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End-of-life incineration of nanocomposites: new insights on nanofillers partitioning into by-products and biological outcomes of airborne emission and residual ash

J. Pourchez[1*], C. Chivas-Joly[2], C. Longuet[3], L. Leclerc[1], G. Sarry[1], and J-M. Lopez-Cuesta[3]

[1] Mines Saint-Etienne, Univ Lyon, Univ Jean Monnet, INSERM, U 1059 Sainbiose, Centre CIS, F - 42023 Saint-Etienne France

[2] LNE, Nanocomposites and Nanometrology, 29 Avenue Roger Hennequin, 78197 Trappes Cedex, France

[3] C2MA, Ecole des Mines d'Alès, 6 Avenue de Clavières, 30319 Alès Cedex, France

* Corresponding author: Jérémie POURCHEZ

École Nationale Supérieure des Mines de Saint-Etienne

158 cours Fauriel, CS 62362

42023 Saint-Etienne Cedex 2. FRANCE.

Email address: pourchez@emse.fr - Telephone number: +33477420180

Supplementary Information File

Part 1 – detailed experimental protocol for the physico-chemical characterization

SEM measurements

SEM images have been performed with a Zeiss ULTRA-Plus equipped of a Field Emission Gun (FEG) microscope and in-Lens SE detector. All images have been carried out through secondary electrons collected by InLens detector. From images, the nanoparticle area-equivalent diameter, $D_{area\text{-}eq.}$, is determined and this measure and is defined as the diameter of a sphere that would have a projected surface similar to the projected image of nanoparticle to be measured.

DLS measurements

All materials were received in powder form as agglomerated nanoparticles. Nanoparticles powder were dispersed in deionized water (MilliQ) and characterized by DLS measurement. All dispersions were prepared with a final concentration of 120 µg/mL and sonicated to break agglomerated nanoparticles. The hydrodynamic diameter of nanoparticles in each suspension was measured by Dynamic Light Scattering (DLS) with a Zetasizer Nano ZS from Malvern equipped with a Helium-Neon laser (4 mW - 632.8 nm). DLS was used to obtain information about the hydrodynamic diameter of nanoparticles, the average size distribution (Z-average) and the polydispersity index (PDI). Around 250 µL of sample were deposited onto the cell as dispersion and were irradiated by a laser. Each acquisition was processed by cumulants method.

Zeta potential measurements

The optimal dispersion of nanoparticles on a substrate depends on zeta potential of the initial suspension. The nanoparticles are well dispersed when the suspension is stable and then absolute value of the zeta potential is higher than 30 mV. For each sample, the measurements of zeta potential as a function of pH were carried out by keeping constant ionic strength with sodium perchlorate solution, perchloric acid and sodium hydroxide of concentration 0.1 mol/L.

AFM measurements

AFM images were collected in air using silicon cantilevers from nanosensors operating in intermittent contact mode (cantilevers of 125 µm in length, resonance frequency of 204-497 kHz, spring constant of 10-130 N/m, and a nominal tip radius of curvature of 5-10 nm). Images were obtained with a specific discrete scanning mode: for each image pixel, the stage move to the targeted position, it waits a very short time for setup stabilization (few milliseconds to tens of milliseconds). Then, the AFM controller acquires the topographical and phase signals at high frequency (about 250 kHz).

Chemical analysis

The chemical analysis of the impurities was determined by inductively coupled plasma spectroscopy (ICP-AES, Jobin-Yvon Activa-S coupled with Jobin Yvon AS 421 autosampler, Horiba, Palaiseau, France). The mineralization consisted in suspending 250 mg of powder in 10 mL of hydrochloric acid (HCl 6M), the mix was agitated and heated for approximately 8h at 90 °C. After complete evaporation, samples were diluted in 2M HCl before ICP analyses of the amount of Al, Fe, Na and Si using adequate controls.

X-Ray diffraction

X-Ray Diffraction (XRD) analyses of nanoparticles were performed on a Bruker X-ray diffractometer using Cu Kα radiation.

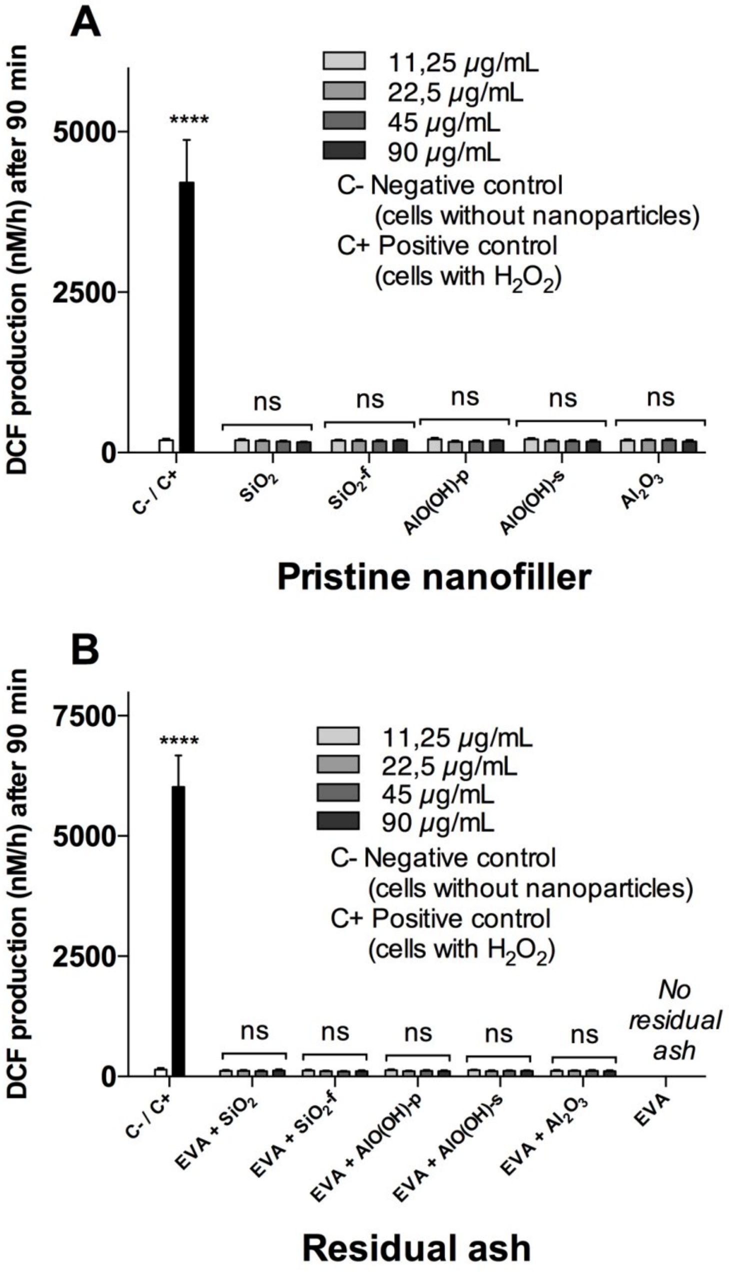
BET measurements (Specific surface area)

The specific surface area was measured by nitrogen absorption, performed on a Micromeritics ASAP2020 system. The powdered samples were put into the sample holder (here 0.5g) and heated to 250 °C in vacuum for at least 16 h until constant weights.

Part 2 – Results on the oxidative stress production

The oxidative stress was negligible after 90 min but also after 24h of contact between cells and the pristine nanofillers (Figures SI-1A and SI-2A), but also for the contact with residual ash (Figures SI-1B and SI-2B) and PM in aerosols (Figures SI-1C and SI-2C). For this biological outcome, no significant difference was noticed between all the samples tested (pristine nanofillers and incineration by-products) compared to the negative control (*i.e.* macrophages incubated without nanoparticles).

Figure SI-1: Reactive oxygen species generation (expressed as a function of DCF production) by macrophages after a 90 min exposure to pristine nanofiller (graph A), residual ash (graph B) or fly ash (graph C). Negative control (noted C-): cells without nanoparticles. Positive control (noted C+): cells with H_2O_2. Significantly different from negative control ****: $p<0.0001$, ns: not significantly different ($p>0.05$).



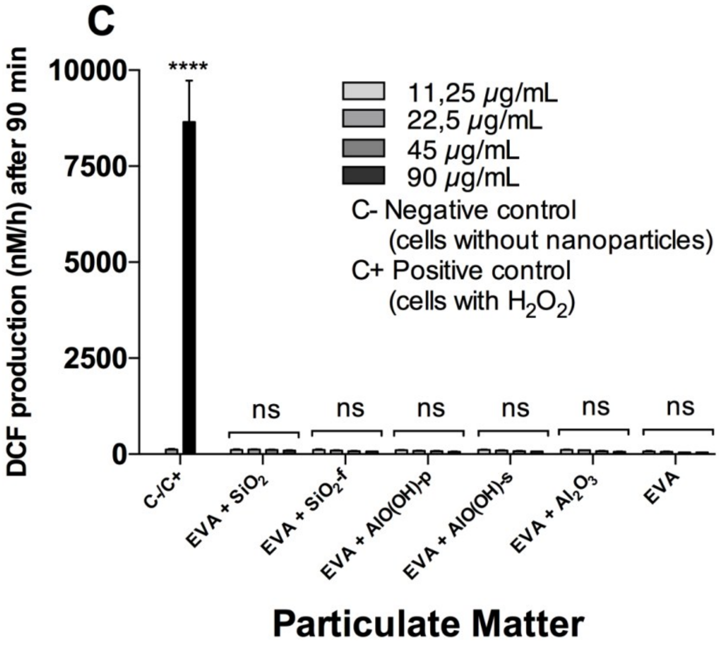
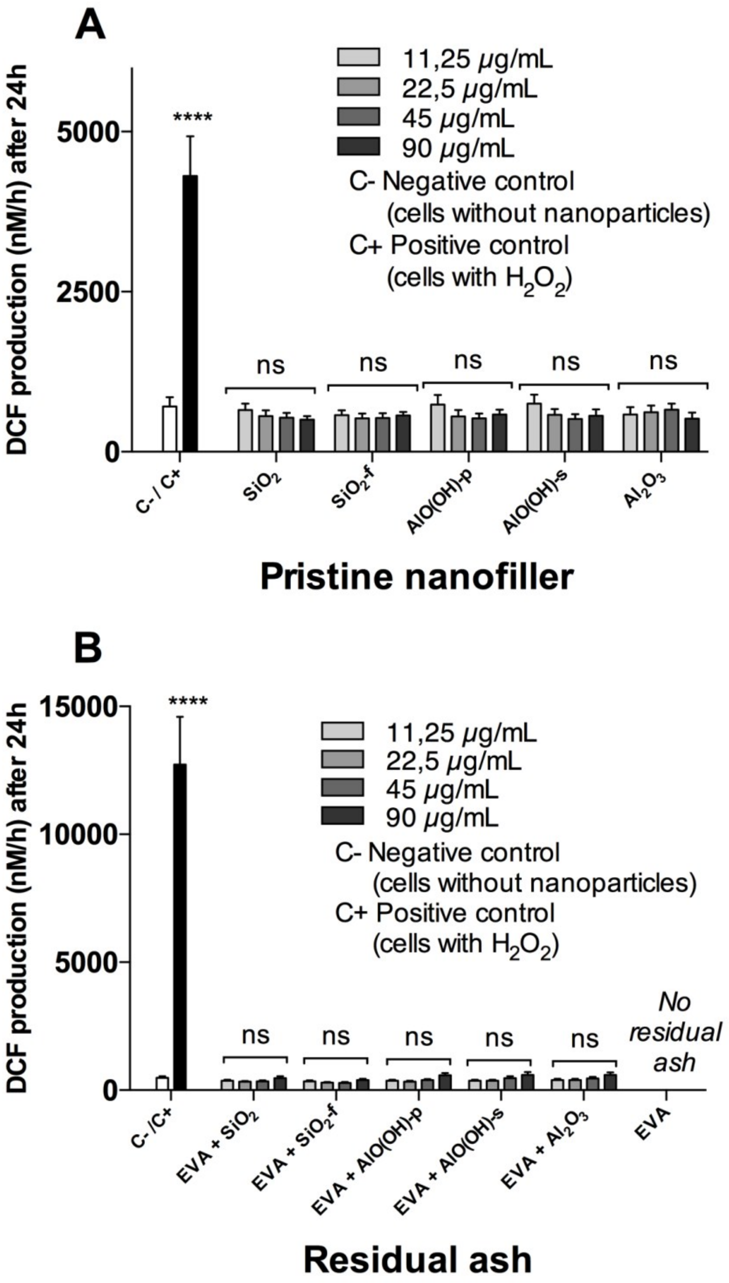
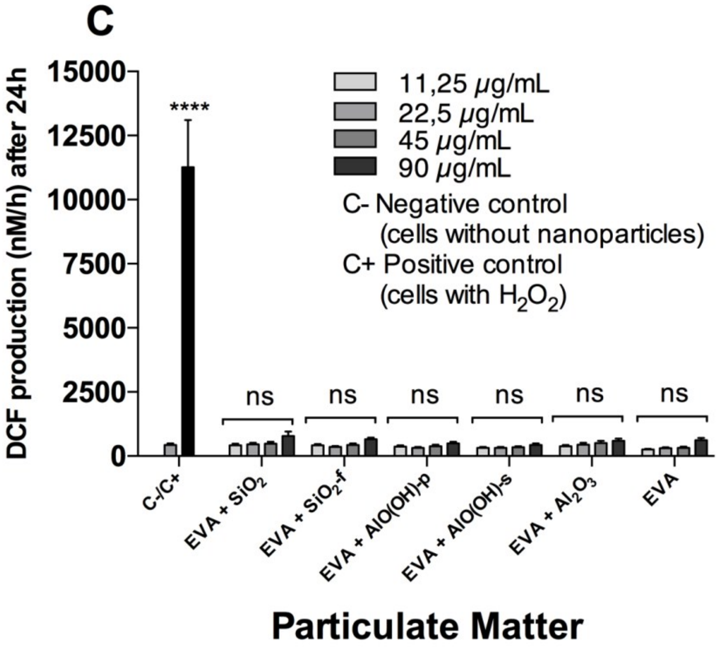

C
DCF production (nM/h) after 90 min
10000
7500
5000
2500
0
****
11,25 μg/mL
22,5 μg/mL
45 μg/mL
90 μg/mL
C- Negative control
(cells without nanoparticles)
C+ Positive control
(cells with H2O2)
ns
ns
ns
ns
ns
ns
C-/C+
EVA + SiO2
EVA + SiO2-f
EVA + AlO(OH)-p
EVA + AlO(OH)-s
EVA + Al2O3
EVA
Particulate Matter

Figure SI-2: Reactive oxygen species generation (expressed as a function of DCF production) by macrophages after a 24 hours exposure to to pristine nanofiller (graph A), residual ash (graph B) or fly ash (graph C). Negative control (noted C-): cells without nanoparticles. Positive control (noted C+): cells with H_2O_2. Significantly different from negative control ****: p<0.0001, ns: not significantly different (p>0.05).




C
DCF production (nM/h) after 24h
15000
12500
10000
7500
5000
2500
0
****
ns
ns
ns
ns
ns
ns
11,25 μg/mL
22,5 μg/mL
45 μg/mL
90 μg/mL
C- Negative control
(cells without nanoparticles)
C+ Positive control
(cells with H2O2)
C-/C+
EVA + SiO2
EVA + SiO2-f
EVA + AlO(OH)-p
EVA + AlO(OH)-s
EVA + Al2O3
EVA
Particulate Matter