Supporting Information

CNC/AgNP hybrids as safer-by-design biocides in paints

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S1 - Preparation and characterization of H2 hybrid using hydrazine

Synthesis of H2 hybrid using hydrazine (N_2H_4). Firstly, the CNCs (10 g, 0.062 mol) were surface-functionalized with NH_2 chelating groups by silanization with 3-aminopropyltriethoxysilane (APTES, 0.683 g, 0.74 mL, 5% molar with respect to CNC, 3.08 10^{-3} mol), according to the protocol proposed by Khanjanzadeha et al.[40] Such a surface modification was performed in toluene (300 mL) at reflux, under stirring at 120 °C for 24 h. The functionalized CNCs (i.e., A-CNC) were centrifuged (5000 rpm for 10 min), washed in ethanol and then dialyzed against deionized water twice. The procedure is schematically represented in Figure S1.1. The successful surface modification of CNCs has been verified by recording photoluminescence spectra (Figure S1b). Then, a saturated solution of $AgNO_3$ in an ethanol/dimethylsulfoxide mixture (100 mL at 50/50 v/v) was mixed with the A-CNCs. The resulting suspension was placed under stirring at room temperature for 4 h and then centrifuged (5000 rpm for 5 min) and washed several times in the ethanol/dimethylsulfoxide mixture. Finally, the chemical reduction of Ag^+ ions was performed using hydrazine (N_2H_4): two drops of hydrazine were added to the complexed A-CNC/Ag to only reduce the silver ions effectively complexed on the CNC surface. At the end of each ethanol wash, the A-CNCs complexed with Ag^+ ions and the residual ethanol were separated and two drops of hydrazine were added to the supernatant to check the absence of free Ag^+ ions. The final product was recovered by centrifugation and finally washed in ethanol and water (5000 rpm for 5 min in ethanol and 11000 rpm for 5 min in water). The CNC concentration in the final

suspension was 10 g/L. A schematic representation of the experimental protocol is reported in Figure S1a. The samples were stored at 4°C in the dark.

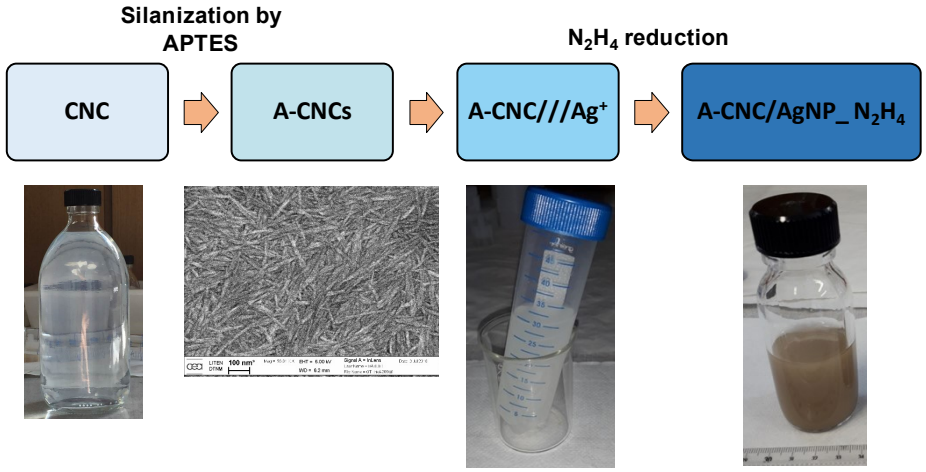
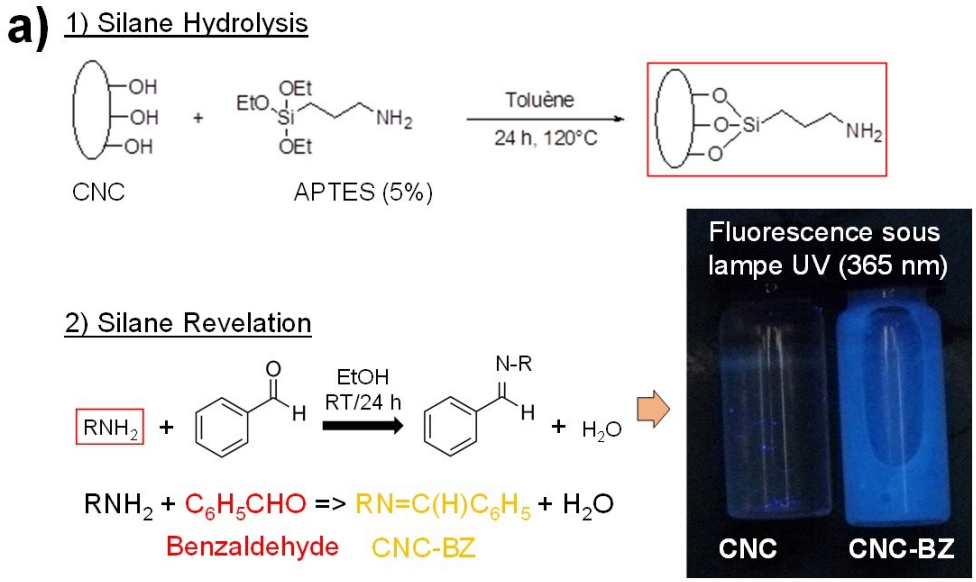


Figure S1.1 Schematic representation of the experimental protocol for the synthesis of A-CNC/AgNP_N_2H_4 hybrids (i.e., H2 hybrid).

Photoluminescence spectra for surface modification of CNC by APTES. To verify the effective surface modification of CNC by APTES, photoluminescence measurements were performed on A-CNC sample. The photoluminescence spectra were recorded at room temperature with a double grating excitation and emission spectrometers (Fluorolog-3 model FL3-22, Horiba Jobin Yvon-Spex) coupled to a R928 Hamamatsu photomultiplier. The excitation sources were a Xe arc-lamp (450 W).

To reveal the presence of amino groups grafted onto the nanocellulose provided by the silanol modification, the further product was reacted with benzaldehyde in ethanol and stirred at room temperature for 24 h to form the aromatic imine analogue, as depicted in figure S2a. The new produced species is exhibiting strong fluorescence upon excitation at 375 nm, as reported in the emission-excitation spectra for the A-CNC (Figure S2b). Untreated nanocellulose is not showing such a strong fluorescent pic. Therefore, the latter photoluminescent measurements provide information regarding firstly, the reactivity of amino moieties provided by the modified nanocellulose with benzaldehyde, and secondly, that the silanol modification has been successful. The fluorescence emission observed at 507 nm under excitation at 375 nm results from the increase of the conjugated system anchored onto the nanocellulose. The observation of such fluorescent phenomenon could simply be detected under UV lamp irradiation (excitation at 365 nm).



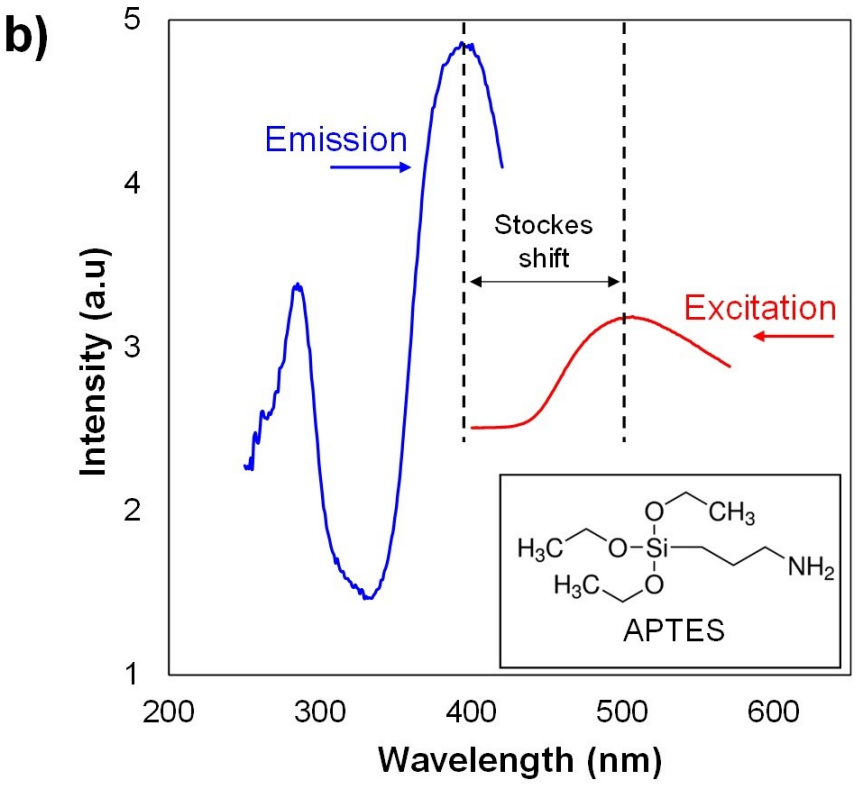


Figure S2 a) Silanol modification of CNCs with benzaldehyde in ethanol; b) Emission-excitation spectra for the A-CNC

S2 - Detailed syntheses of H3 hybrids

Synthesis of H3 hybrid with AgBr. The experimental protocol for the synthesis of this hybrid system was adapted from the one proposed by Sambhy et al.[21] The CNCs (10 g, 0.062 mol) were surface-functionalized with pyridyl chelating groups by silanization, working with 2-(4-pyridylethyl)triethoxysilane (0.831 g, 5% molar with respect to CNC, 3.08 10^{-3} mol). Such surface modification was performed in toluene (300 mL) at reflux, under stirring at 120 °C for 24 h. The functionalized CNCs in suspension, named as P-CNCs, were centrifuged (5000 rpm for 10 min) and washed in ethanol and deionized water twice. The resulting P-CNCs were resuspended in nitromethane (200 mL) with 1-bromopentane (0.466 g, 0.38 mL, 5% molar with respect to CNC, 3.08 10^{-3} mol) and placed under stirring at 60 °C for 24 h to produce de quaternized analogue species. The resulting P^+-CNCs suspension was then centrifuged (5000 rpm for 10 min) and washed in ethanol and deionized water twice. Then, 10 g of P^+-CNCs were dispersed in nitromethane (100 mL). An amount of 0.452 g of $AgNO_3$ was dissolved in 5 mL of 1:1 dimethylsulfoxide/nitromethane mixture (2.66 mmol). Both the P^+-CNCs and the $AgNO_3$ solutions were cooled to 0 °C in an ice bath. The $AgNO_3$ solution was then added dropwise to the stirring P^+-CNCs solution for 15 min. The mixture was stirred for 1 h at room temperature to form AgBr/CNC complex species. The resulting hybrid suspension was centrifuged (5000 rpm for 10 min) and washed in diethylether, ethanol and finally in deionized water twice. The final hybrid sample was stored in the dark at 4°C.

S3 - Characterization of CNC/AgNP hybrids

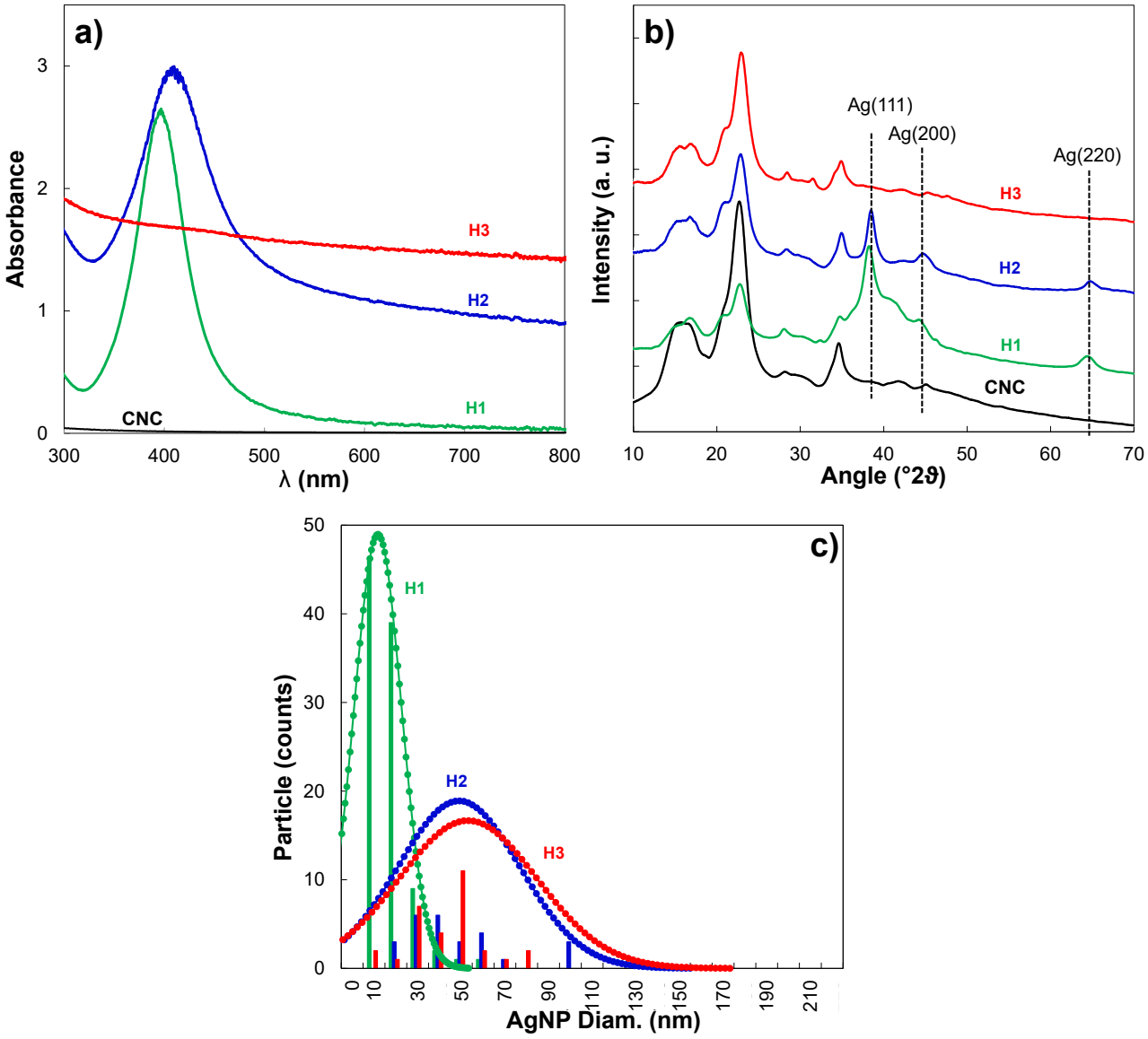


Figure S3. a) UV-Vis spectra of diluted hybrid suspensions; b) XRD diffractograms of native CNC compared to the H1, H2 and H3 powder hybrid systems; and c) Size distributions of AgNP Feret's diameter fixed on CNC obtained using ImageJ software from analysis of images acquired by scanning transmission electron microscopy.

S4. Biocidal efficiency of paints

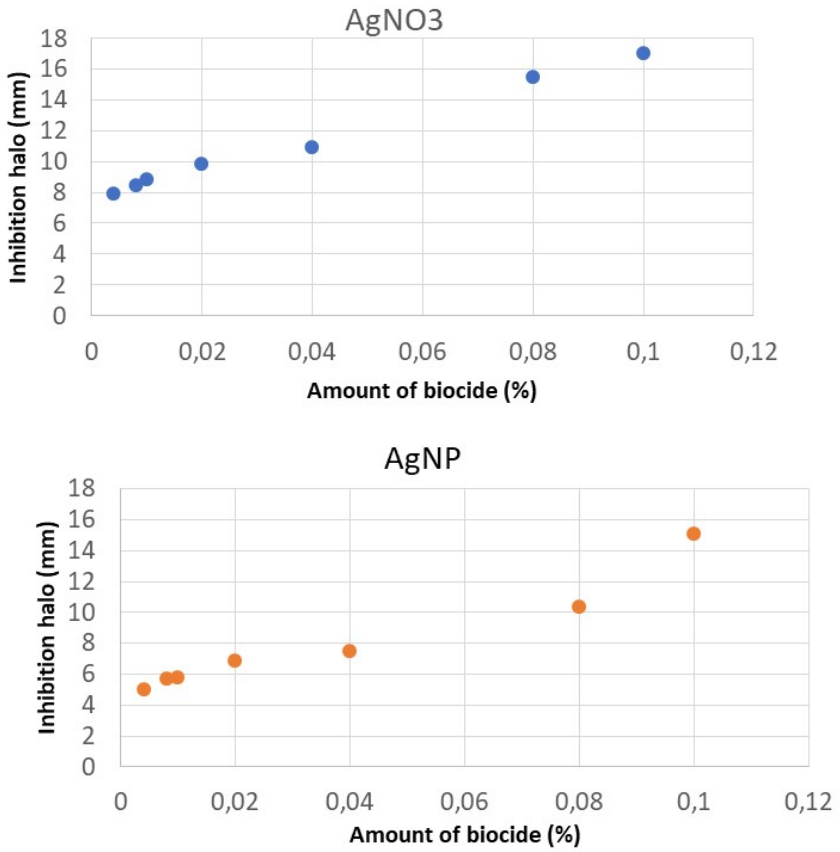


Figure S4.1: Reference of the biocide activity tests. The dose response assayed with a range of AgNPs and AgNO3 from 0.004% to 0.1 %

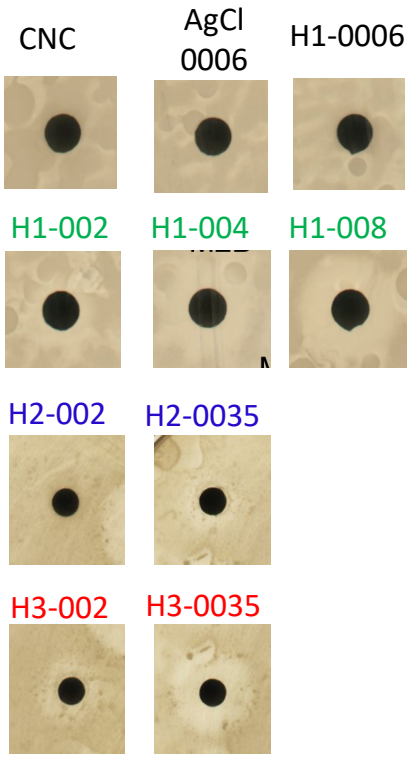


Figure S4.2. Diffusion disk images of paints formulates with different CNC/AgNP hybrid systems at various Ag contents.

S5. SEM-EDS characterization on paints

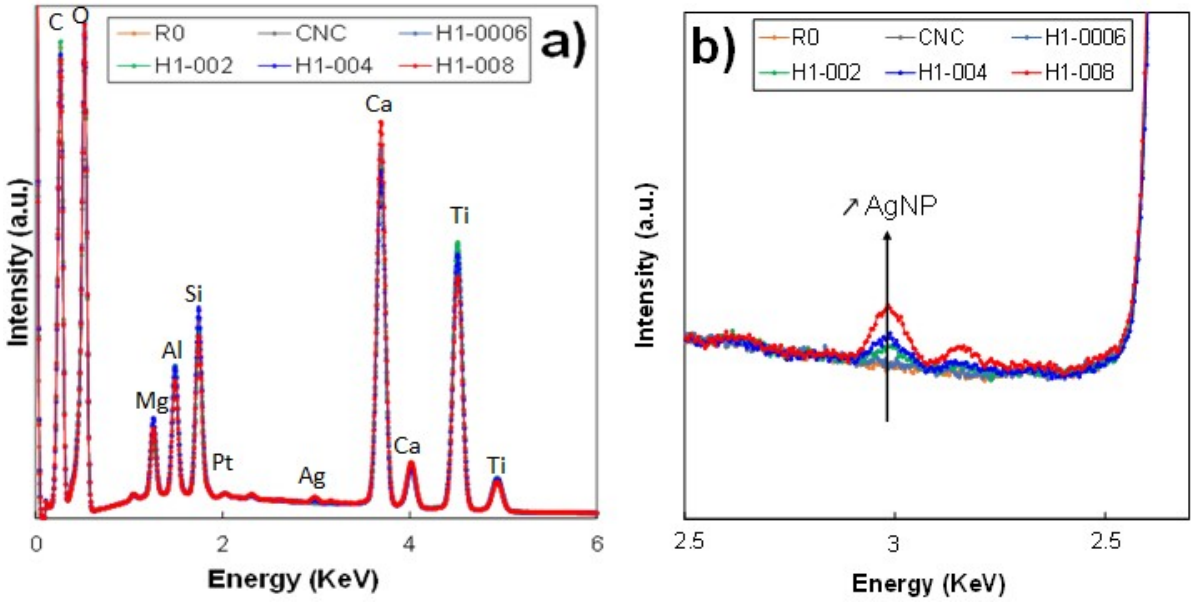


Figure S5. a) SEM-EDS measurements on paint formulated at various Ag content using hybrid H1. b) Increase of intensity of the EDS characteristic peak of silver with the increase of the AgNP content in the dry-state paint.

S6. Photographs of dry paints before and after artificial weathering

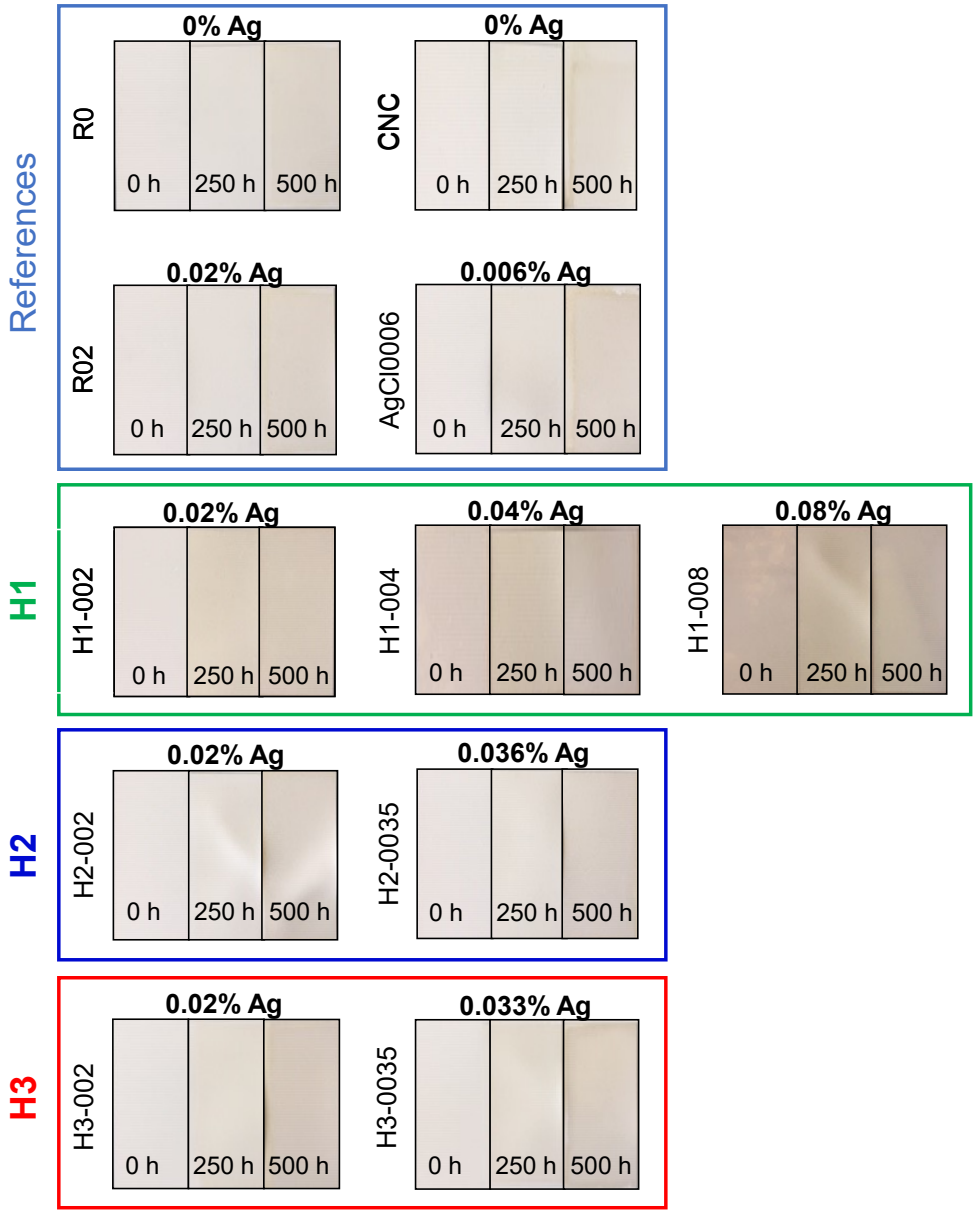


Figure S6. Pictures of paints without biocide (R0), paints with CNCs, paints with organic commercial biocide (R02), paints with commercial AgCl biocide (AgCl0006), paints with H1 hybrid (H1-002, H1-004 and H1-008), paints with H2 hybrid (H2-002 and H2-0035) and paints with H3 hybrid (H3-002 and H2-0035) before and after 250 hours and 500 hours of exposure to artificial weathering.

S7: EDX analysis obtained from SEM images

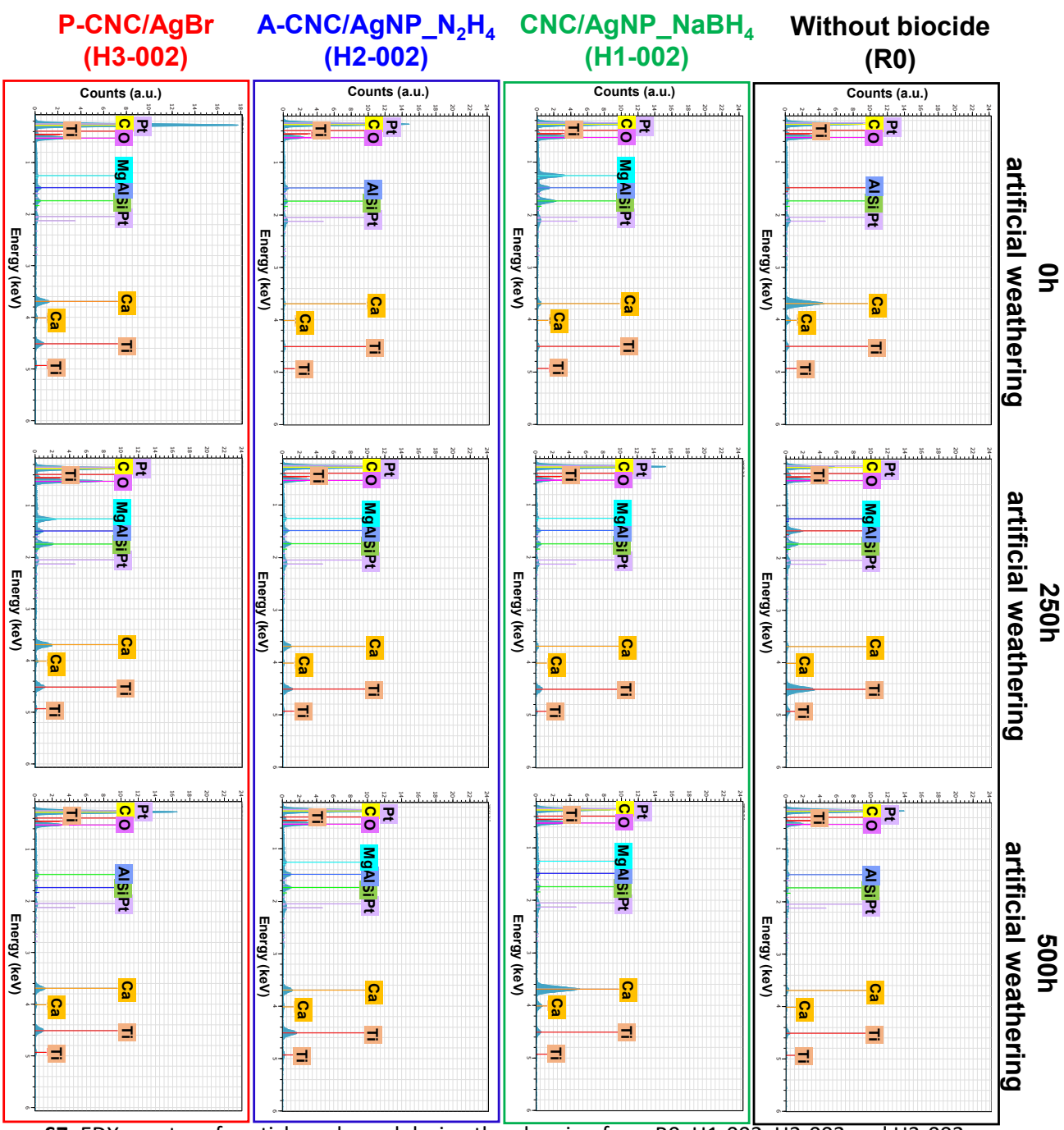


Figure S7. EDX spectra of particles released during the abrasion form R0, H1-002, H2-002 and H3-002 paints before artificial weathering and after 250 and 500 hours of UV exposure.

S8: Potential pulmonary impact of abraded paints particles and NP

Detailled materials and methods

Cell culture. The human type II alveolar epithelial cell line A549 was obtained from ATCC and cultivated in RPMI 1640 GlutaMAX$^{(TM)}$ medium (Gibco, 61870) supplemented with 10% Fetal Bovine Serum (FBS) decomplemented (Gibco, 10270-106) and 1% penicillin-streptomycin (Gibco, 15140-122) (cell culture medium) at 37 °C in a humidified atmosphere containing 5% CO_2 (Sanyo-18AIC). A549 cells were seeded in 75 cm^2 tissue culture flasks (Falcon, 353136), with 10^5-10^6 cells/flask. At 90% confluency, A549 cells were trypsinized (Gibco, 25300), and seeded in 96-well plates black/clear (Falcon #353219) with 25 600 cells/well, (250 µL of culture medium/well) for exposures. Cells were grown for 24h before exposure to suspensions. Cells were exposed for 24h before assays. After exposure cell culture media were harvested for further analysis.

Cytotoxicity. Cytotoxicity was assessed with PrestoBlue™ Cell Viability Reagent (Invitrogen, A13262). Resazurin, is a cell-permeable nontoxic active ingredient. On entering live cells, the cellular reducing environment reduces the blue and non-fluorescent resazurin into red and highly fluorescent resorufin. Viable cells continuously convert resazurin to resorufin, increasing the overall fluorescence and color of the media surrounding the cells.

Wells are emptied and 100 µL of PrestoBlue reagent 10% is added per well. After 30 minutes at 37°C, fluorescence is analyzed (excitation à 555 nm; emission à 585 nm) with absorbance-based plate reader (Infinite M200, Tecan). Percentage of viability is calculated with the fluorescence signal ratio between exposed and unexposed cells.

Oxidative stress. Oxidative stress was assessed with CellRox® Green Reagent (Invitrogen, C10444), which binds to DNA upon oxidation and is designed to measure reactive oxygen species (ROS) in live cells. These cell-permeable reagents exhibit strong fluorogenic signal.

Two microliters of CellRox et 2,5 mM are added to each well. After 30 minutes at 37°C, wells are washed with l'HBSS 1x +/+ (Gibco, 14025-050) and fluorescence is measured (excitation à 485 nm; emission à 520 nm) with a plate reader (Infinite M200, Tecan). Oxidative stress is calculated with the fluorescence signal ration between exposed and unexposed cells. Cells exposed to 1 mM TBHP (Hydroperoxyde de tert-butyle) was used as a chemical positive control, and TiO_2 P25 NP at 100 µg/mL as a NP positive control.

Pro-inflammatory cytokines release in cell culture media. Pro-inflammatory cytokines, Il-1β, IL-6, IL-8 and TNF-α releases were measured in 50 µL cell culture media using a commercially available ELISA multiplex kit (Mesoscale discovery, V-PLEX Human Proinflammatory Panel I 4-Plex, K15053D-2) and a multiplex reader (Mesoscale discovery, Sector Imager 24000) according to supplier recommendations. Cells exposed to 20 µg/mL Lipopolysaccharides (LPS, Sigma-Aldrich, L2880) were used as a positive inflammatory control.

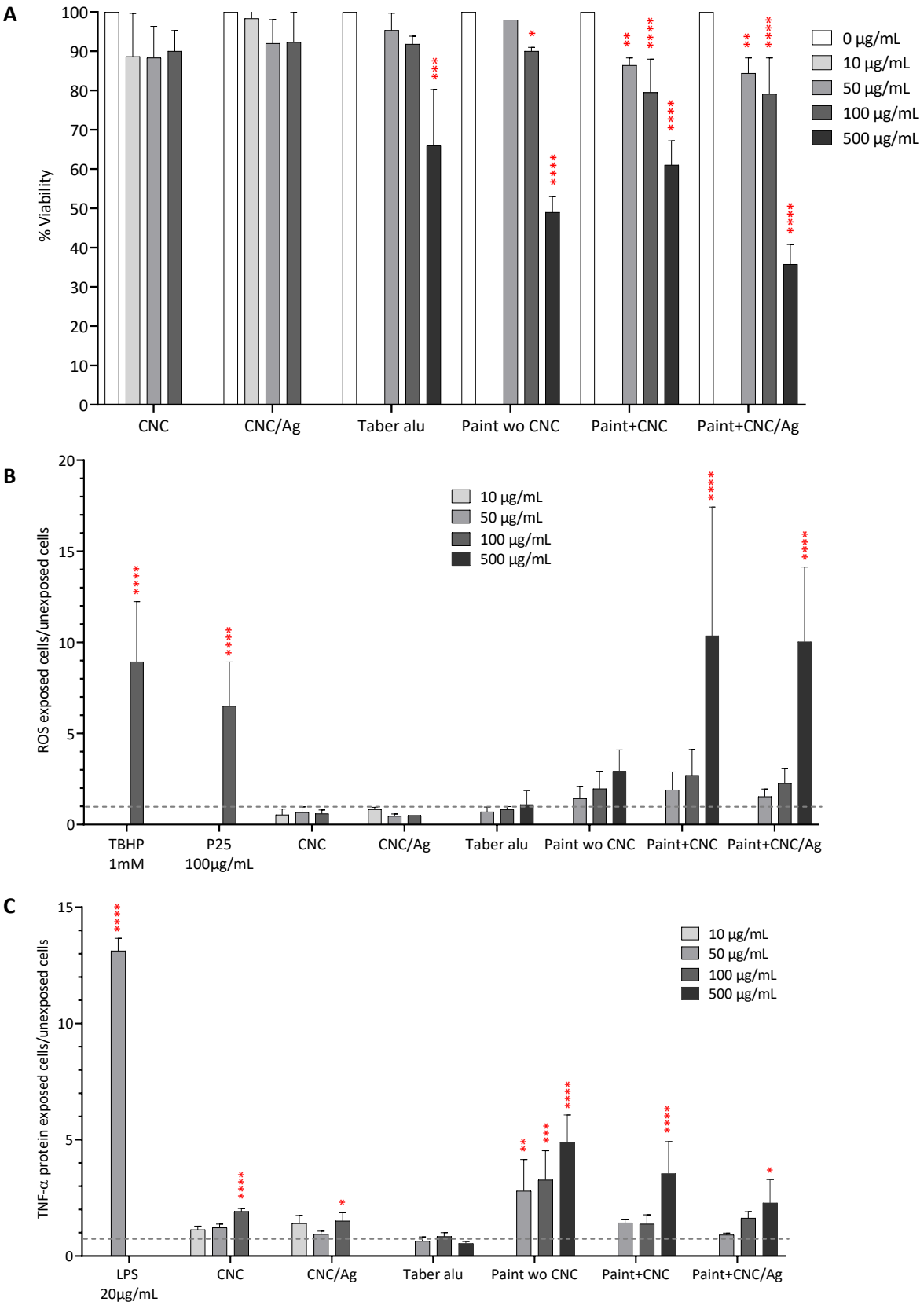

A
% Viability
0 µg/mL
10 µg/mL
50 µg/mL
100 µg/mL
500 µg/mL
CNC
CNC/Ag
Taber alu
Paint wo CNC
Paint+CNC
Paint+CNC/Ag
B
ROS exposed cells/unexposed cells
TBHP 1mM
P25 100µg/mL
C
TNF-α protein exposed cells/unexposed cells
LPS 20µg/mL

Figure S8. A) Mean percentage of cell viability (exposed cells/unexposed cells) with Blue Alamar assay + standard deviation (SD). B) Reactive Oxygen Species generation assessed with CellRox probe, expressed as mean ratios of exposed cells to unexposed cells + SD. C) TNF-α protein expression ratio from exposed cells to unexposed cells + SD. Statistical analysis were performed with 2 ways ANOVA and a Dunnett post-hoc. $p < 0.05$ is *, $p > 0.01$ is **, $p < 0.001$ is **** and $p < 0.0001$ is ****. CNC: cellulose nanocrystals, CNC/Ag: CNC hydride with silver, Taber alu: substrate for abrasion, Paint wo CNC: paint without CNC, Paint+CNC: paint with CNC, Paint+CNC/Ag: paint with hydrids CNC/Ag, TBHB: tert-butyl hydroperoxide as a positive control for ROS generation, P25 is TiO2 nanoparticles as a positive nanoparticle control for ROS generation, LPS: Lipopolysaccharide as a positive control for inflammation.