indicate the energy of He ions backscattered from the respective atoms located at the sample surface. The solid line is a simulated spectrum using the RUMP code [2], assuming the calculated sample composition, as well as a layer roughness amounting to approx. 25% of the layer thickness (including the contributions of both the sample surface and the HOPG-film interface).

5. XPS Analysis

As explained in the main text, the Ag and Cu spectra indicate a metallic Ag and a mostly metallic Cu.

Both the Mg2p (Fig. 2e) and Mg1s (Fig. 2f) spectra present a single, broad peak resulting from the superposition of different components. The analysis of these photoemission lines is then based on the peak intensity derived from the C1s (Fig. 2c) and O1s (Fig. 2d) core levels and assuming that three possible stable Mg chemical states are separately contributing to the Mg lineshape (MgO, Mg(OH)₂ and MgCO₃). To estimate an initial intensity of the different Mg components, we have to consider the C1s and O1s data.

The C1s spectrum (Fig. 2c) presents two distinct peaks at 290.6 and 285.4 eV BE. They are attributed to the $(CO_3)^{2-}$ group (blue line) [3] and to hydrocarbon contaminations (orange line) [3,4], respectively. Since Cu and Ag are metallic, it is reasonable to assume that all the $(CO_3)^{2-}$ groups are bound to Mg to from MgCO₃. We thus imposed that the weighted areas (i.e. the area normalized to both the atomic sensitivity factor [5] and the IMFP [6]) of the blue components in the Mg 1s and Mg 2p peaks have to be equal to the one of the blue $(CO_3)^{2-1}$ fit in the C1s spectrum. Moreover, since for each C atom in MgCO₃ there are 3 atoms of O, the weighted area of the blue component (532.9 eV BE) in the O1s spectrum was also fixed to be three times the weighted area of the blue $(CO_3)^{2}$ component in the C1s spectrum. The remaining areas of the two components in the O1s data. *i.e.* the clear shoulder displayed in red at 530 eV BE, relative to the O^{2-} ions of MgO, and the (OH)- group of Mg(OH)₂ displayed in green at 532 eV BE, are then obtained with the fitting procedure. With these assumptions, we also obtain a very good agreement of each Mg component BE with the values reported in the literature.[3,4,7,8] We identify the relative concentrations of the three Mg chemical states to be 42%, 39% and 19% for MgO, Mg(OH)₂ and MgCO₃, respectively, as reported in Table 1. In the table, the BE errors in the 3^{rd} column have to be considered stochastic errors and are computed by considering both the fitting procedure errors on the Voight function center and the error propagation upon small variations of the peak areas, kept fixed during the fitting procedure. The 5th column of the table indicates the relative peak area with respect to the total area of all the components and, in the case of Mg peaks, represent the relative concentration of each compound inside the Mg. The total atomic ratio between 0 and Mg reads 1.75 ± 0.10 , a value compatible with the RBS results and with the total XPS weighted area integration.

6. Porosity Estimation

The porosity of the film was estimated from the combined analysis of the RBS and XPS results, under the assumption that the chemical state of the surface and of the bulk of the film are the same. This assumption is reasonable, since SCBD deposited films have been shown to be obtained by pile up of single nanoparticles that maintains their chemical and physical structure [9]. RBS measurements allow to directly quantify the number of atoms N_i per unit of area A for each element of the sample (N_i/A , 3^{rd} row of Table S1). Even though RBS cannot perform a chemical analysis of the sample, from XPS it is possible to retrieve the relative concentration of every Mg compound (4th row). The total number of atoms per unit of area, for each compound, is consequently reported in the last row of Table S1.

For each compound $i \in \{Ag, Cu, MgO, Mg(OH)_2, MgCO_3\}$, the total volume V_i can be expressed in terms of the total deposited mass M_i and element density ρ_i , or by using the molecular mass m_i and the total number of "molecules" N_i :

$$V_i = \frac{M_i}{\rho_i} = \frac{N_i m_i}{\rho_i}$$

From the total volume V_{TOT} it is then possible to obtain the expected thickness t assuming the film did not show any porosity

$$V_{TOT} = A t = \sum V_i \rightarrow t = \sum \frac{(N_i/A) m_i}{\rho_i}$$

By using the values of N_i/A reported in Table S1 and the literature values for ρ_i and m_i , we obtain t = 68 nm. The porosity of the film is then

$$\phi = 1 - \frac{t}{t_{exp}} = 1 - \frac{68}{85 \pm 5} = 0.20 \pm 0.04$$

where t_{exp} is the experimental thickness of the porous film, obtained by partially covering a flat Si substrate with an aluminum foil, co-depositing it together with the HOPG and measuring it through AFM. The acquired image on the edge of the film is reported in Fig. S6, together with the pixel height distribution (vertical axis in log-scale) and a typical line profile. Even though the edge is not very sharp, it is possible to appreciate that the film thickness is homogeneous for the 20 µm visible in Fig..

N _i /A	Ag	Cu		Mg	
[at./m ²]	metallic Ag	metallic Cu	MgO	Mg(OH) ₂	MgCO ₃
RBS	6,50E+20	6,62E+20		1,47E+21	
XPS	1 N _{Ag}	$1 \ N_{Ag}$	$0,42 N_{Mg}$	0,39 N _{Mg}	0,19 N _{Mg}
Tot	6,50E+20	6,62E+20	6,19E+20	5,75E+20	2.80E+20

Table S1 Absolute elemental content (as obtained from RBS) of a 85 nm thick sample of AgCuMg503020 and relative concentration of each Mg compound (obtained from XPS). The last row reports the computed values of N_i / A for each constituent of the NP film.



Fig. S6 AFM image of the film profile at the edge of the mask. The darker zone is the flat Si substrate whereas the brighter zone is the deposited film of AgCuMg503020 NPs. The edge where the film terminate is not sharp (about 500 long) but this does not impede the correct identification of the film thickness. The vertical axis of height distribution reported in the top inset is in logarithmic scale. The bottom inset reports a typical line scan of the image.

7. MD Simulation Results

The energy of the metals as a function of the temperature is plotted in Fig. S7, for the two simulation runs. In this plot we can observe the liquid-to-solid transition of the metal that is located at about 650 K.

The plots showing the number of contacts between Ag, Cu and MgO as a function of the temperature are shown in Fig. S8 and S9 for the two simulation runs, respectively. Since the initial states of the MD simulations is the mixture of Ag and Cu at 800 K, these graphs should be interpreted starting from right (800 K) to left (400 K), as the cooling process which lead to the NPs formation. It is possible to appreciate the phase segregation of Cu and Ag around 600-650K as a sudden decrease of the Ag-Cu interactions, for both the simulations. On the other hand, the contacts with the MgO box remains almost constant during the whole simulation.



Fig. S7 Total energy of the metal as a function of the temperature for the two MD simulation runs.



Fig. S8 Number of contacts between Ag and Cu (left) and percentage of contacts of the two metals with the MgO box (right), as a function of the temperature, for the first run.



Fig. S9 Number of contacts between Ag and Cu (left) and percentage of contacts of the two metals with the MgO box (right), as a function of the temperature, for the second run.



Fig. S10 Renderings of the initial and final configurations of the MD simulations for the two runs. Orange spheres represent Cu atoms whereas light-blue spheres represent Ag atoms. MgO is not visible.

The initial and final configurations of the two MD simulation runs are plotted in Fig. S10. Cu is depicted in orange, Ag is depicted in light-blue and MgO is not displayed. From these renderings it is possible to appreciate the segregation between Cu and Ag and the partial core-shell structure, with many Cu atoms in the outer part of simulation box.

8. Human Cytotoxicity Results



Fig. S11 Bright-field images of HeLa and A549 cells after 24 h of incubation on controls dishes (panel a and b) and on AgCuMg503020 (ACM532) NP-coated dishes (c and d). Scale bar is 150 μ m. Panel e: corresponding viability test plot, data shown as mean ± SD (n=3), with error bars representing the standard deviation.

9. Bibliography

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