Description of document:	Consumer Product Safety Commission (CPSC) Study of silver nanowire release from touchscreen displays 2023(?)
Requested date:	2019
Release date:	31-May-2023
Posted date:	30-June-2025
Source of document:	FOIA Request U.S. Consumer Product Safety Commission 4330 East West Highway Bethesda, MD 20814 Fax: 301-504-0127 E-mail: CPSCFOIARequests@cpsc.gov CPSC FOIA e-FOIA Public Access Link Website FOIA.gov

United States
Consumer Product Safety Commission

May 31, 2023

Via WatchDox

RE: Freedom of Information Act Request #19-F-00108: A copy of CPSC reports and memos pertaining to the CPSC study/review of silver nanowire release from touchscreen displays. This also relates to contracts: CPSC-Q-16-0084 or CPSCS160060.

Thank you for your Freedom of Information Act (FOIA) request seeking the above-referenced information from the U.S. Consumer Product Safety Commission (CPSC). In response to your request, please find copies of the CPSC's contracts and memorandums are enclosed.

CPSC considered the foreseeable harm standard when reviewing these records.

If you need any further assistance, or you would like to discuss any aspect of your request, please contact me, or one of CPSC's FOIA Public Liaisons, Korinne Super (ksuper@cpsc.gov) or Cooper Gerus (cgerus@cpsc.gov), via email or at 1-800-638-2772.

Fees. We are not charging you fees in this instance to cover the costs to the CPSC in processing this request, performing the file searches, and preparing the information. Thank you for your interest in consumer product safety.

Sincerely,

ABIOYE OYEWOLE

Digitally signed by ABIOYE OYEWOLE
Date: 2023.05.31 09:53:07 -04'00'

Abioye Oyewole for Michelle Pitts
Chief FOIA Officer
Office of the General Counsel
Division of Information Access
mpitts@cpsc.gov

SIINN Nanowires – Final report (AWD00707 – P0018495)

Executive Summary

Although there has been a substantial effort to understand the human and environmental impacts of engineered nanomaterials, there is a serious lack of knowledge about the specific hazards of silver nanowires. Silver nanowires are high aspect ratio (length to width ratio) nanomaterials, and this raises concern about their unique properties and their interactions with living systems. This project investigated the exposure and toxicity of silver nanowires, with a specific focus on consumer protection. Together, the researchers in the consortium will investigate the release of silver nanowires from touchscreen displays, the toxicity of the silver nanowires to human and ecosystem health, and the mechanisms by which silver nanowires induce toxicity. A goal of this research is to understand the intrinsic characteristics that influence silver nanowire fate and effects in organisms and ecosystems, so that silver nanowires can be synthesized in ways that minimize harm to people and the environment. Results of this work indicate that diameter is an important characteristic that affects silver nanowire toxicity to cells, where thinner diameter nanowires are able to be crumpled up inside the cell and easier to process and eliminate. In contrast, thicker diameter nanowires were found to maintain their rigid structure after cellular uptake and subsequently induced toxicity to the cell resulting in apoptosis. The finding that diameter affects silver nanowire toxicity has not been previously reported, and this finding is relevant to making silver nanowire-enabled technologies safer for people and the environment.

Description of the project and its objectives

The SIINN Silver Nanowires Consortium was an international research collaboration with laboratories located in France and the United States. The overarching goal of this project was to understand how different silver nanowire dimensions influence the toxicity of silver nanowires to people and organisms and find ways to make silver nanowire technology safer for people and organisms. Collaborators in the project worked together to coordinate silver nanowire synthesis and characterization, human and environmental toxicity testing, and silver nanowire release from products and recycling. Using the exact same silver nanowires for experimentation across all

laboratories allows for better comparison of experimental results within the consortium, and coordinated work across laboratories improves our understanding of silver nanowire toxicity as it applies to real world exposure scenarios and to international regulations and perspectives.

The specific objective of the project were:

Task 1 (University of Lille): Evaluating and minimizing toxicant release nanowire-enabled products.

Nanowire-enabled touchscreens will be exposed to aqueous solutions, stimulated by solar radiation and mechanical stresses, and the release of ions or other potential toxicants will be measured. The researcher will develop quantitative criteria of toxicant release from commercial devices that are under controlled use or aging conditions. The experiments for the release and recycling of silver nanowires will be conducted in coordination with the human and environmental studies, and this will maximize the ability of the research to inform silver nanowire synthesis and reduce potential hazards of silver nanowires to people and the environment.

Task 2 (University of Grenoble – Alps): Silver nanowire dermal toxicity

The aim of this activity is to assess the cellular toxicity of silver nanowires with well-controlled physical and chemical properties including different lengths, coatings and morphology. Silver nanowire toxicity will be assessed in cultured human skin epithelial cells along multiple axes of materials properties: stability with respect to silver ion (Ag+) release, diameter, length, and surface chemistry. In addition, the researchers will identify silver nanowires with minimal cytotoxicity as defined by established assays for cell viability and toxicity.

Task 3 (University of Florida): Silver nanowire environmental toxicity

Silver nanowires released from products may make their way to the environment. The potential hazard to environmentally relevant organisms will be evaluated through cellular and whole organism silver nanowire toxicity studies, bioaccumulation and trophic transfer of silver nanowires. These studies in conjunction with Task 2 will enable selection and development of nanowires with less potential for harm to people or the environment.

A description of the results

Previous research on high aspect ratio nanomaterials has focused on the impact of material length on toxicity, but the Silver Nanowires Consortium has shown that nanowire width is also a relevant factor that influences silver nanowire toxicity. The result that thinner silver nanowires are less toxic to cells was observed across multiple cell lines, murine fibroblasts and rainbow trout gill (RTgillW1) and gut cell lines (RTgutGC). These toxicity patterns have been observed across multiple cell lines. The finding that diameter affects silver nanowire toxicity has not been previously reported, and this finding is relevant to making silver nanowire-enabled technologies safer for people and the environment.

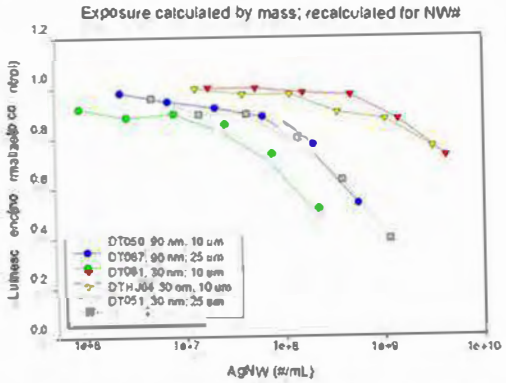


Figure 1: Cell viability of rainbow trout gill cells exposed to silver nanowires of varying length and diameter

High resolution XRF and tomographic imaging of murine fibroblasts, performed at the University of Grenoble Alps, revealed that silver nanowires with a thinner diameter become crumpled after they are internalized into the cell, whereas silver nanowires with a thicker diameter maintain their rigid structure inside the cell. Crumpled (thin diameter) and rigid (thick diameter) silver nanowires undergo very different cellular processing pathways once they are inside the cell. Thin and crumpled silver nanowires are more easily processed by the cell, while thick and rigid silver nanowires can puncture internal cellular compartments and react with intracellular proteins and acids to induce toxicity to the cell (figure 2a and 2b).

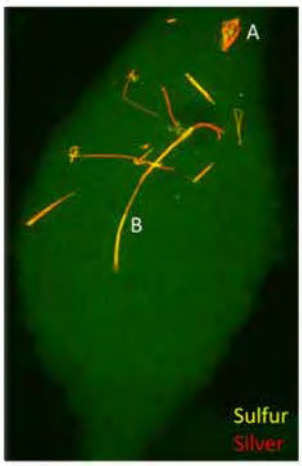


Figure 2: X ray tomographic image of murine fibroblast exposed to thick and thin silver nanowires. Nanowires are shown to be A) crumpled inside cellular compartments and B) protruding from cellular compartments.

The project was impacted by COVID19, and the environmental team at the University of Florida were not able to complete whole organism studies.

Impact, especially on EHS related challenges

New fundamental insights were gained into nanowire properties that influence biological interactions, and this will improve our ability to minimize potential hazards of silver nanowires to living systems. New insights were also be gained regarding testing concepts for minimizing toxicity through information and material exchange amongst the consortium participants. Results of this work could help emerging nanowire-enabled products to be more safe and sustainable for people and the environment.

List of scientific publications for the project duration

Lehmann, S.G., et al., *Crumpling of silver nanowires by endolysosomes strongly reduces toxicity.* Proc Natl Acad Sci U S A, 2019. **116**(30): p. 14893-14898.

Omaña-Sanz, B., et al., *An electrochemical method to rapidly assess the environmental risk of silver release from nanowire transparent conductive films.* NanoImpact, 2020. **18**: p. 100217.

Toybou, D., et al., *A toxicology-informed, safer by design approach for the fabrication of transparent electrodes based on silver nanowires.* Environmental Science: Nano, 2019. **6**(2): p. 684-694.

Environmental Science Nano

PAPER

View Article Online
View Journal | View Issue

Cite this: Environ. Sci.: Nano, 2019, 6, 684

A toxicology-informed, safer by design approach for the fabrication of transparent electrodes based on silver nanowires

Djadidi Toybou,[ab] Caroline Celle,[a] Catherine Aude-Garcia,[c] Thierry Rabilloud *[c] and Jean-Pierre Simonato *[a]

Received 14th August 2018,
Accepted 10th October 2018

DOI: 10.1039/c8en00890f

rsc.li/es-nano

Fabrication of silver nanowires (AgNWs) with fine and independent control of both the diameter (from 30 to 120 nm) and length (from 5 to 120 μm) by concomitant addition of co-nucleants and temperature control is demonstrated, and used for the preparation of size standards. Percolating random networks were fabricated using these standards and their optoelectronic properties were measured and compared with regard to the nanowire dimensions. The transparent electrodes appear suitable for various applications and exhibit excellent performances (e.g. 16 ohm sq^{-1} at 93% transparency), with haze values varying from 1.6 to 26.2%. Besides, *in vitro* toxicological studies carried out on murine macrophages with the same size standards revealed that AgNWs are weakly toxic (no toxicity observed below 50 μg mL^{-1} Ag), in particular compared to other silver nanoparticles. Short AgNWs (4 μm) appeared to be slightly more toxic than longer AgNWs (10 and 20 μm). Conversely, long AgNWs (20 μm) induced a more prolonged pro-inflammatory response in murine macrophages. These results contribute, in a safer by design approach, to promoting the use of short AgNWs. The global knowledge dealing with the combination of nanowire dimensions associated with optoelectronic performances and related toxicity should encourage the rational use of AgNWs, and guide the choice of the most adequate AgNW dimensions in an integrated approach.

Environmental significance

The use of silver nanowires is a promising alternative for the fabrication of transparent electrodes. They will be increasingly introduced in consumer devices. The relationship between the nanowires' dimensions and their toxicity is still insufficiently studied. We demonstrate a straightforward method to prepare silver nanowires with controlled dimensions. We propose a global approach, starting from tailor-made nanowires, based on a study of both their physical properties when used as random percolating networks, which is of interest from the technological point of view, and their toxicological behaviour toward murine macrophages, which is of interest from the health, safety & environment point of view. These results contribute, in a "safer by design approach", to promoting the use of short AgNWs.

Introduction

The recent development of nanomaterials has generated a wave of hope in many fields. The potential of nanomaterials appears boundless, either due to their intrinsic properties (*e.g.* quantum dots and fullerenes), their additional properties for bulk materials (*e.g.* nanocomposites), or when they are used in the form of assemblies (*e.g.* carbon nanotube based cables). A characteristic and high-potential example of nanomaterial assembly is the fabrication of random networks of metallic nanowires, in particular, silver nanowires (AgNWs).[1–3] Simply put, it is possible to generate random networks of metallic nanowires, above the percolation threshold, which conduct electricity through the nanowire-based metallic lattice and concurrently let the light pass through the empty spaces between the nanowires. This has been widely studied during the last decade and has raised the maturity of this technology up to the industrial level. Many applications using AgNWs have been investigated, including touch screens, light-emitting devices, transparent film heaters and others.[4–12] This system has many advantages such as very low sheet resistance with high transparency, flexibility, and low-cost processing under ambient conditions.

Though many important advances have been realized in this field, some key points remain to be tackled, in particular, toxicity issues for safe industrial use. Since the expected

[a] Univ. Grenoble Alpes, LITEN DTNM, CEA, F-38054 Grenoble, France. E-mail: jean-pierre.simonato@cea.fr
[b] Univ. Grenoble Alpes, ISTERRE, CNRS, F-38000, Grenoble, France
[c] Univ. Grenoble Alpes, Chemistry and Biology of Metals, CEA BIG, CNRS UMR 5249, F-38054 Grenoble, France. E-mail: thierry.rabilloud@cea.fr

properties are application-dependent and rely on the AgNWs' morphology,[13,14] it would be of great interest to be able to tailor the shape of the nanowires in order to obtain optimal optoelectronic performances for each use. Conventional parametric studies[15,16] as well as other more specific methods[17,18] have demonstrated efficient routes for modifying both the length and diameter, but not independently. An ultrasonic method was also proposed to select the desired length by cutting AgNWs; however, this method leads to poor control over size distribution and generates large amounts of by-products.[19]

The fabrication of AgNW size standards would also allow toxicological studies to be performed on definite species, in particular to determine whether the two main dimensions (*i.e.* diameter and length) play a critical role in toxicity. This is of tremendous importance since AgNWs will increasingly be introduced in technological devices and consumer products, and thus data are awaited to assess the real toxicity of these nanomaterials. Although several reports have begun to tackle this topic,[20–26] much more remains to be done.

In this article, we propose a straightforward method to modulate independently the diameter and the length of nanowires in a wide range, allowing us to fabricate size standards, *i.e.* AgNW samples with definite lengths and diameters. It has permitted us to perform a direct comparison of the optoelectronic properties of transparent electrodes made with these different nanowires, and to realize comparative experiments on toxicity with macrophages.

Materials and methods

Synthesis and purification of AgNWs

In a typical synthesis, $AgNO_3$ (0.68 g) was dissolved in EG (40 mL) at a slow stirring rate in a round flask. In another flask, PVP (average mol. wt 40 000, 1.77 g), NaCl, and the co-nucleant were dissolved in EG (80 mL) at 120 °C. The solution was cooled to room temperature and then slowly added to the first flask within 8 min. The mixture was finally heated at the reaction temperature and cooled down at ambient temperature. The purification of the AgNWs was realized by decantation according to a published procedure.[27]

Characterization of the mean dimensions of AgNWs

The morphology analysis of AgNWs was performed using a Leo 1530 SEM. Measurement of the diameter and length dimensions was realized with the ImageJ software. The statistical studies were performed on 100 to 200 nanowires for each sample.

Preparation of AgNW electrodes

The AgNW-based solutions were used for the fabrication of electrodes after dilution in methanol at 0.2 mg mL^{-1} concentration. The silver concentration was measured using an atomic absorption spectrometer (Perkin Elmer AAnalyst 400). The deposition was performed on 2.5 × 2.5 cm^2 heated substrates (70 °C) with an automatic SonoTek spray-coater. The performances of the electrodes were measured after cooling, without any post-treatment.

Performance measurements

Total transmittance and haze values were measured with a UV-visible spectrometer (Varian Cary 5000) equipped with an integrating sphere. The sheet resistance was set as the mean value of at least 5 measurements by using a four-pin probe with a Loresta resistivity meter (EP MCP-T360).

Toxicological assays

Nanomaterials. As control nanomaterials, spherical silver nanoparticles (<100 nm) coated with PVP40 were purchased from Sigma-Aldrich (catalogue number 758329) as a 5 wt% dispersion in ethylene glycol. Working solutions were prepared by dilution in deionized water. The characterization of these nanoparticles has been published previously by some authors of this article.[28] Additional controls consisted of silica-based nanomaterials, either colloidal silica[29] or crystalline silica (reference materials BCR66 and BCR70, purchased from Sigma-Aldrich).

Cell lines. The mouse macrophage cell line RAW 264.7 was obtained from the European Cell Culture Collection (Salisbury, UK). The cells were cultured in RPMI 1640 medium + 10% fetal bovine serum. The cells were seeded at 200 000 cells per ml and harvested at 1 000 000 cells per ml. For treatment with nanomaterials, the cells were seeded at 500 000 cells per ml. They were treated with nanomaterials on the following day and harvested after a further 24 hours in culture.

Neutral red uptake assay. This assay was performed according to a published protocol.[30] Cells grown in 12-well plates and treated or not with nanomaterials were incubated for 1 h with 40 μg mL^{-1} neutral red (final concentration, added from a 100× stock solution in 50% ethanol–water). At the end of the incubation period, the medium was discarded and the cell layer was rinsed twice with PBS (5 min per rinse). The PBS was removed, and 1 mL of elution solution (50% ethanol and 1% acetic acid in water) was added per well. The dye was eluted for 15 min under constant agitation, and the dye concentration was read spectrophotometrically at 540 nm. The results were expressed as % of the control cells (untreated).

Cytokine production and persistence experiments. The tumour necrosis factor (TNF-α), interleukin 6 (IL-6) and monocyte chemoattractant protein-1 (MCP-1) concentrations in the culture supernatants of cells exposed for 24 h to AgNWs and control nanomaterials were measured using the cytometric bead array (CBA) mouse inflammation kit (BD Pharmingen, France) according to the manufacturer's instructions. The measurements were performed on a FACSCalibur flow cytometer and the data were analysed using CellQuest software (Becton Dickinson). Bacterial lipopolysaccharide (LPS) was used as a positive control (200 ng mL^{-1}, 24 h). At the end of the exposure period, the medium was removed and saved

for cytokine measurements. Fresh complete culture medium (without nanomaterials) was added to the cells and left for 36 h. The medium was then removed and saved, and fresh complete culture medium was added for a final 36 h period. Thus for each nanomaterial, 3 cytokine assays were performed, covering the 24 h exposure period and two post-exposure 36 h time windows.

Results and discussion

Controlled synthesis of AgNWs: key factors for independent tuning of length and diameter

The synthesis of AgNWs has been extensively studied since the first report of Xia *et al.*[31] Among different routes to fabricate these nanowires, the most studied process has been the *in situ* reduction of silver salts by the reductive polyol method. This solvothermal process usually includes the use of ethylene glycol as the main solvent, silver nitrate as a cheap silver source, a nucleant with or without co-nucleant(s) to initiate the growth of nanowires and PVP (polyvinylpyrrolidone) as the capping agent which allows orientation of the uniaxial growth.[32] It has already been demonstrated that AgNWs with different lengths and diameters can be obtained "on demand" thanks to the conditions extracted from parametric studies; however, it has not been demonstrated so far that both the diameter and the length could be tuned independently. In other words, modification of the process to adjust one dimension has concomitantly a strong effect on the other dimension (*e.g.* protocol adjustment to modify the mean diameter will strongly impact the mean length of the AgNWs).

It has been previously reported in the literature that the use of a co-nucleant besides NaCl, the molar mass of PVP and synthesis temperature may strongly affect NW morphologies.[33] In particular, halide ions are known to modify the nanowire diameter.[34–36] Bromide ions proved to be efficient in reducing the diameter, but at the same time they also altered the length of AgNWs.[37–39] To begin this study, the influence of F^-, Cl^-, Br^- and I^- as co-nucleants was investigated and compared to a standard synthesis protocol.[40,41]

The impact of each halide ion on the morphology of as-synthesized AgNWs is presented in Fig. 1. Compared to the standard synthesis without a co-nucleant (Fig. 1a, pink cross, 63 ± 10 nm diameter/8 ± 3 μm length), each added halide ion (molar ratio, 1 : 1 NaCl:KX) has a noticeable effect on the final morphology of AgNWs. An exception is the case of iodide-modulated synthesis which leads only to irregular nanoparticles. The results obtained with KBr or NaBr as a co-nucleant appeared very close. To confirm this point, NaCl and KCl were also used as nucleants (standard conditions, 0.7 mmol L^{-1}), and as shown in Fig. 1(a), AgNWs with similar morphologies (51 ± 7 nm diameter and 6 ± 1 μm length) were produced. This means that using KCl as a co-nucleant in an equimolar ratio with NaCl gives the same result as doubling the amount of NaCl; thus alkali counter-cations do not play a significant role in the reaction mechanism. Nevertheless, it must be noticed that both the diameter and length are affected in the same way when the chloride quantity changes, as already reported elsewhere.[42] Fluoride ions (Fig. 1a – blue triangle) increased the length from 8 ± 3 μm to 12 ± 5 μm, and the diameter to 71 ± 10 nm. Whereas the effects of fluoride and iodide ions have not been reported extensively, diameter shrinkage with the use of a bromide-based co-nucleant is in fair agreement with the literature.[37–39,43]

To further investigate the effect of bromide as a co-nucleant, we performed experiments with varying concentrations of KBr, from 0 to 0.7 mmol L^{-1}. The results are presented in Fig. 1b. It appears that the diameter decreases from 63 ± 10 nm down to 20 ± 3 nm with increasing amount of bromide ions, while the length increases from 6 ± 3 to 12 ± 5 μm. Nanowires with 15 nm diameter can also be obtained at higher concentrations of bromide, but the amount of metallic by-products becomes excessive. As shown here, and in agreement with literature reports, adjustment of the KBr

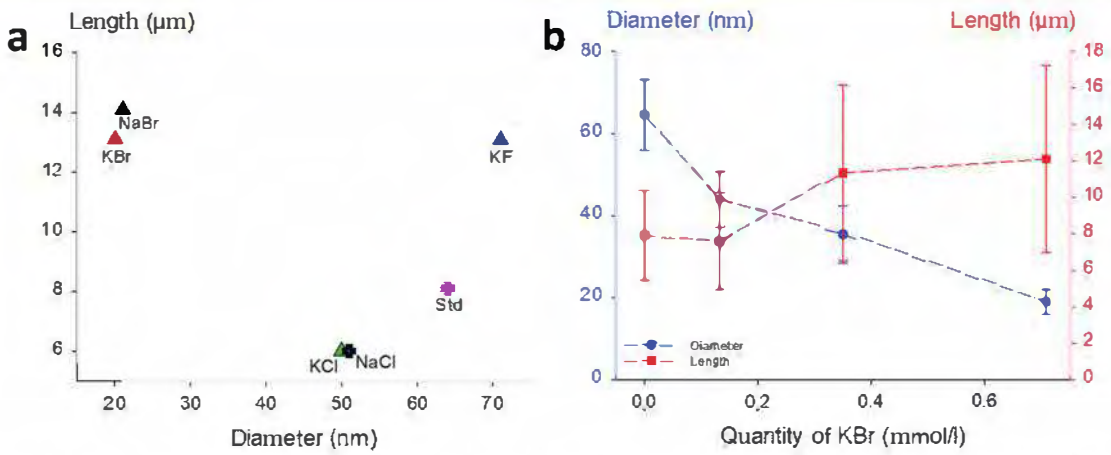


Fig. 1 Influence of several halide ions on the morphology of AgNWs. (a) Lengths of AgNWs synthesized by a standard protocol[41] (pink dot, NaCl as the only nucleant) and modified procedures with additional halide salts as co-nucleants (KBr, NaBr, KCl, NaCl, and KF). (b) Effect of KBr concentration on diameters and lengths of AgNWs.

quantity leads to a fine control of the diameters but changes significantly the lengths.

Thus, in our goal to fabricate "on demand" AgNW size standards with defined lengths and diameters, we carried out experiments by setting the KBr amount at 350 mmol L^{-1} to control the diameter, and we introduced NaCl in various amounts in order to modulate the length. The results plotted in Fig. 2a show that when the chloride concentration was varied between 0.5 and 2.5 mmol L^{-1}, we obtained a collection of AgNWs with lengths ranging from 28 ± 9 down to 4 ± 2 μm. For higher concentrations of chloride, nanoparticles were obtained as the sole product. These results demonstrate that by adequate choice of the nature of co-nucleants and by fine adjustment of their concentrations, the variation of length is actually possible while keeping the diameter almost unchanged (Fig. 2b). This is a straightforward route to select the desired length at constant diameter. The mean length modification can be ascribed to the increased (or decreased) number of seeds, which depends on the molar ratio between chlorine and silver. This is consistent with the report from Buhro and colleague[44] who demonstrated, in the early stage of the polyol synthesis, the impact of chloride concentration on the generation of nucleation sites.

Whereas diameters below 50 nm are desirable for most optoelectronic applications requiring a low haze value, in some specific uses like photovoltaics, larger diameters are expected.[5] In our effort to modulate length and diameter independently, we looked at a protocol to modify the diameter while keeping the length mostly unchanged. It was previously demonstrated by Unalan and colleagues that polyol synthesis carried out at low temperatures generates thick wires (with diameters higher than 300 nm) and that temperature plays a crucial role in the nanowire formation.[15] We used our standard protocol at various temperatures. The results are presented in Fig. 2c and d. We observed that the mean diameter decreased drastically, from 90 ± 10 nm down to 50 ± 8 nm, when the temperature was raised from 150 to 180 °C. At the same time, the mean length of the AgNWs remained

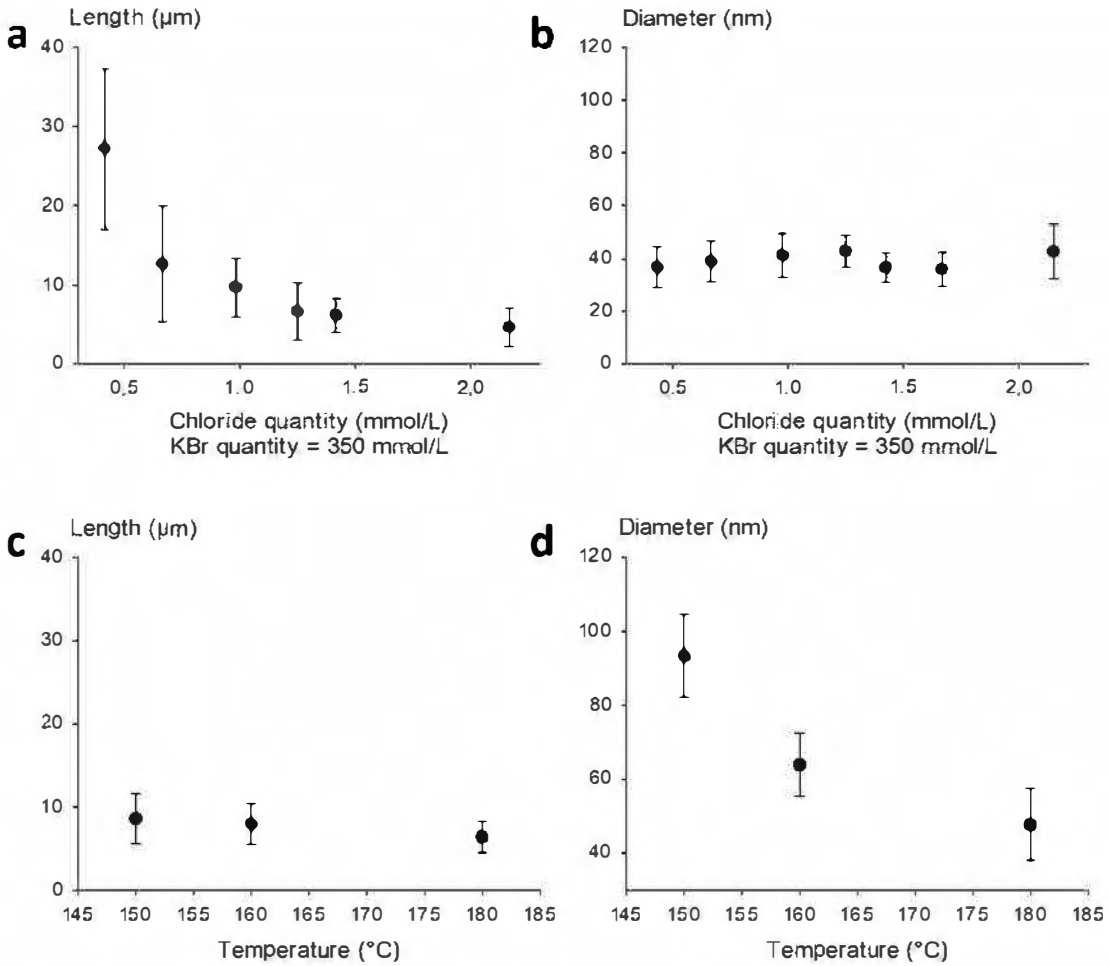


Fig. 2 Tuning of the length of thin AgNWs by NaCl concentration and reaction temperature. (a) Length as a function of the chloride concentration, with a constant KBr concentration of 350 mmol L^{-1}. Increasing the quantity of chloride decreases the mean length of AgNWs. (b) The diameter of AgNWs remains constant around 40 nm while the length decreases from 28 μm to 3 μm. The KBr concentration is set at 350 mmol L^{-1}. (c) The length remains almost constant while the reaction temperature is modified. (d) Diameter tuning by reaction temperature modification, where increasing the temperature induces a diameter decrease.

almost constant. This demonstrates that it is also possible to change the diameter of AgNWs while keeping the length mostly unchanged by selecting the adequate reaction temperature.

Fabrication of AgNW size standards. Thanks to the protocols presented hereinbefore, we were able to prepare different AgNW size standards, as shown in Fig. 3. Combinations of any lengths or diameters in the range of 5–120 μm and 30–120 nm, respectively, were achievable. This gives access to calibrated samples of nanowires. Histograms showing the distribution in diameters of three different samples of 10 μm long AgNWs with various mean diameters are presented in Fig. 3a, and histograms of three different populations with different lengths of 40 nm diameter AgNWs are shown in Fig. 3b. In each case, the overlap between two adjacent statistical populations was calculated to be below 15%. SEM images are also presented to illustrate the major differences obtained between the different samples in Fig. 3c; they show undoubtedly how both diameters and lengths can be tuned according to specific synthetic protocols. The main criteria to tune the synthesis of the AgNW standards are schematized in Fig. 3d. We also verified that the proposed conditions are scalable. We performed experiments using up to 5 L semi-batch reactors, which allowed us to confirm that the synthesis of AgNWs with pre-determined dimensions can be carried out "on demand".

Optoelectrical performances of transparent electrodes: impact of AgNWs' dimensions on haziness. When assembled in the form of random networks above the percolation threshold, it is known that these materials can provide a relevant alternative to TCOs (transparent conductive oxides) for the fabrication of transparent conductive materials. In this case, the properties of interest (*e.g.* transparency and sheet resistance) are measured at the macroscopic scale, but depend strongly on the nanosized building blocks. We thus fabricated transparent electrodes using various AgNW standards with aspect ratios (*i.e.* length/diameter ratios) ranging from 55 to 1000. For the sake of clarity, the different AgNW mean dimensions are expressed as DaLb, with a and b indicating the mean diameter in nm and the mean length in μm, respectively. The transparency, conductivity and haze factor (*i.e.* the diffused part of the transmitted light) of the electrodes were measured and compared.

A typical plot of the transmittance as a function of the sheet resistance is presented in Fig. 4a. This series of dots was measured using D30L10 AgNWs. As expected, for these dimensions and for highly purified nanowires, excellent optoelectrical performances were obtained, with for instance 97% transparency for a 50 ohm sq^{-1} sheet resistance. This compares very well with the state-of-the-art for metallic nanowire-based technology, and also with ITO or any other

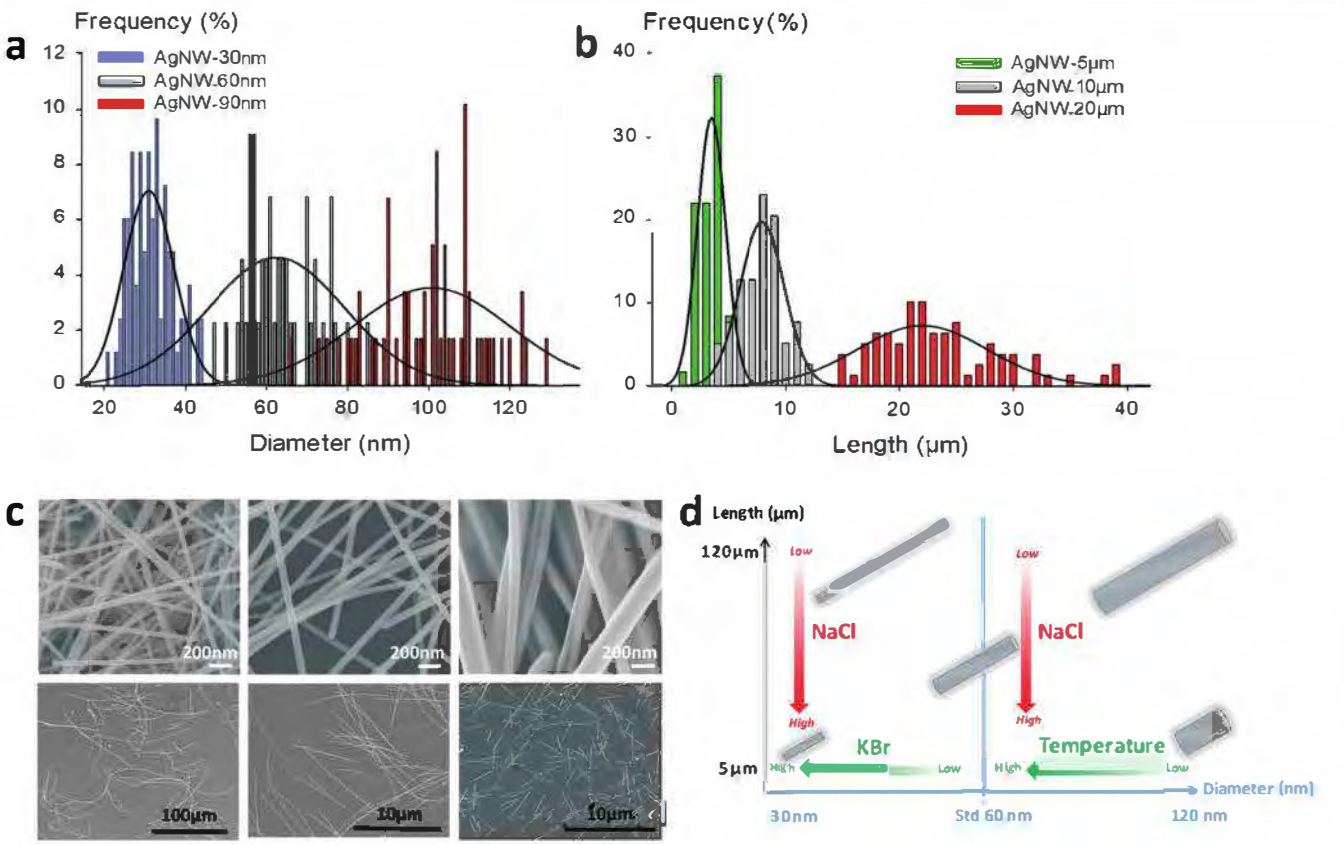


Fig. 3 Independent tuning of the diameter and length of silver nanowires. (a) Histograms of three different mean diameters for 10 μm long AgNWs. (b) Histograms of three different mean lengths for 40 nm diameter AgNWs (c) SEM pictures of various lengths and diameters. Top, from left to right: mean diameters of 30 nm, 60 nm, and >100 nm; bottom, from left to right: mean lengths of >100 μm, 10 μm, and 5 μm. (d) Schematic diagram to control the morphology of AgNWs.

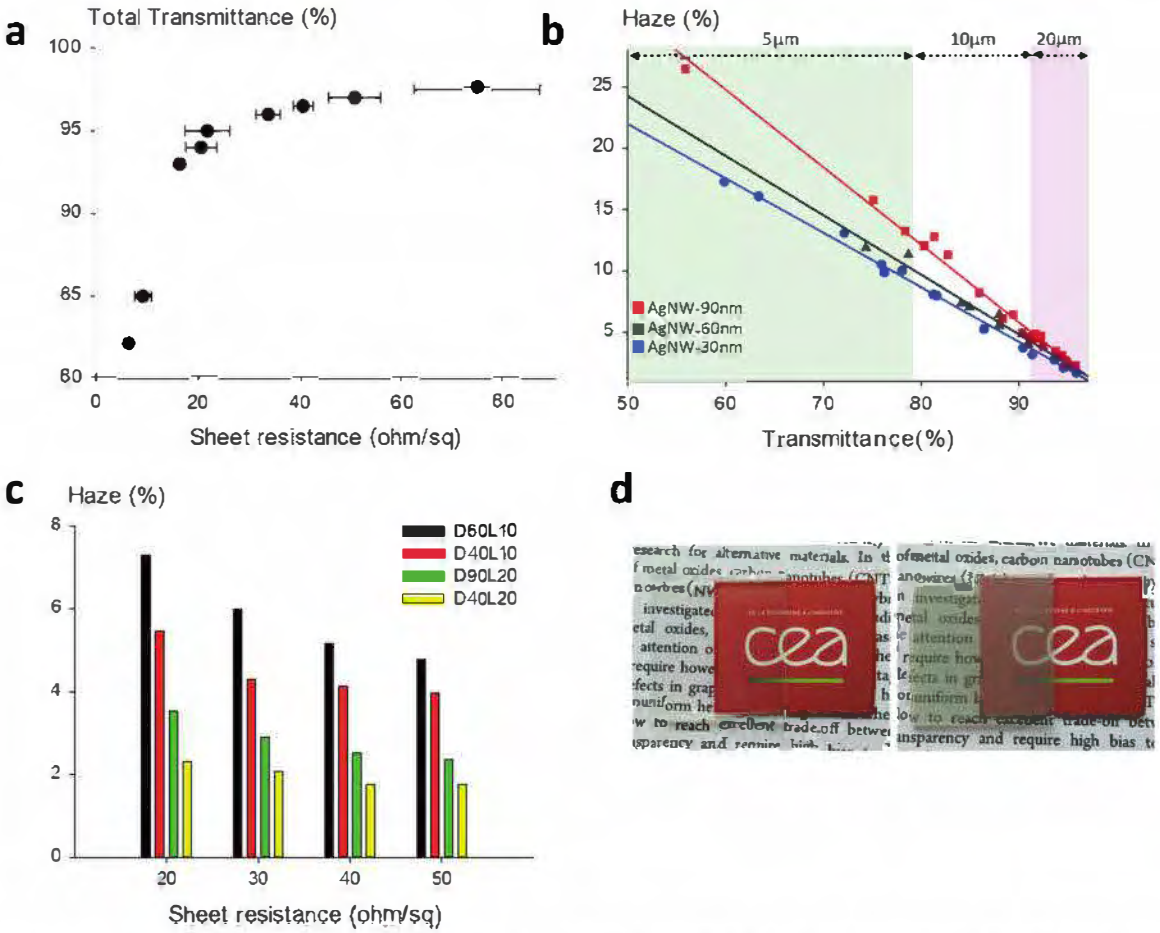


Fig. 4 Optoelectrical properties of transparent electrodes fabricated with various AgNW size standards. (a) Total transmittance as a function of the sheet resistance for D30L10 AgNWs. (b) Haze values for 30, 60 and 90 nm diameter AgNWs as a function of the total transmittance. The green part represents measurements made on 5 μm long AgNWs, the white part for 10 μm length and the pink part for 20 μm length. The solid lines correspond to linear regressions for each diameter. (c) Comparison of the haze level on given sheet resistances for standard AgNWs (D60L10), thin and short (D40L10), thick and long (D90L20) and thin and long nanowires (D40L20). (d) Left, a 96% transparent electrode with a very low haze value (1.6%); right, a 60% transparent electrode with high haziness (13.0%).

kind of transparent electrode. We chose to limit the field of study to nanowires with at most 20 μm length because longer AgNWs are much more difficult to synthesize, to handle, to formulate and to deposit with high homogeneity on a large substrate area using low-cost solution deposition techniques. In Fig. 4b, the haze values are presented as a function of the total transmittance for AgNWs with different diameters and for different lengths (5, 10 and 20 μm long). This graph shows a linear relationship between the haze value and the total transmittance regardless of the length. The dimension effect is very important since the haze values vary from 16% down to less than 2%. Very low haziness, compatible with optoelectronic applications, is demonstrated for small diameters, which is consistent with previous studies,[45–47] and very interestingly, it is also shown that even with 90 nm diameter AgNWs, it is possible to achieve low haze values for long nanowires. The haze factor reported in the literature for sub-15 nm diameter silver nanowires reached very low values below 1% at 94.5% transmittance.[48] However, reducing the diameter of AgNWs below the mean free path of electrons (~40 nm) is risky due to charge carrier scattering at the interface and to material instability under operational stress. The histogram in Fig. 4c shows haze values at given sheet resistances for AgNWs with various lengths and diameters. When the densities of AgNWs are decreased (*i.e.* increasing sheet resistances), the haze value is lowered drastically regardless of the size standards. The impact of the length is obvious since 20 μm long nanowires are much less light diffusive than 10 μm long nanospecies. For instance, in the case of 40 nm diameter AgNWs, the haze value is more than doubled when the length is reduced from 20 μm down to 10 μm. This is expected by percolation theory. Indeed, for finite size scaling in stick percolation dealing with large size systems, the critical number density (N_c) of sticks required for percolation is given by $N_c \times L^2 = 5.637$ where L is the length of nanowires.[49] This indicates that for a two-fold increase of the length of the AgNWs, the required N_c to reach percolation is divided by four.[17,50] As shown by De *et al.* and by Lagrange *et al.*, percolation theory dominates the electrical behaviour of networks associated with low density.[51,52] However, it should be

emphasized that in our examples, the electrodes are fabricated with densities far above N_c, but the length effect is still clearly perceptible. The diameter effect is also visible for both 10 μm and 20 μm long AgNWs, and as expected, small diameters lower the haziness. Pictures of two electrodes are included in Fig. 4d to illustrate the pronounced optical difference (transparency and haziness) for different densities of AgNWs.

Toxicity of AgNW size standards. Even if silver toxicology is well known,[53,54] the toxicity of silver-based nanomaterials is difficult to predict because of their different shapes, which can contribute to different biological responses regarding the plurality of microorganisms. It is described in the literature that low-aspect ratio nanosilver species such as nanoparticles exhibit toxicity mainly relying on silver ion release, either extracellularly (for bacteria) or due to internalization and dissolution in the lysosomes for animal cells.[28,55,56] For high aspect ratio silver nanomaterials, like AgNWs, the toxicity effect may be driven by dissolution on the one hand; on the other hand, the form factor may contribute largely, as AgNWs exhibit a fibre shape having similarities with asbestos. Different toxicity approaches are currently ongoing to decipher possible mechanisms in order to avoid toxicity caused by the aspect ratio, and some research studies are carried out to establish safety thresholds like for asbestos.[57–59]

In this study, we investigated the basic responses of murine macrophages in the presence of AgNWs. We chose macrophages because they are in charge of removing nanomaterials, and because they are also the main actors at play in insoluble fibre toxicity. Thanks to the fabrication of AgNW size standards described above, toxicological experiments were performed on macrophages to discriminate the effects of the diameter and length.

Previous studies dealing with the potential toxic effect of AgNWs on lung and macrophage models revealed a length-dependent toxicity for both *in vitro* and *in vivo* studies, with short nanowires causing less inflammatory responses.[20,57,60–62] These different studies compared various lengths; however, a possible diameter effect was not directly tackled by comparative experiments, or both diameters and lengths were changed at the same time.

Prior to toxicity experiments on macrophages, we evaluated the contribution of dissolved species. The dissolution of relevant morphologies of nanowires was monitored over a period of 24 h in medium only under working conditions. The AgNWs were compared to silver nanoparticles coated with PVP with a mean diameter close to standard AgNWs (≈60 nm) and citrate-coated silver nanoparticles which are known to dissolve quickly. The results are shown in Fig. 5a.

Regardless of their dimensions, AgNWs exhibit similar dissolution rates. Silver nanoparticles coated with PVP dissolved slightly faster than the nanowires. For all the AgNWs, the solubilized part of silver was found to be low (<10%), even after 24 h. The corresponding Ag^+ concentration was lower than the known silver ion cytotoxicity (1 μg mL^{-1}); hence subsequent toxicity measurements on macrophages were considered relevant to evaluate the potential intrinsic toxicity of AgNWs.

AgNWs show low and length-dependent toxicity for macrophages. Cytotoxicity assays were performed on RAW264.7 murine cells, and the neutral red uptake assay was used to determine the potential adverse effects for each morphology of AgNWs. Various morphologies were chosen to check independently the potential diameter or length effects. For instance, we used AgNWs with the diameter dimension set at 40 nm with various lengths (4, 10 and 20 μm), and 10 μm long

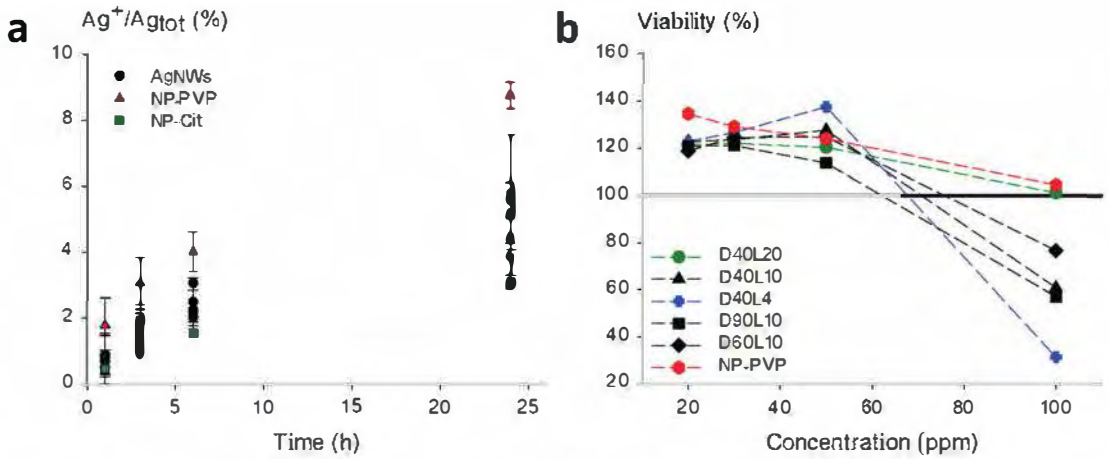


Fig. 5 Dissolution rate of the studied nanomaterials and cell viability of RAW264.7 murine cells treated with the nanomaterials. (a) Dissolution monitored for 24 h at 37 °C in RPMI medium containing only the nanomaterials at 2.5 μg mL^{-1} concentration except for less concentrated citrate-coated nanoparticles (0.4 μg mL^{-1}). PVP-coated nanoparticles (NP-PVP, red triangle) dissolve faster than nanowires (AgNWs, black dot). The total dissolution of all the nanomaterials is less than 10% in the medium. (b) The cells were incubated for 24 h with different concentrations of the nanomaterials (from 20 to 100 ppm). The shortest nanowires (D40L4) exhibit a higher toxicity, AgNWs with 10 μm length (D40L10, D60L10, and D90L10) exhibit a similar toxicity regardless of the diameter dimension, and long nanowires with 20 μm length (D40L20) and PVP-coated nanoparticles (NP-PVP) show a very low toxicity. The LC50 of the short nanowires, which exhibit the highest toxicity, is higher than 80 ppm.

AgNWs with various diameters (40, 60 and 90 nm). No decrease of cellular activity up to high doses (50 ppm nanomaterials) was observed, as evidenced in Fig. 5b. The shortest AgNWs (length of 4 μm) presented the lowest IC50 at 100 ppm concentration, followed by the three other nanowires with 10 μm length. Longer AgNWs (20 μm length) did not present a cellular activity decrease under our experimental conditions. This length effect can be related to the uptake ability of macrophages. Phagocytosis of short nanowires (4 μm) can be easily completed, leading to silver ion release inside the cells, as with the well-described silver nanoparticle toxicity. The 10 μm long nanowires, with different diameters, show a similar inhibition behaviour. The quasi non-toxicity of long AgNWs (20 μm length) can probably be ascribed to the fact that the nanowires were not internalized, as the threshold length of phagocytosis was exceeded. These results suggest that AgNWs induce cytotoxicity in a length-type-dependent manner, which is consistent with a reported study on RAW264.7 treated with carbon nanotubes of different lengths.[63] These results are in accordance with the literature, with cytotoxicity being correlated with the degree of cell uptake and the amount of ionic silver due to intracellular dissolution.[62,64]

Long AgNWs induce a slight but persistent pro-inflammatory profile. Macrophages are known for their capacity of phagocytizing particulate substances, such as pathogenic agents but also particulate chemicals (*e.g.* oxidized lipoprotein particles in the case of atherosclerotic foam cells). They also play a strong role in immune reaction modulation through their ability to release various signalling molecules such as pro- or anti-inflammatory cytokines, depending on the nature of the phagocytized particles. They also trigger these mechanisms upon frustrated phagocytosis (*e.g.* in the asbestos case), which makes them an attractive choice to study possible pro-inflammatory responses. To evaluate this response, we measured two major inflammatory cytokines: interleukin 6 (IL-6) and tumour necrosis factor alpha (TNF-α) at the highest non-toxic concentration (50 ppm). The inflammatory responses due to the nanowires were compared, on the one hand, to that of amorphous and crystalline silica particles (LS30, BCR66, and BCR70) known to induce a low but well-documented inflammatory response[65–68] and on the other hand, to bacterial lipopolysaccharide (LPS) as a positive control known to induce very strong pro-inflammatory responses.

Fig. 6a shows the relative amount of secreted TNF-α by macrophages in response to the different nanowires. After 24 h, we can notice a similar production rate for all the morphologies of AgNWs. However, D40L20 is the only AgNW that induces a slightly higher TNF-α production than silica. For IL-6 (Fig. 6b), the production induced by AgNWs was very low and always lower than those induced by silica. Overall, the bar charts for IL-6 and TNF-α show a globally low secretion for each cytokine after a 24 h exposure to nanomaterials, representing less than 1% of the LPS-induced production. These results show that the AgNWs induce a weak increase

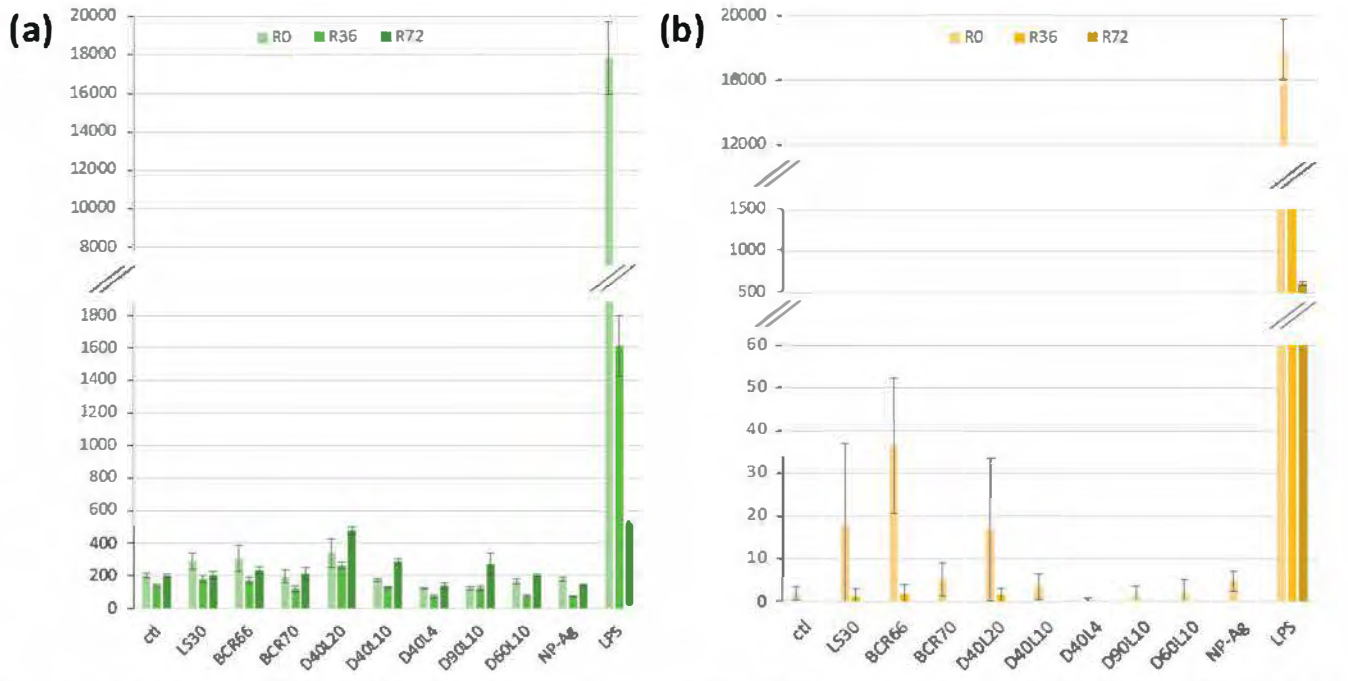


Fig. 6 Pro-inflammatory cytokine secretion after treatment with nanomaterials (R0) and persistence results at 36 h (R36) and 72 h (R72). The secretion level was compared to LPS as a positive control and silica-based nanomaterials (LS30, BCR66, and BCR70) as the nanomaterial control. (a) TNF-α secretion is very low (the same level as the control) for all AgNWs, and lower than the silica control (BCR66), except for D40L20. (b) The IL-6 secretion of cells treated with AgNWs is similar regardless of the length or diameter, and at the same level as the negative control. For both IL-6 secretion and TNF-α secretion, the comparative secretion with LPS is very low (<1%).

in cytokine release, which correlated positively with the length of the wires, as expected for fibre-like materials.

One of the major mechanisms at play in persistent fibre/particle toxicity is the presence of a prolonged pro-inflammatory response over time. In order to test this possibility by our *in vitro* system, we studied the evolution of the production of TNF-α and IL-6 over time after a 24 h exposure to nanomaterials (Fig. 6). LPS-induced TNF-α release was both acute and very transient. Compared to the control, none of the short nanowires, the amorphous silica or the silver nanoparticles induced any significantly higher TNF-α release. Only the small crystalline silica (BCR66) and the long nanowires (20 μm) induced a weak but sustained TNF-α release. Although the shape of the curves is different for IL-6, with a prolonged LPS-induced release, the same trend can be observed, with only the small crystalline silica (BCR66) and the long nanowires inducing a prolonged IL-6 release compared to the control. These results suggest that the long nanowires have an inflammatory effect that is sustained over time. To the best of our knowledge, this is the first reported study on the persistence of the inflammatory response *in vitro*.

Our results are in line with those obtained using *in vivo* models[20,26,60] and suggest that simple *in vitro* models can be predictive of the inflammatory potential of nanomaterials. In addition, these *in vitro* systems are much cheaper and ethically more acceptable than *in vivo* experiments.

Taken collectively, all these data allow us to propose a "safer by design" approach for the use of AgNWs, depending on the applications. Different performances for both electrical and optical aspects (including haziness) can be achieved, depending on the density and on the dimensions of AgNWs. According to the toxicological results, a rational approach would be to foster the use of nanowires up to 10 μm length since they can combine high optoelectrical performances and minimal biological effects.

Conclusions

By careful modifications of the polyol process, in particular, precisely defined halide ion concentrations and temperature setting, independent fine tuning of the diameter and length of AgNWs has been successfully demonstrated. Size standards were prepared accordingly, and used for the fabrication of transparent electrodes. Excellent optoelectrical performances were obtained and the haze value was varied from 1.6 to 26.2%. In the meantime, we performed *in vitro* toxicological assays on murine macrophages. The AgNWs were found to be weakly toxic for macrophages and showed a length-dependent toxicity, with the toxicity decreasing with length. Conversely, the cytokine release assays showed a weak but significant pro-inflammatory potential for long nanowires (20 μm), and the observed persistence of the response, although at weak levels, calls for attention when devising the rules for the use of AgNWs in consumer products and in the recycling of such products. This study set out to take a holistic view of AgNWs, with a clear relationship between AgNW dimensions and both their optoelectronic performances, when used in the form of random percolating networks, and their toxicity and pro-inflammatory potential. Since AgNWs will certainly be increasingly introduced in commercial devices in the short term, it seems important to have a global vision of these nanomaterials, which will ineluctably encourage the rational use of AgNWs in a "safer by design" approach.

Conflicts of interest

There are no conflicts of interest to declare.

Acknowledgements

This work was supported by the Labex Serenade (no. ANR-11-LABX-0064) funded by the "Investissements d'Avenir" French Government program of the French National Research Agency (ANR) through the AMIDEX project (no. ANR-11-IDEX-0001-02). In particular, the authors thank Pr L. Charlet for coordinating the "nanosilver topic", and they are grateful for the funding of D. Toybou's PhD grant.

References

1 D. S. Hecht, L. Hu and G. Irvin, *Adv. Mater.*, 2011, 23, 1482–1513.
2 S. Ye, A. R. Rathmell, Z. Chen, I. E. Stewart and B. J. Wiley, *Adv. Mater.*, 2014, 26, 6670–6687.
3 D. Langley, G. Giusti, C. Mayousse, C. Celle, D. Bellet and J.-P. Simonato, *Nanotechnology*, 2013, 24, 452001.
4 H. Hosseinzadeh Khaligh, K. Liew, Y. Han, N. M. Abukhdeir and I. A. Goldthorpe, *Sol. Energy Mater. Sol. Cells*, 2015, 132, 337–341.
5 T. Sannicolo, M. Lagrange, A. Cabos, C. Celle, J.-P. Simonato and D. Bellet, *Small*, 2016, 12, 6052–6075.
6 T. Sannicolo, D. Muñoz-Rojas, N. D. Nguyen, S. Moreau, C. Celle, J.-P. Simonato, Y. Bréchet and D. Bellet, *Nano Lett.*, 2016, 16, 6052–6075.
7 S.-Y. Chou, R. Ma, Y. Li, F. Zhao, K. Tong, Z. Yu and Q. Pei, *ACS Nano*, 2017, 11, 11368–11375.
8 W. Lan, Y. Chen, Z. Yang, W. Han, J. Zhou, Y. Zhang, J. Wang, G. Tang, Y. Wei, W. Dou, Q. Su and E. Xie, *ACS Appl. Mater. Interfaces*, 2017, 9, 6644–6651.
9 T. Sannicolo, N. Charvin, L. Flandin, S. Kraus, D. T. Papanastasiou, C. Celle, J.-P. Simonato, D. Muñoz-Rojas, C. Jiménez and D. Bellet, *ACS Nano*, 2018, 12, 4648–4659.
10 S. Choi, J. Park, W. Hyun, J. Kim, J. Kim, Y. B. Lee, C. Song, H. J. Hwang, J. H. Kim, T. Hyeon and D.-H. Kim, *ACS Nano*, 2015, 9, 6626–6633.
11 Y. Atwa, N. Maheshwari and I. A. Goldthorpe, *J. Mater. Chem. C*, 2015, 3, 3908–3912.
12 D. Doganay, S. Coskun, S. P. Genlik and H. E. Unalan, *Nanotechnology*, 2016, 27, 435201.
13 C. Preston, Y. Xu, X. Han, J. N. Munday and L. Hu, *Nano Res.*, 2013, 6, 461–468.

14 C. Preston, Z. Fang, J. Murray, H. Zhu, J. Dai, J. N. Munday and L. Hu, *J. Mater. Chem. C*, 2014, 2, 1248–1254.
15 S. Coskun, B. Aksoy and H. E. Unalan, *Cryst. Growth Des.*, 2011, 11, 4963–4969.
16 N. V. Nghia, N. N. K. Truong, N. M. Thong and N. P. Hung, *Int. J. Mater. Chem.*, 2012, 2, 75–78.
17 S. M. Bergin, Y.-H. Chen, A. R. Rathmell, P. Charbonneau, Z.-Y. Li and B. J. Wiley, *Nanoscale*, 2012, 4, 1996.
18 Y. Shi, H. Li, L. Chen and X. Huang, *Sci. Technol. Adv. Mater.*, 2005, 6, 761–765.
19 M. J. Large, J. Burn, A. A. King, S. P. Ogilvie, I. Jurewicz and A. B. Dalton, *Sci. Rep.*, 2016, 6, 25365.
20 K. F. Chung, J. Seiffert, S. Chen, I. G. Theodorou, A. E. Goode, B. F. Leo, C. M. McGilvery, F. Hussain, C. Wiegman, C. Rossios, J. Zhu, J. Gong, F. Tariq, V. Yufit, A. J. Monteith, T. Hashimoto, J. N. Skepper, M. P. Ryan, J. Zhang, T. D. Tetley and A. E. Porter, *ACS Nano*, 2017, 11, 2652–2664.
21 L. D. Scanlan, R. B. Reed, A. V. Loguinov, P. Antczak, A. Tagmount, S. Aloni, D. T. Nowinski, P. Luong, C. Tran, N. Karunaratne, D. Pham, X. X. Lin, F. Falciani, C. P. Higgins, J. F. Ranville, C. D. Vulpe and B. Gilbert, *ACS Nano*, 2013, 7, 10681–10694.
22 R. M. Silva, J. Xu, C. Saiki, D. S. Anderson, L. M. Franzi, C. D. Vulpe, B. Gilbert, L. S. Van Winkle and K. E. Pinkerton, *Part. Fibre Toxicol.*, 2014, 11, 52.
23 M. Charehsaz, S. Coskun, H. E. Unalan, R. Reis, S. Helvacioglu, A. K. Giri and A. Aydin, *Toxicol. Environ. Chem.*, 2017, 99, 837–847.
24 S. Sweeney, I. G. Theodorou, M. Zambianchi, S. Chen, A. Gow, S. Schwander, J. Zhang, K. F. Chung, M. S. P. Shaffer, M. P. Ryan, A. E. Porter and T. D. Tetley, *Nanoscale*, 2015, 7, 10398–10409.
25 R. Cui, Y. Chae and Y.-J. An, *Chemosphere*, 2017, 185, 205–212.
26 L. D. Scanlan, R. B. Reed, A. V. Loguinov, P. Antczak, A. Tagmount, S. Aloni, D. T. Nowinski, P. Luong, C. Tran, N. Karunaratne, D. Pham, X. X. Lin, F. Falciani, C. P. Higgins, J. F. Ranville, C. D. Vulpe and B. Gilbert, *ACS Nano*, 2013, 7, 10681–10694.
27 C. Mayousse, C. Celle, E. Moreau, J.-F. Mainguet, A. Carella and J.-P. Simonato, *Nanotechnology*, 2013, 24, 215501.
28 C. Aude-Garcia, F. Villiers, V. Collin-Faure, K. Pernet-Gallay, P.-H. Jouneau, S. Sorieul, G. Mure, A. Gerdil, N. Herlin-Boime, M. Carrière and T. Rabilloud, *Nanotoxicology*, 2016, 10, 586–596.
29 B. Dalzon, C. Aude-Garcia, V. Collin-Faure, H. Diemer, D. Béal, F. Dussert, D. Fenel, G. Schoehn, S. Cianférani, M. Carrière and T. Rabilloud, *Nanoscale*, 2017, 9, 9641–9658.
30 G. Repetto, A. del Peso and J. L. Zurita, *Nat. Protoc.*, 2008, 3, 1125–1131.
31 Y. Sun, Y. Yin, B. T. Mayers, T. Herricks and Y. Xia, *Chem. Mater.*, 2002, 14, 4736–4745.
32 Y. Sun, B. Mayers, T. Herricks and Y. Xia, *Nano Lett.*, 2003, 3, 955–960.
33 P. Zhang, I. Wyman, J. Hu, S. Lin, Z. Zhong, Y. Tu, Z. Huang and Y. Wei, *Mater. Sci. Eng., B*, 2017, 223, 1–23.
34 P. Zhang, Y. Wei, M. Ou, Z. Huang, S. Lin, Y. Tu and J. Hu, *Mater. Lett.*, 2018, 213, 23–26.
35 L. Hu, H. S. Kim, J.-Y. Lee, P. Peumans and Y. Cui, *ACS Nano*, 2010, 4, 2955–2963.
36 C. Chen, L. Wang, G. Jiang, J. Zhou, X. Chen, H. Yu and Q. Yang, *Nanotechnology*, 2006, 17, 3933.
37 K. Zhang, Y. Du and S. Chen, *Org. Electron.*, 2015, 26, 380–385.
38 B. Li, S. Ye, I. E. Stewart, S. Alvarez and B. J. Wiley, *Nano Lett.*, 2015, 15, 6722–6726.
39 E.-J. Lee, Y.-H. Kim, D. K. Hwang, W. K. Choi and J.-Y. Kim, *RSC Adv.*, 2016, 6, 11702–11710.
40 C. Celle, C. Mayousse, E. Moreau, H. Basti, A. Carella and J.-P. Simonato, *Nano Res.*, 2012, 5, 427–433.
41 C. Mayousse, C. Celle, E. Moreau, J.-F. Mainguet, A. Carella and J.-P. Simonato, *Nanotechnology*, 2013, 24, 215501.
42 W. M. Schuette and W. E. Buhro, *Chem. Mater.*, 2014, 26, 6410–6417.
43 R. R. da Silva, M. Yang, S.-I. Choi, M. Chi, M. Luo, C. Zhang, Z.-Y. Li, P. H. C. Camargo, S. J. L. Ribeiro and Y. Xia, *ACS Nano*, 2016, 10, 7892–7900.
44 W. M. Schuette and W. E. Buhro, *ACS Nano*, 2013, 7, 3844–3853.
45 Y. Liu, Y. Chen, R. Shi, L. Cao, Z. Wang, T. Sun, J. Lin, J. Liu and W. Huang, *RSC Adv.*, 2017, 7, 4891–4895.
46 J. Lee, P. Lee, H. Lee, D. Lee, S. S. Lee and S. H. Ko, *Nanoscale*, 2012, 4, 6408.
47 X. Yu, X. Yu, J. Zhang, L. Chen, Y. Long and D. Zhang, *Nano Res.*, 2017, 10, 3706–3714.
48 H.-W. Jang, Y.-H. Kim, K.-W. Lee, Y.-M. Kim and J.-Y. Kim, *APL Mater.*, 2017, 5, 080701.
49 J. Li and S.-L. Zhang, *Phys. Rev. E*, 2009, 80, 040104.
50 J. Han, S. Yuan, L. Liu, X. Qiu, H. Gong, X. Yang, C. Li, Y. Hao and B. Cao, *J. Mater. Chem. A*, 2015, 3, 5375–5384.
51 S. De, T. M. Higgins, P. E. Lyons, E. M. Doherty, P. N. Nirmalraj, W. J. Blau, J. J. Boland and J. N. Coleman, *ACS Nano*, 2009, 3, 1767–1774.
52 M. Lagrange, D. P. Langley, G. Giusti, C. Jiménez, Y. Bréchet and D. Bellet, *Nanoscale*, 2015, 7, 17410–17423.
53 K. M. Newton, H. L. Puppala, C. L. Kitchens, V. L. Colvin and S. J. Klaine, *Environ. Toxicol. Chem.*, 2013, 32, 2356–2364.
54 C. Hogstrand, F. Galvez and C. M. Wood, *Environ. Toxicol. Chem.*, 1996, 15, 1102–1108.
55 A. Pratsinis, P. Hervella, J.-C. Leroux, S. E. Pratsinis and G. A. Sotiriou, *Small*, 2013, 9, 2576–2584.
56 M. van der Zande, R. J. Vandebriel, E. Van Doren, E. Kramer, Z. Herrera Rivera, C. S. Serrano-Rojero, E. R. Gremmer, J. Mast, R. J. B. Peters, P. C. H. Hollman, P. J. M. Hendriksen, H. J. P. Marvin, A. A. C. M. Peijnenburg and H. Bouwmeester, *ACS Nano*, 2012, 6, 7427–7442.
57 R. M. Silva, J. Xu, C. Saiki, D. S. Anderson, L. M. Franzi, C. D. Vulpe, B. Gilbert, L. S. Van Winkle and K. E. Pinkerton, *Part. Fibre Toxicol.*, 2014, 11, 52.
58 A. Schinwald, T. Chernova and K. Donaldson, *Part. Fibre Toxicol.*, 2012, 9, 47–47.

59 A. Schinwald, F. A. Murphy, A. Prina-Mello, C. A. Poland, F. Byrne, D. Movia, J. R. Glass, J. C. Dickerson, D. A. Schultz, C. E. Jeffree, W. MacNee and K. Donaldson, *Toxicol. Sci.*, 2012, 128, 461–470.
60 A. Schinwald and K. Donaldson, *Part. Fibre Toxicol.*, 2012, 9, 34.
61 A. Schinwald, T. Chernova and K. Donaldson, *Part. Fibre Toxicol.*, 2012, 9, 47–47.
62 I. G. Theodorou, K. H. Müller, S. Chen, A. E. Goode, V. Yufit, M. P. Ryan and A. E. Porter, *ACS Biomater. Sci. Eng.*, 2017, 3, 2336–2347.
63 D. Liu, L. Wang, Z. Wang and A. Cuschieri, *Nanoscale Res. Lett.*, 2012, 7, 361.
64 X. Wang, Z. Ji, C. H. Chang, H. Zhang, M. Wang, Y.-P. Liao, S. Lin, H. Meng, R. Li, B. Sun, L. V. Winkle, K. E. Pinkerton, J. I. Zink, T. Xia and A. E. Nel, *Small*, 2014, 10, 385–398.
65 C. M. Sayes, K. L. Reed and D. B. Warheit, *Toxicol. Sci.*, 2007, 97, 163–180.
66 D. Warheit, T. McHugh and M. Hartsky, *Scand. J. Work, Environ. Health*, 1995, 19–21.
67 M. V. D. Z. Park, I. Lynch, S. Ramírez-García, K. A. Dawson, L. de la Fonteyne, E. Gremmer, W. Slob, J. J. Briedé, A. Elsaesser, C. V. Howard, H. van Loveren and W. H. de Jong, *J. Nanopart. Res.*, 2011, 13, 6775–6787.
68 E.-J. Park, J. Roh, Y. Kim and K. Choi, *J. Health Sci.*, 2011, 57, 60–71.

Progress report – Scientific Rapporteur

Project Acronym:
NanoWIR2ES

Runtime of the project:
☐ 1st year ☒ 2nd year ☐ 3rd year

Project Coordinator:
Annette Hofmann

Project Rapporteur:
Evangelia Sarantopoulou

1 Project objectives and work progress

The main outputs and the work progress outlined in the report are in line with the work plan of the project. However, the progress in WP3 ("Degradation of NW-Enabled Thin-Films and Devices" M1-M36) for the second year of project implementation is somewhat limited and vaguely described (page 9 of the report). Also, the work carried out over the past year by partner 2 (involved in WP2, M4-M24 *electron microscopy characterization of long-term aged Ag NW* and WP4 (M4-M25, *Ag NWs for cytotoxicity studies*) is not explicitly clarified.

2 Impact

The dissemination activities are in line with the project work plan. They mainly include the contribution in dedicated sessions at international conferences, participation in summer school and design of the project's website. It is also mentioned in the report, that the findings on the "Safety-by-Design" are summarized in a paper that will be published soon. Entries on NANOhub or enanoMapper databases and other relevant databases for nano risk and nanosafety are not included yet.

3 Transnational collaboration and project management

The transnational collaboration is via standard tools (internet video communication), and website while for face-to-face meetings the participation of NanoWIR2ES partners in international conferences sections was managed.

4 Deviations to the original project plan

Significant deviations are related to the tasks of Partner 4 (WP7 and WP8) due to equipment failure and/or limitations. However, there is not a clear and convincing plan to solve these issues and avoid possible interference with achieving the future deliverables and milestones in time.

Leaching studies WP9 (M7-M36), conducted by partner two are transferred to the third year. Moreover, it is not clear whether the funding agency approves the contract of Benjamin Gilbert ("as an invited researcher in the SERENADE network has been requested for another two years, until 2020.").

5 Final comments - Recommendations

There is not a clear and convincing plan to solve the issues related due to equipment failure and limitations in WP7 and WP8. Moreover, there are concerns regarding "the degradation of NWs enabled thin films and devices"(WP3) study, as only one nanowire enabled device has been found up to now. All the above could affect the completion of tasks/objectives.

Progress report – Scientific Rapporteur

Project Acronym:
NanoWIR2ES

Runtime of the project:
☐ 1st year ☒ 2nd year ☐ 3rd year

Project Coordinator:
Dr. Annette Hofmann

Project Rapporteur:
Dr. Evangelia Sarantopoulou

1 Project objectives and work progress

The NanoWIR2ES did not progress in close cooperation with all partners as outlined in the original plan. Besides the changes related to WP3, the progress of the work is in line with the work plan originally proposed with minor modifications occurred mainly in the timing and in the applied techniques. However, the status of WP3: Task 2 (M12) (on the "NW release under simulated disposal conditions") and of the D3.1 is not obvious enough. The planned actions to solve issues concerning the evaluation of "the degradation of NW-enabled thin films and devices" (WP3, conducted by Leitat) and how will not interfere with fulfilling the future deliverables and milestones in time need further analysis.

2 Impact

Dissemination activities are in line with the project work plan. They include the participation in two international conferences. Entries on NANOhub or enanoMapper databases are not included yet, as first inputs will be implemented during the second year. There is not precise information concerning the project website (e.g. updates what could also give the public access to information e.g. regarding the technology development of Ag NW toward safer options). Publications or submissions are not incorporated in the report.

3 Transnational collaboration and project management

The advantage of the transnational collaboration has a sound research impact at international level. However, since one of the partners is not financially supported is not clear enough how the project can proceed without obstacles in communication and teamwork proved to be very useful to meet the challenges in the individual WPs.
The project management follows well-established methods. Two full sessions at international conferences were organized and at both conferences, "one-day workshop type extended group meeting" was held. But, it is not obvious if a consortium meeting carried out within the reporting period.

4 Deviations to the original project plan

The significant modifications of the initial proposal, because the funding agency does not financially support one partner, have been approved by ERA-NET SIINN call office. Nevertheless, the planned actions to solve issues regarding the evaluation of "the degradation of NW-enabled thin films and devices" and the status of WP3: Task 2 (M12) and the D3.1 (Procurement of commercial NW touch screen device(s), M6) need further specification.

The experimental work planned for the subsequent period will be continued without deviations and following the project's contract given that "The study on degradation of NW-enabled thin films (WP3) is not associated with a precise slot." However, further explanation is needed. According to the originally proposed plan the "Simulated NW release from Flexible NW-enabled devices" (T3.2, month 6–month 36) and the study for "Enabling Ag NW release" "will enhance the effort of WP 9 to remove Ag NW from discarded devices" (month 7 – month 36).

5 Final comments - Recommendations

The principal objectives of NanoWIR2ES during the second reporting period have been successfully met. However, there are concerns regarding "the degradation of NWs enabled thin films and devices (WP3)" and potential interference with managing the planned deliverables and milestones in time.

Progress report

Please, note:

- The same font and style should be used for the whole report (Times New Roman, 11pt, single spaced).
- Adhere to the given page limits.
- All of the following sections have to be filled in.

This report must be submitted to the SIINN Call Office (siinn2014@fct.pt) within 60 days of the due date.

Project Acronym:

NanoWIR2ES

Project Full Title:

NanoWire Intelligent Re-design and Recycling for Environmental Safety

Runtime of the project:

☐ 1st year ☒ 2nd year ☐ 3rd year

Period covered:

From 01/04/2017 to 31/03/2018

Contact Data (Project Coordinator)

Name	Annette Hofmann
Organisation	University de Lille Sciences et Technologies
Country	France
Address	Sciences de la Terre, bâtiment SN5, Av. Paul Langevin, 59655 Villeneuve d'Asc
Phone	+33 320 43 41 49
E-mail	annette.hofmann@univ-lille1.fr

Partners

Number	Country	Organisation	Principal Investigator[1]	Other personnel[2]
1 Coordinator	France	Université de Lille Sciences et Technologies (ULille)	Annette Hofmann	Brenda Omana-Sanz Sophie Sobanska Ludovic Lesven Tech. personnel
2	Spain	Acondidionamien to Tarrasense (LEITAT)	Vincent Jamier	Tech. Personnel
3	France	Université Grenoble Alpes (UGA)	Laurent Charlet	Sylvia Lehmann Benjamin Gilbert Djadidi Toybou Caroline Celle Jean-Pierre Simonato
4	US	University of Florida, Gainsville (UFG)	Chris Vulpe	Devrah Arndt Tech personnel

[1] The Principal Investigator (PI) is the point of contact of the partner for the corresponding Funding Organisation
[2] Name of other personnel participating in the project

1 Project objectives and work progress

The project covers several aspects of safety and safety improvement of Ag-nanowire in view of designing products with minimized potential exposure and health risks for consumers. The fields addressed in year 2 (see Gantt chart) are in line with the work plan described in the joined proposal. Following fields were covered: (i) materials design, synthesis and purification, (ii) intrinsic toxicity of silver nanowires (AgNW) comprising studies on cellular toxicity, environmental toxicity and elaboration of metrics for safer-by-design AgNW geometries, (iii) release and recovery studies of silver from conducting films, (iv) outreach for commercially relevant nanowire enabled devices.

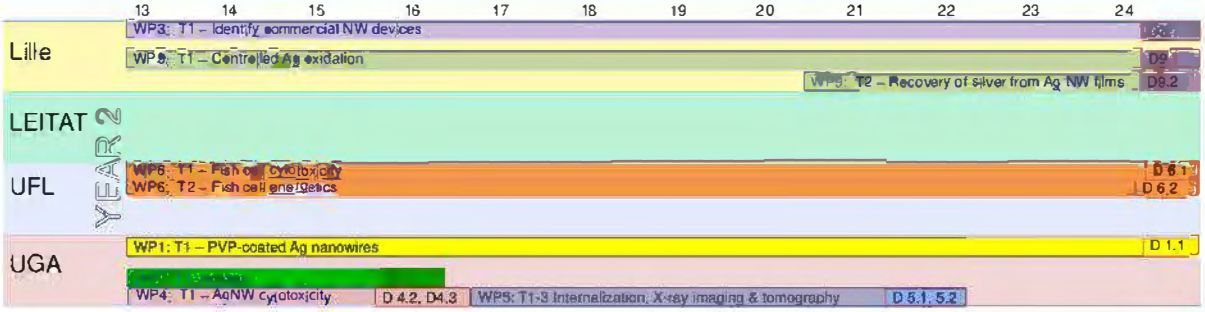


Table 1 : Institutional abbreviations: Lille is the University of Lille (A. Hofmann); LEITAT is the Spanish Technological Center specialized in production technologies in Barcelona (V. Jamier); UFL is the University of Florida – Gainsville (C. Vulpe); UGA is the University of Grenoble Alps (L. Charlet). **D** = Deliverable, **WP** = Work Package, **T** = Talk

Outline of work carried out over the past year by each partner

Partner 3 Grenoble-France (CEA & UGA) - Djadidi Toybou, Caroline Celle, Jean-Pierre Simonato, Laurent Charlet, Benjamin Gilbert

Materials design (WP1)

The synthesis and characterisation of AgNW with well-controlled physical and chemical properties, has been continued. While in year 1, special emphasis was placed on synthesis of **AgNW with different dimensions,** work in year 2 was dedicated to the study of the **reproducibility** of the controlled synthesis of AgNW's, to the improvement of the **purification** of the NW suspensions and to the evaluation of **optoelectric** performances.

Following the parametric studies reported during the last period, reproducibility of NW synthesis was evaluated for four batches of AgNWs with specific aspect ratios. AgNW of 4, 8 and 10 um length and 30, 44 and 55 nm diameter were synthesized twice under same conditions. A very good level of reproducibility in terms of diameter and length was obtained thus reflecting high control on the methodology.

Because toxicity assays (WP 4-8) need AgNW suspensions of high grade purity, a special purification method was developed capable of removing the Ag nanoparticles that form during NW synthesis and that are still present in the NW suspension after standard purification. By applying successive settling steps in methanol solvent, a highly purified AgNW suspension is obtained with less than 3 % NP.

The high grade AgNW suspensions were spray coated onto polymer substrate for studying NW dimension impact on optoelectrical properties. Indeed, transparency and conductivity of the flexible electrodes are influenced by length and width of the NW used (Fig. 1). We showed that the high quality of optoelectrical properties already achieved with our standard AgNW, could be further tuned by modulating NW dimensions. Highly purified silver nanowire suspensions exhibiting very good optoeletrical properties have been provided to all partners involved in toxicological studies.

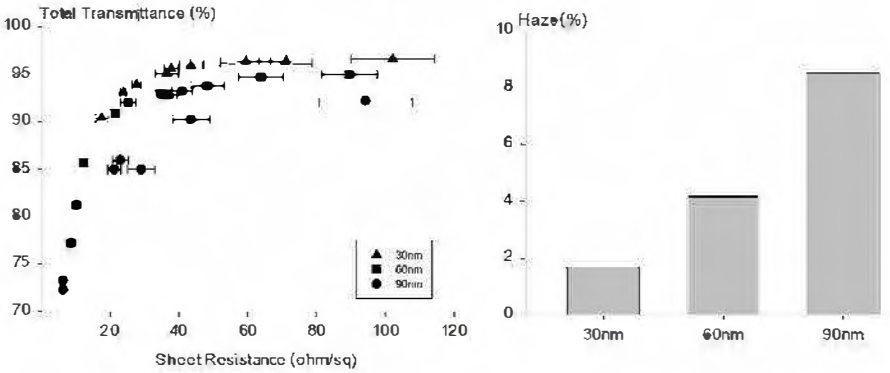


Figure 1. Total transmittance as a function of the sheet resistance of transparent conductive electrodes made of AgNWs with a length of 10µm and diameters ranging from 30 to 90 nm. Associated haze factor.

Deliverables from year 2 are (a) the high level control on the synthesis of AgNW with specific dimensions, (b) an improved purification method for NW suspensions and (c) fine tuning of opto-electric parameters for flexible AgNW electrodes (D1.1).

Partner 3 Grenoble-France (UGA) - Sylvia Lehmann, Benjamin Gilbert, Laurent Charlet
Internalization of AgNW in dermal cells, viability assays, optical & X-ray microscopy studies (WP 2, 4, 5)

Keratocytes and fibrobasts, the major cells of the epidermis and dermis layers of skin, have been used as model skin cells in AgNW exposure experiments. Cytotoxicity assays were mainly conducted in year 1 (MTT, RNU, ATP measurements). Additional experiments conducted in year 2 targeted AgNW cell injury factors of the oxidative-stress hierarchie with following probes : CM-H2DCFDA, a fluorescent probe for general ROS, JC-1, a probe for the determiation of mitochondrial membrane potential, ψ_m. Fluo-4AM used to probe Ca^{2+} release, and propidium iodide (PI) to trace loss of membrane integrity of apoptotic cells.
The toxicity tests were designed as a function of AgNW dimensions. NWs were synthesized and made available by CEA-Grenoble partner WP1. The development of an AgNW suspension purification method conducted by this group has proven essential to ensure reproducible toxicity results.

While the factors favoring **NW internalization**, the relation between **internalization and cell viability**, and the **toxicity** of Ag ions compared to nanowires have been addressed in year 1, the second year was dedicated to characterising the mechanisms of toxicity produced by internalized AgNW. It was shown that ROS production occurs when AgNW are internalized but that it is dose dependent. Also, thin and thick NW (30- and 90-nm diameter) had clearly distinguishable toxicity effect. Assays for mitochondrial membrane potential and Ca^{2+} release confirmed the major influence of NW diameter on the cell response.

Optical- and X-ray microscopy studies initiated in year 1 were continued during the second year. We used a combination of synchrotron X-ray imaging and microchemical analysis methods at beamlines ID-16A and ID-21 at the ESRF to study the intracellular location, morphology and chemistry of 30-nm and 90-nm diameter 10-µm AgNW administered to murine fibroblasts. Holographic X-ray nano-imaging generated 2D projection images (phase contrast maps) and stereographic images with ~40-nm resolution. Full 3D reconstructions with ~80-nm resolution were obtained from holotomography. Nanofocus X-ray fluorescence (XRF) of the same specimens provided 2D elemental maps with approximately 40-nm resolution. Microfocus XRF of duplicate samples provided 2D elemental maps that could be complemented with Ag L_{III}-edge X-ray absorption spectra to determine silver speciation.

Key findings from these studies show that AgNW fate and cellular toxicity is determined by the interplay of the membrane forces generated during NW internalization, the bending stiffness of the AgNW and the strength of the plasma membrane. Although 30- and 90-nm diameter AgNW are readily internalized, the thinner NW are mechanically crumpled by the forces of endocytosis while the thicker nanowires puncture the enclosing membrane, release silver ions and lysosomal contents to the

cytoplasm thereby initiating oxidative stress. This finding extends the fiber pathology paradigm and will enable the manufacture of safer nanowire enabled consumer products (Fig. 2).

The main objective of WPs 4 and 5, i.e. to understand the mechanism of cytotoxicity of AgNW and to tailor AgNW with lower risk of celluar injury has been reached. Also, we were able to show that the proposed safer AgNWs do not deteriorate the performance of the target technology (Fig. 1). Results of this study are summarised in a manuscript that is about to be submitted to a highly quoted international journal of research.

Deliverables from year 2 comprise (a) Ag+ release and toxicity effect (D2.1), (b) the role of AgNW diameter in cell injury (D4.2, D5.1, D5.2), (b) mechanical forces and chemical reactions excerted on AgNW inside the cell (D4.3), (c) new AgNW dimensions for safer NW enabled technology (final goal).

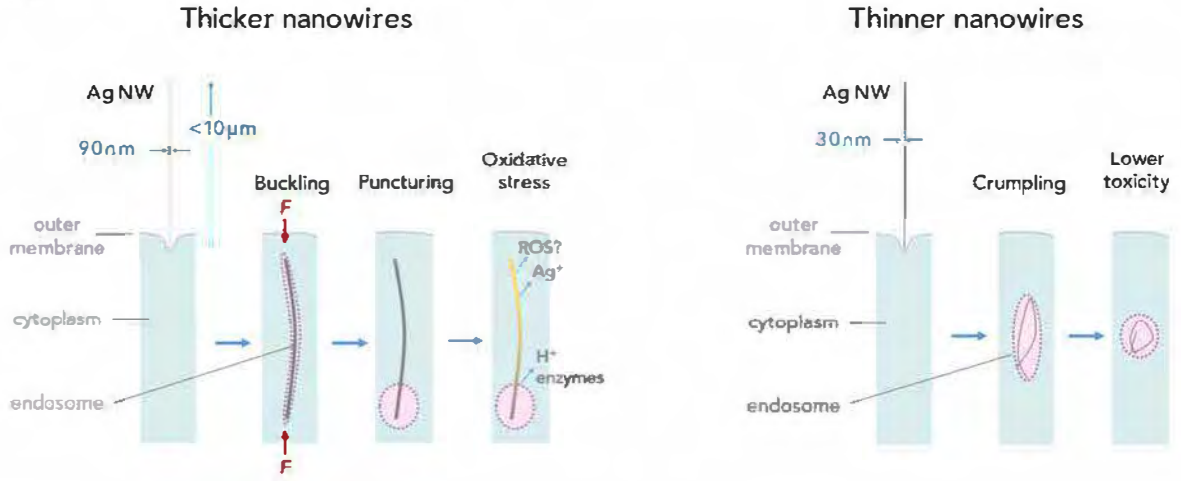


Figure 2. Diagram showing the biomechanical mechanism for silver nanowire (Ag NW) toxicity. Thicker and stiffer Ag NW are more cytotoxic because they have a greater tendency to puncture endolysosome membranes following internalization.

Partner 4 (Florida, US) - Devrah Arndt, Chris Vulpe, Benjamin Gilbert
Cellular, organismal and environmental toxicity (WP 6, 7, 8)

Cytotoxicity studies on rainbow trout gill and intestinal cells started in year 1 were continued and refined. Partner 3 CEA Grenoble (WP1) synthesized Ag NW of different dimensions for these studies. A major result of this year's studies is that AgNW concentration and dimensions do influence the toxicity to rainbow trout cells.

Results of metabolic assays (Seahorse XFe24 Analyzer) conducted on rainbow trout cells indicate that thick silver nanowires (90 nm diameter) significantly reduce basal metabolism and ATP production in gill and gut cells at higher concentrations (above 120 ug/mL). Thin silver nanowires (30 nm diameter) had no effect on basal metabolism or ATP production in rainbow trout gill cells (Figure 3a and 3b) while proton leakage was decreased by thin silver nanowires and increased by thick silver nanowires (Figure 3c). The reserve capacity makes up the energetic reserve that can be used by the cell in times of stress, and all treatments of silver nanowires significantly reduced the reserve capacity of trout cells (Figure 3d). These results are consistent with the findings in WP 4 and 5 and provide more insights into the mechanism through which NW geometry plays a major role in the cell toxicity.

Difficulties were encountered with the standard cell culture media (L15) that reacted with the $AgNO_3$ in the toxicity control assay, thereby alleviating the toxic effect of the silver ion. Therefore an in-house made media (L15-ex) was developed with decreased salt concentrations and without amino acids. Using this media, interference of media components with silver ions was resolved, and $AgNO_3$ exposure tests could be used again as a toxicity reference.

AgNW exposures in L15-ex media revealed differential toxicity of AgNWs regarding length, where long nanowires induced significantly more toxicity to rainbow trout cells than short nanowires. These results are somewhat contradictory to other findings (WP4) and will be investigated further. It is suspected that this observation is due to different synthesis and purification procedures used to make short versus long AgNWs.

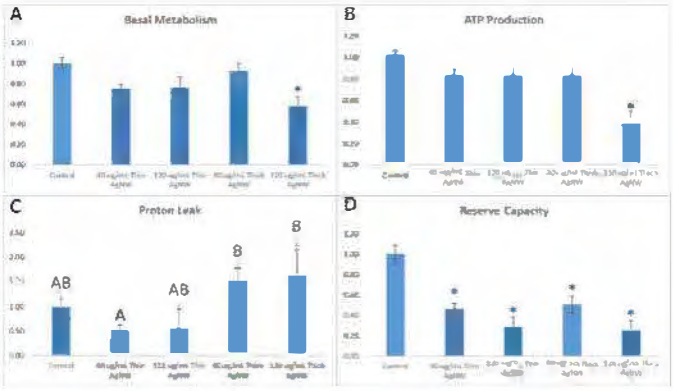


Figure 3 : Impact of thick and thin AgNWs on rainbow trout gill cells. A) basal metabolism, B) ATP production, C) proton leak, and D) reserve capacity. * denotes significant at $p < 0.05$. A/B markers in C. denote significant difference at $p < 0.05$ for comparisons between all treatments (in addition to comparisons between treatments and the control).

Deliverables of year 2 comprise (a) aqueous environmental factors can modulate AgNW toxicity (D6.1.2), (b) development of a specific cell culture media compatible with silver (D6.1.2), and (c) AgNW thickness influences cellular metabolism (D6.2.1).

Partner 1 Lille, France (ULille) - Brenda Omana, Annette Hofmann, Ludovic Lesven, Sophie Sobanska, Benjamin Gilbert

Release and recovery studies of silver from conducting films (WP9)

Silver NW are emerging materials entering the production of ultra thin conducting films for advanced electronic, optic and textile materials and devices. Here we are developping an electrochemical **method for reclaiming Ag from Ag NW in transparent electrode technology** with the aim to reduce the risk of **toxicologically critical Ag compounds** entering an e-Waste stream, and to develop ways to recover and reuse nano Ag, using sustainable methodology.

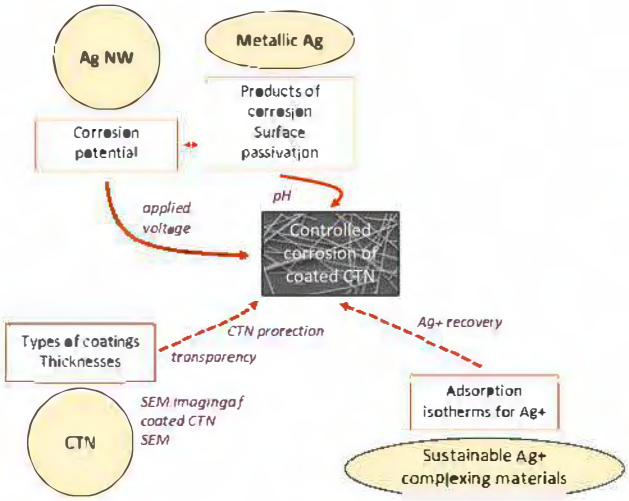


Figure 4 : While the red boxes represent processes and technology critical for the development of a Ag reclamation technique, the arrows represent the specific parameters that need to be fine-tuned. Full red lines indicate that we have already determined optimal parameters while dashed lines indicate that experimental work is still in progress. Initial experiments have already shown that corrosion of CTN coated with polysiloxane resin was possible under some conditions. Initial conditions will be modulated in further experiments (pH, applied electric potential, coating thickness) to explore the field of reactivity and applicability.

In year 1, we showed that the option of a fuel cell for Ag recovery, using oxygen from air as a reductant, was difficult to set up despite thermodynamically possible, because of the omni-presence of dioxygen in all compartments. In year 2, we have not developped the fuel cell approach further, but used an external power supply to provoke Ag corrosion. Conversely, we have focused on all other key elements needed to extract Ag from Conductive Transparent Networks (CTN) electrochemically.

Figure 4 illustrates the factors needed to corrode AgNW under optimal conditions, the constraints on corrosion due to polymeric coatings used by industrials to protect the CTN as well as our desire to closely couple CTN corrosion and Ag^+ recovery and storage.

Deliverables from year 2 : (a) Ag NW corrosion potential (D 9.1), (b) Ag corrosion products under atmospheric conditions (D 9.1), (c) sustainable materials for Ag^+ adsorption (D 9.2)

2 Impact[3]

Safety-by-Design

The key finding of this project from years 1 and 2 is the demonstration of a robust link between Ag NW diameter and toxicity in mouse, fish and human cell lines and the discovery of a new mechanism of nanowire toxicity associated with the puncturing of endolysosomes. This work demonstrates success in coupling **NW risks and NW design**, and the determination of **safest design for minimum risk solutions** while maintaining **technical performance** is being reached. The findings are anticipated to have high impact on the manufacturing approaches for consumer devices that incorprorate Ag-NW. The findings are summarized in a paper to be published soon. We expect a strong **impact on NM safety issues, safety by design development and eco-friendly high end novel products**.

Framework for Ag NW life-cycle risk assessment and mitigation

Over year 2, the project partners consolidated the **framework around Ag NW** exposure assessment, toxicity mechanisms and effects on human health and environment.

Ag NW environmental and cellular exposure studies have been conducted in direct link with NW material design studies, metrics that can act as toxicity indicator parameters have been finetuned (WP1, WP4-6). The effort of linking toxicity studies to material design and physical / chemical mechanisms of toxicity continued in year 2. The important linkages between NW diameter and length, cell internalization and cell viability have been further confirmed.

The differential toxicity of AgNWs in aqueous environments of different ionic strengths (fresh- and saltwater environments) has been clearly indentified via the use of different culture media, leading us to also think culture media as a tool to quantify enviromnmental toxicity.

Data Upload

During the May-June 2018 Safer Nanodesign summer school (see below), co-PI's Gilbert and Charlet attended a tutorial hosted by Nikolay Kochev, co-PI of eNanoMapper, to learn how to upload experimental nanotoxicity data into EU databases. We will use the Excel templates to capture the cytotoxicity and ecotoxicity data and upload them.

End-of-life silver capture

The technique implementing controlled release of silver from coated AgNW CTNs has progressed (WP1, WP9). Although we are not yet certain to achieve complete recovery of silver from CTNs in this recycling effort, it appears that the electrochemical technique can provide metrics for releasability of Ag^+ from coated CTNs, for safety and environmental impact, and for a **"design for recycling"** approach.

Education and Outreach

Co-Investigators Charlet and Gilbert participate in the annual Safer NanoDesign summer school in Archamps, France. The website for the 2018 school is at:
http://www.esi-archamps.eu/Thematic-Schools/Discover-bioHC/SAFERNANO
During this school, the NANOWIR²ES project is used as an example for assessing the potential nanotoxicity of engineered nanomaterials and the use of collaboration between materials scientists,

[3] Include link and details for any mentioned publication, report, patent or communication.

geoscientists and toxicologists to re-design materials to reduce toxicity during use and at the end of a product life.

Dissemination of results:

253rd ACS - Annual spring meeting, San Francisco 2-6 April 2017

We organized a dedicated session that included talks that described the NANOWIR^{2}ES project and recent results to an international audience.

Session: Chemical Principles of Environmental, Cellular & Organismal Nanotoxicology.
Organisers : C. Celle, L. Charlet, B. Gilbert, S. Lehman, J. Simonato, C. Vulpe.

Orals
Devrah Arndt, Toybou, D, Simonato, JP, Celle, C, Gilbert, B, Charlet, L, Vulpe, C, Lehmann, S. Impact of silver nanowire length and diameter on rainbow trout RTgillW1 and RTgutGC cell lines.
Sylvia Lehmann, Gilbert B, Viau M, Toybou D, Simonato JP, Cell C, Maffeis, T, Charlet L. Toward safer silver nanowires by design: Modulation of characteristics and evaluation of dermal toxicity.
Caroline Celle, Thomas Sannicolo, Djadidi Toybou, Anthony Cabos, Jean-Pierre Simonato. Flexible transparent film heaters based on random networks of silver nanowires: synthesis, characterization and integration into devices.

Colloquium, Department of Physics, U W-Madison, Jan 26th 2018
Benjamin Gilbert et al. Nanowire technology and toxicity *Oral*

E-MRS Fall Meeting Warsaw, Poland, September 18-21 2017
Djadidi Toybou, Caroline Celle, Laurent Charlet, Jean-Pierre Simonato. A Suitable method to integrate silver nanowires on common optoelectronic applications. *Oral*

Regional Southeastern SETAC Meeting, Brunswick, Georgia, USA. September 28-30, 2017.
Devrah Arndt, Djadidi Toybou, Benjamin Gilbert, Sylvia Lehmann, Laurent Charlet, Brenda Omana, Annette Hofmann, Christopher Vulpe. Altering silver nanowire dimensions to reduce toxicity and achieve safety by design. *Oral.*

ISMET General Meeting Lisbon, Portugal. October 3-6, 2017
Brenda Omana Sanz, Hofmann, A., Lesven, L., Sobanska, S., Gilbert, B., Charlet, L. Electrochemical silver removal and recovery from nanowires in fuel cell. *Poster*

National SETAC Meeting, Minneapolis, Minnesota, USA. November 12-16, 2017.
Devrah Arndt, Sylvia Lehmann, Djadidi Toybou, Caroline Cell, Benjamin Gilbert, Christopher Vulpe. Differential toxicity of silver nanowires with different dimensions: Achieving safety by design. *Poster.*

Quarterly Reports submitted to the Consumer Product Safety Commission (US funding agency)
Devrah Arndt, Christopher Vulpe. Updates on the progress of the work.
Submitted March 26th, 2017; Submitted June 26th, 2017; Submitted September 26th, 2017;Submitted December 26th, 2017.

3 Transnational collaboration and project management

Management and communication
The NANOWIR^{2}ES project uses a range of communication methods to coordinate the research. The full group meets quarterly using internet video communication (Zoom). Topical subgroups and collaborations communicate more frequently to coordinate sample and data exchange and other issues.

In addition, we convene special sessions at international conferences as an opportunity for face-to-face meetings and to disseminate the work to the broader nanosafety and related communities. In April 2017, we organized a session at the American Chemical Society meeting in San Francisco, USA. In October 2018 we will organize 2 sessions at the Reunion des Science de la Terre meeting in Lille, France. We seek to have all project partners join these sessions and have offered financial support for attendance from LEITAT, Spain (Partner 2).

To improve communication around the project, a **website** has been built, presenting the consortium, main project objectives and research. It is used by all consortium members for communication about the project.
http://nanowir2es.univ-lille.fr/

Added value of transnational collaboration
The consortium brings together scientists of very **different disciplines**, in particular material scientists, biologists, ecotoxicologists and geochemists. The integrated investigation of Ag NW undertaken in this project, combining synthesis, design, risk and recyclability, is bringing forward **ecodesign strategies for nanomaterials and** will benefit industrial applications of new generation products.

Grenoble partner with CEA group LITEN plays a central role in the project as they provide all consortium members with Ag NW materials, synthesized specifically to meet the specific needs of the various experiments. The whole project is built on this very **tight national and transnational collaboration** between Grenoble and all partners.
Added value of collaboration between partners is found at all levels of the project.
Research on Ag NW toxicity conducted by US partner 4 and by Grenoble partner 3 is complementary with respect to the techniques employed, targeted cells, and fields investigated (environmental versus human health).The transnational collaboration of the two research groups of renown international level is most beneficial to the advancement of the project and to strong **research impact**.
In the field of material science and recycling, the collaboration between geochemists of the Lille group and Grenoble/CEA is very effective. Tools are being developed to **link** NW thin film design and the possibilty of Ag+ release. Barcelona partner 2 (LEITAT) is now provided with a commercially available AgNW product and will be able to conduct the Ag leaching study in simulated landfill. Their expertise in environmental release is **favorably linking** to the degradation and recycling research conducted by Lille partner 1.

4 Deviations to the original project plan

Preceding the start of the project, several changes to the initial proposal were made, described in a letter of statement sent to the ERA-NET SIINN call office in September 2015 and a modification to proposal joined to the consortium agreement and signed in March 2016.
Major changes concerned the **synthesis of Ag nanowires** which was taken over by Dr. Simonato's group at CEA LITEN in Grenoble, collaborating with Professor Charlet, and providing NW at no charge to the NanoWIR2ES participants. Dr. Caroline Celle in Simonato's group is implicated in all aspects of synthesis.

Objectives in **WP3** concerning the **leaching of NW enabled thin films and devices** is conducted by **Leitat**, at the discretion of their financial capabilities. We have now identified one commercially available product containing a Conductive Transparent Network with silver (CTN). This product has been acquiered by the Lille partner and was shipped to Leitat. The leaching studies are planned for year 3.

Work packages WP4 and WP5 in the NanoWIR2ES project, conducted by **Partner 3 UGA,** were limited to 2 years in the initial proposal because the funding for Sylvia Lehmann only extended until February 2018. Partner 3 has reached the principal objectives of their project which have been summarized in a scientific paper that is about to be submitted. The contract of Benjamin Gilbert as an invited researcher in the SERENADE network has been requested for another 2 years, until 2020. Partner 3 will remain an active member in the NanoWIR2ES consortium until the end of the project in 2019.

Partner 4 UFG is facing a number of analytical challenges. The Seahorse XF Bioinstrument, allowing the observation of parameters of cell metabolism recently stopped working and has been sent to the manufacturer for repair (delay of WP6.2). The single particle ICPMS technique was proposed for the quantification of cellular uptake in WP7 (T7.1) and for determining tissue distribution (T7.2). The application of this technique is limited because the AgNWs in our study are too large (lengths of 10-25 µm) for homogeneous transport by the nebulizer in the instrument. Partner 4 is uncertain wether this issue can be resolved within time and money constraints. It was previously determined that AgNWs could be easily imaged using darkfield microscopy. Unfortunately, the confocal microscope available at UFG is not equipped with darkfield capabilities. Also, silver nanowires are not functionalized, so it is not possible to attach a fluorescent probe to the nanowires for visualization. Partner 4 is exploring additional resources to accomplish the goals of WP7. WP8 is delayed because results from toxicity assays in WP6 and uptake studies in WP7 are expected to inform the design of the exposures for RNAseq analysis (previous results from toxicity and uptake studies will indicate the optimal time course and exposure concentrations for gene expression analysis).

The research actions by **Partner 1 ULille** are focusing on the development of an electrochemical cell for the oxydative extraction of the Ag from CTN. Here the thickness of the protective coating of AgNW CTNs is a crucial factor conditionning the extend of recovery of silver. It appears that even thick coatings cannot inhibit Ag^+ release completely. This finding motivates partner 1 to consider the technique for silver recovery also as a tool for describing the risk of Ag^+ release during environmental chemical degradation of a device containing AgNW CTNs. In year 3, this aspect will be explored in parallel to the principal goal of recovering Ag^+ from CTNs.

Use of resources
Resources have been used as intended initially. Partners 1 and 4 are the only partners that received funding for the NanoWIR2ES project. The post-doctoral researchers hired at the start of the project in 2016 (Brenda Oman a-Sanz, Devrah Arndt) have been maintained in their positions. Both partners have spent resources for attending conferences in San Francisco-USA (April 2017), Lisbon-Portugal (October 2017), and Minneapolis, MN, USA (November 2017).

Partner 1 has allocated resources for consumables for the experimental work, for electron-microscopy machine time and for chemical analyses. Resources for partner 4 have been allocated to cell culture maintenance, cytotoxicity assays, RNAseq analysis software, and aquarium set up and maintenance for rainbow trout.

5 Final comments

Objectives for the coming year are following the key goals initially defined. Main objectives are summarized below:

Intrinsic toxicity of silver nanowires (Ag NW) comprising studies on cellular toxicity, biological effects of exposure, environmental toxicity:

Cytotoxicity assays using AgNWs of different lengths and diameters are completed. The following objectives will be addressed in the following year:

1. Toxicity of AgNWs to whole organisms (WP6.3) will be completed. AgNW toxicity will be explored in chorionated and de-chorionated embryos and in rainbow trout fry with static renewal exposure designs.

2. Time course exposures for uptake (WP7.1) and bioaccumulation (WP7.2) of AgNWs in cells and tissues will be completed using confocal darkfield microscopy. Access to confocal darkfield microscopy is currently being explored and may require travel to an enabled facility.

3. RNA extraction and library creation for RNAseq experiments and analysis (WP8).

Release & recovery studies of silver from conducting films

Standard condition have been defined for the recovery of silver in AgNW CTNs. In the coming year following goals will be reached :

1. Extend of Ag release from AgNW CTN will be quantified as a function of thickness of coating, electrochemical cell potential and pH (D.9.1), leading to a tool to measure AgNW weatherability in CTN's.

2. Sustainable materials for Ag+ sorption have been identified. Adsorption isotherms will be determined at several pHes and the best suited material will be retained for integration in the silver release reactor.

3. Findings in the above will be summarised in an international research journal.

4. The differential anodic stripping voltametry method for the semi-quantitative measurement of Ag^+ concentrations, set-up in year 2, will be improved.

Outreach for commercially relevant nanowire enabled devices:

One commercial item has been purchased during year 2. The effort to find other products will be continued.

Silver release in simulated landfill conditions

Data will be acquired for commercial AgNW containing products (D 3.2). At minimum one device, already indentified, will be investigated.

Progress report

Please, note:

- The same font and style should be used for the whole report (Times New Roman, 11pt, single spaced).
- Adhere to the given page limits.
- All of the following sections have to be filled in.

This report must be submitted to the SIINN Call Office (siinn2014@fct.pt) within 60 days of the due date.

Project Acronym:

NanoWIR2ES

Project Full Title:

NanoWire Intelligent Re-design and Recycling for Environmental Safety

Runtime of the project:

☐ 1st year ☒ 2nd year ☐ 3rd year

Period covered:

From 01/04/2017 to 31/03/2018

Contact Data (Project Coordinator)

Name	Annette Hofmann
Organisation	University de Lille Sciences et Technologies
Country	France
Address	Sciences de la Terre, bâtiment SN5, Av. Paul Langevin, 59655 Villeneuve d'Asc
Phone	+33 320 43 41 49
E-mail	annette.hofmann@univ-lille1.fr

Partners

Number	Country	Organisation	Principal Investigator[1]	Other personnel[2]
1 Coordinator	France	Université de Lille Sciences et Technologies (ULille)	Annette Hofmann	Brenda Omana-Sanz Sophie Sobanska Ludovic Lesven Tech. personnel
2	Spain	Acondidionamien to Tarrasense (LEITAT)	Vincent Jamier	Tech. Personnel
3	France	Université Grenoble Alpes (UGA)	Laurent Charlet	Sylvia Lehmann Benjamin Gilbert Djadidi Toybou Caroline Celle Jean-Pierre Simonato
4	US	University of Florida, Gainsville (UFG)	Chris Vulpe	Devrah Arndt Tech personnel

[1] The Principal Investigator (PI) is the point of contact of the partner for the corresponding Funding Organisation
[2] Name of other personnel participating in the project

1 Project objectives and work progress

The project covers several aspects of safety and safety improvement of Ag-nanowire in view of designing products with minimized potential exposure and health risks for consumers. The fields addressed in year 2 (see Gantt chart) are in line with the work plan described in the joined proposal. Following fields were covered: (i) materials design, synthesis and purification, (ii) intrinsic toxicity of silver nanowires (AgNW) comprising studies on cellular toxicity, environmental toxicity and elaboration of metrics for safer-by-design AgNW geometries, (iii) release and recovery studies of silver from conducting films, (iv) outreach for commercially relevant nanowire enabled devices.

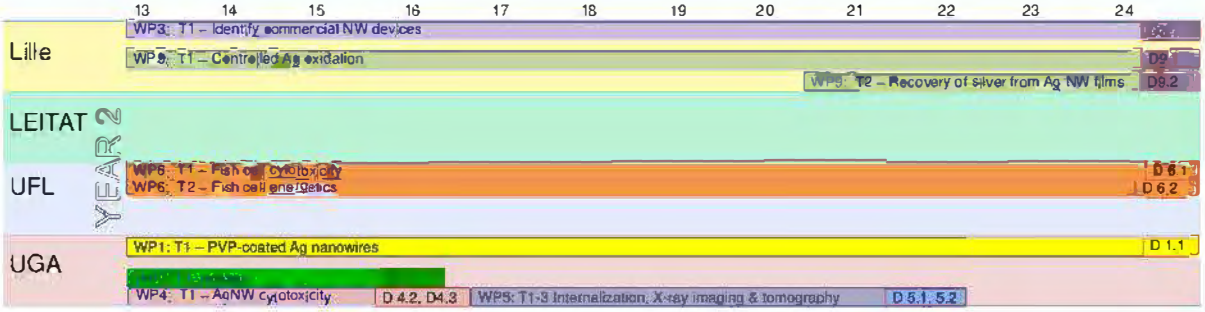


Table 1 : Institutional abbreviations: Lille is the University of Lille (A. Hofmann); LEITAT is the Spanish Technological Center specialized in production technologies in Barcelona (V. Jamier); UFL is the University of Florida – Gainsville (C. Vulpe); UGA is the University of Grenoble Alps (L. Charlet). **D** = Deliverable, **WP** = Work Package, **T** = Talk

Outline of work carried out over the past year by each partner

Partner 3 Grenoble-France (CEA & UGA) - Djadidi Toybou, Caroline Celle, Jean-Pierre Simonato, Laurent Charlet, Benjamin Gilbert

Materials design (WP1)

The synthesis and characterisation of AgNW with well-controlled physical and chemical properties, has been continued. While in year 1, special emphasis was placed on synthesis of **AgNW with different dimensions,** work in year 2 was dedicated to the study of the **reproducibility** of the controlled synthesis of AgNW's, to the improvement of the **purification** of the NW suspensions and to the evaluation of **optoelectric** performances.

Following the parametric studies reported during the last period, reproducibility of NW synthesis was evaluated for four batches of AgNWs with specific aspect ratios. AgNW of 4, 8 and 10 um length and 30, 44 and 55 nm diameter were synthesized twice under same conditions. A very good level of reproducibility in terms of diameter and length was obtained thus reflecting high control on the methodology.

Because toxicity assays (WP 4-8) need AgNW suspensions of high grade purity, a special purification method was developed capable of removing the Ag nanoparticles that form during NW synthesis and that are still present in the NW suspension after standard purification. By applying successive settling steps in methanol solvent, a highly purified AgNW suspension is obtained with less than 3 % NP.

The high grade AgNW suspensions were spray coated onto polymer substrate for studying NW dimension impact on optoelectrical properties. Indeed, transparency and conductivity of the flexible electrodes are influenced by length and width of the NW used (Fig. 1). We showed that the high quality of optoelectrical properties already achieved with our standard AgNW, could be further tuned by modulating NW dimensions. Highly purified silver nanowire suspensions exhibiting very good optoeletrical properties have been provided to all partners involved in toxicological studies.

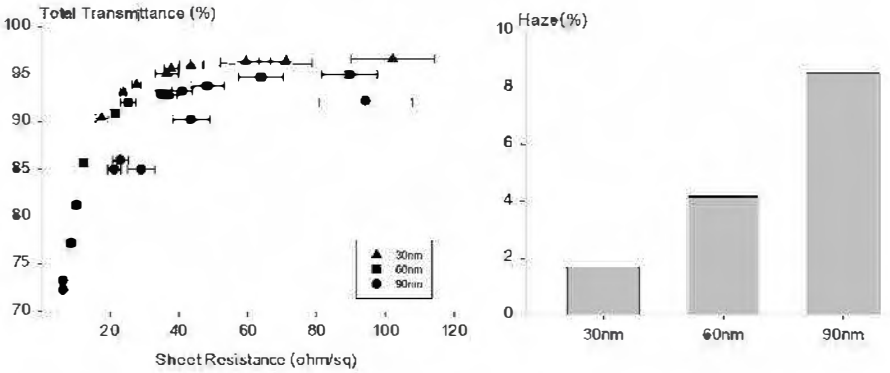


Figure 1. Total transmittance as a function of the sheet resistance of transparent conductive electrodes made of AgNWs with a length of 10µm and diameters ranging from 30 to 90 nm. Associated haze factor.

Deliverables from year 2 are (a) the high level control on the synthesis of AgNW with specific dimensions, (b) an improved purification method for NW suspensions and (c) fine tuning of opto-electric parameters for flexible AgNW electrodes (D1.1).

Partner 3 Grenoble-France (UGA) - Sylvia Lehmann, Benjamin Gilbert, Laurent Charlet
Internalization of AgNW in dermal cells, viability assays, optical & X-ray microscopy studies (WP 2, 4, 5)

Keratocytes and fibrobasts, the major cells of the epidermis and dermis layers of skin, have been used as model skin cells in AgNW exposure experiments. Cytotoxicity assays were mainly conducted in year 1 (MTT, RNU, ATP measurements). Additional experiments conducted in year 2 targeted AgNW cell injury factors of the oxidative-stress hierarchie with following probes : CM-H2DCFDA, a fluorescent probe for general ROS, JC-1, a probe for the determiation of mitochondrial membrane potential, ψ_m. Fluo-4AM used to probe Ca^{2+} release, and propidium iodide (PI) to trace loss of membrane integrity of apoptotic cells.
The toxicity tests were designed as a function of AgNW dimensions. NWs were synthesized and made available by CEA-Grenoble partner WP1. The development of an AgNW suspension purification method conducted by this group has proven essential to ensure reproducible toxicity results.

While the factors favoring **NW internalization**, the relation between **internalization and cell viability**, and the **toxicity** of Ag ions compared to nanowires have been addressed in year 1, the second year was dedicated to characterising the mechanisms of toxicity produced by internalized AgNW. It was shown that ROS production occurs when AgNW are internalized but that it is dose dependent. Also, thin and thick NW (30- and 90-nm diameter) had clearly distinguishable toxicity effect. Assays for mitochondrial membrane potential and Ca^{2+} release confirmed the major influence of NW diameter on the cell response.

Optical- and X-ray microscopy studies initiated in year 1 were continued during the second year. We used a combination of synchrotron X-ray imaging and microchemical analysis methods at beamlines ID-16A and ID-21 at the ESRF to study the intracellular location, morphology and chemistry of 30-nm and 90-nm diameter 10-µm AgNW administered to murine fibroblasts. Holographic X-ray nano-imaging generated 2D projection images (phase contrast maps) and stereographic images with ~40-nm resolution. Full 3D reconstructions with ~80-nm resolution were obtained from holotomography. Nanofocus X-ray fluorescence (XRF) of the same specimens provided 2D elemental maps with approximately 40-nm resolution. Microfocus XRF of duplicate samples provided 2D elemental maps that could be complemented with Ag L_{III}-edge X-ray absorption spectra to determine silver speciation.

Key findings from these studies show that AgNW fate and cellular toxicity is determined by the interplay of the membrane forces generated during NW internalization, the bending stiffness of the AgNW and the strength of the plasma membrane. Although 30- and 90-nm diameter AgNW are readily internalized, the thinner NW are mechanically crumpled by the forces of endocytosis while the thicker nanowires puncture the enclosing membrane, release silver ions and lysosomal contents to the

cytoplasm thereby initiating oxidative stress. This finding extends the fiber pathology paradigm and will enable the manufacture of safer nanowire enabled consumer products (Fig. 2).

The main objective of WPs 4 and 5, i.e. to understand the mechanism of cytotoxicity of AgNW and to tailor AgNW with lower risk of celluar injury has been reached. Also, we were able to show that the proposed safer AgNWs do not deteriorate the performance of the target technology (Fig. 1). Results of this study are summarised in a manuscript that is about to be submitted to a highly quoted international journal of research.

Deliverables from year 2 comprise (a) Ag+ release and toxicity effect (D2.1), (b) the role of AgNW diameter in cell injury (D4.2, D5.1, D5.2), (b) mechanical forces and chemical reactions excerted on AgNW inside the cell (D4.3), (c) new AgNW dimensions for safer NW enabled technology (final goal).

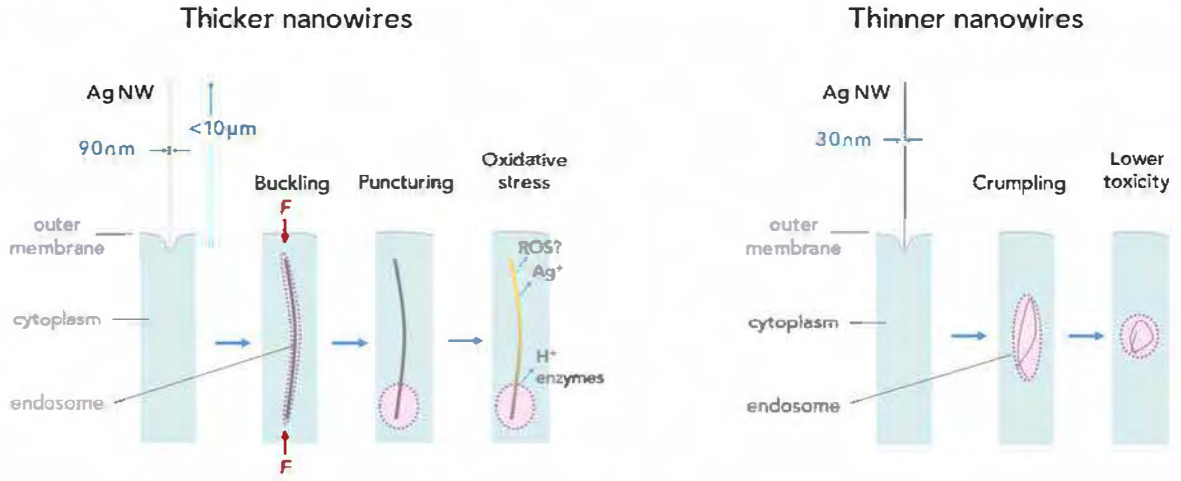


Figure 2. Diagram showing the biomechanical mechanism for silver nanowire (Ag NW) toxicity. Thicker and stiffer Ag NW are more cytotoxic because they have a greater tendency to puncture endolysosome membranes following internalization.

Partner 4 (Florida, US) - Devrah Arndt, Chris Vulpe, Benjamin Gilbert
Cellular, organismal and environmental toxicity (WP 6, 7, 8)

Cytotoxicity studies on rainbow trout gill and intestinal cells started in year 1 were continued and refined. Partner 3 CEA Grenoble (WP1) synthesized Ag NW of different dimensions for these studies. A major result of this year's studies is that AgNW concentration and dimensions do influence the toxicity to rainbow trout cells.

Results of metabolic assays (Seahorse XFe24 Analyzer) conducted on rainbow trout cells indicate that thick silver nanowires (90 nm diameter) significantly reduce basal metabolism and ATP production in gill and gut cells at higher concentrations (above 120 ug/mL). Thin silver nanowires (30 nm diameter) had no effect on basal metabolism or ATP production in rainbow trout gill cells (Figure 3a and 3b) while proton leakage was decreased by thin silver nanowires and increased by thick silver nanowires (Figure 3c). The reserve capacity makes up the energetic reserve that can be used by the cell in times of stress, and all treatments of silver nanowires significantly reduced the reserve capacity of trout cells (Figure 3d). These results are consistent with the findings in WP 4 and 5 and provide more insights into the mechanism through which NW geometry plays a major role in the cell toxicity.

Difficulties were encountered with the standard cell culture media (L15) that reacted with the $AgNO_3$ in the toxicity control assay, thereby alleviating the toxic effect of the silver ion. Therefore an in-house made media (L15-ex) was developed with decreased salt concentrations and without amino acids. Using this media, interference of media components with silver ions was resolved, and $AgNO_3$ exposure tests could be used again as a toxicity reference.

AgNW exposures in L15-ex media revealed differential toxicity of AgNWs regarding length, where long nanowires induced significantly more toxicity to rainbow trout cells than short nanowires. These results are somewhat contradictory to other findings (WP4) and will be investigated further. It is suspected that this observation is due to different synthesis and purification procedures used to make short versus long AgNWs.

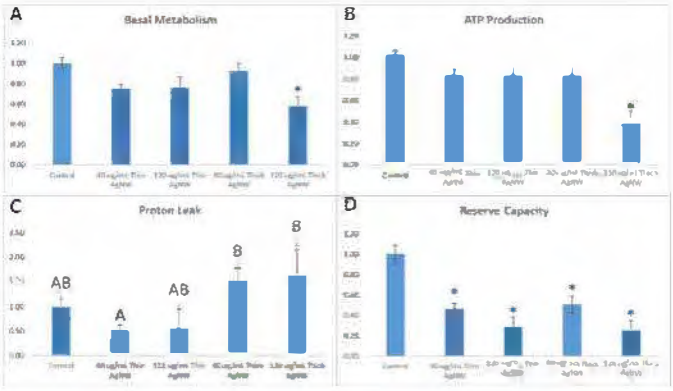


Figure 3 : Impact of thick and thin AgNWs on rainbow trout gill cells. A) basal metabolism, B) ATP production, C) proton leak, and D) reserve capacity. * denotes significant at $p < 0.05$. A/B markers in C. denote significant difference at $p < 0.05$ for comparisons between all treatments (in addition to comparisons between treatments and the control).

Deliverables of year 2 comprise (a) aqueous environmental factors can modulate AgNW toxicity (D6.1.2), (b) development of a specific cell culture media compatible with silver (D6.1.2), and (c) AgNW thickness influences cellular metabolism (D6.2.1).

Partner 1 Lille, France (ULille) - Brenda Omana, Annette Hofmann, Ludovic Lesven, Sophie Sobanska, Benjamin Gilbert

Release and recovery studies of silver from conducting films (WP9)

Silver NW are emerging materials entering the production of ultra thin conducting films for advanced electronic, optic and textile materials and devices. Here we are developping an electrochemical **method for reclaiming Ag from Ag NW in transparent electrode technology** with the aim to reduce the risk of **toxicologically critical Ag compounds** entering an e-Waste stream, and to develop ways to recover and reuse nano Ag, using sustainable methodology.

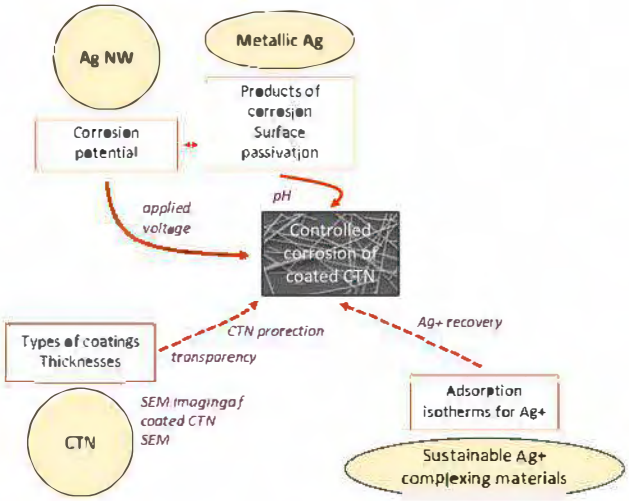


Figure 4 : While the red boxes represent processes and technology critical for the development of a Ag reclamation technique, the arrows represent the specific parameters that need to be fine-tuned. Full red lines indicate that we have already determined optimal parameters while dashed lines indicate that experimental work is still in progress. Initial experiments have already shown that corrosion of CTN coated with polysiloxane resin was possible under some conditions. Initial conditions will be modulated in further experiments (pH, applied electric potential, coating thickness) to explore the field of reactivity and applicability.

In year 1, we showed that the option of a fuel cell for Ag recovery, using oxygen from air as a reductant, was difficult to set up despite thermodynamically possible, because of the omni-presence of dioxygen in all compartments. In year 2, we have not developped the fuel cell approach further, but used an external power supply to provoke Ag corrosion. Conversely, we have focused on all other key elements needed to extract Ag from Conductive Transparent Networks (CTN) electrochemically.

Figure 4 illustrates the factors needed to corrode AgNW under optimal conditions, the constraints on corrosion due to polymeric coatings used by industrials to protect the CTN as well as our desire to closely couple CTN corrosion and Ag^+ recovery and storage.

Deliverables from year 2 : (a) Ag NW corrosion potential (D 9.1), (b) Ag corrosion products under atmospheric conditions (D 9.1), (c) sustainable materials for Ag^+ adsorption (D 9.2)

2 Impact[3]

Safety-by-Design

The key finding of this project from years 1 and 2 is the demonstration of a robust link between Ag NW diameter and toxicity in mouse, fish and human cell lines and the discovery of a new mechanism of nanowire toxicity associated with the puncturing of endolysosomes. This work demonstrates success in coupling **NW risks and NW design**, and the determination of **safest design for minimum risk solutions** while maintaining **technical performance** is being reached. The findings are anticipated to have high impact on the manufacturing approaches for consumer devices that incorprorate Ag-NW. The findings are summarized in a paper to be published soon. We expect a strong **impact on NM safety issues, safety by design development and eco-friendly high end novel products**.

Framework for Ag NW life-cycle risk assessment and mitigation

Over year 2, the project partners consolidated the **framework around Ag NW** exposure assessment, toxicity mechanisms and effects on human health and environment.

Ag NW environmental and cellular exposure studies have been conducted in direct link with NW material design studies, metrics that can act as toxicity indicator parameters have been finetuned (WP1, WP4-6). The effort of linking toxicity studies to material design and physical / chemical mechanisms of toxicity continued in year 2. The important linkages between NW diameter and length, cell internalization and cell viability have been further confirmed.

The differential toxicity of AgNWs in aqueous environments of different ionic strengths (fresh- and saltwater environments) has been clearly indentified via the use of different culture media, leading us to also think culture media as a tool to quantify enviromnmental toxicity.

Data Upload

During the May-June 2018 Safer Nanodesign summer school (see below), co-PI's Gilbert and Charlet attended a tutorial hosted by Nikolay Kochev, co-PI of eNanoMapper, to learn how to upload experimental nanotoxicity data into EU databases. We will use the Excel templates to capture the cytotoxicity and ecotoxicity data and upload them.

End-of-life silver capture

The technique implementing controlled release of silver from coated AgNW CTNs has progressed (WP1, WP9). Although we are not yet certain to achieve complete recovery of silver from CTNs in this recycling effort, it appears that the electrochemical technique can provide metrics for releasability of Ag^+ from coated CTNs, for safety and environmental impact, and for a **"design for recycling"** approach.

Education and Outreach

Co-Investigators Charlet and Gilbert participate in the annual Safer NanoDesign summer school in Archamps, France. The website for the 2018 school is at:
http://www.esi-archamps.eu/Thematic-Schools/Discover-bioHC/SAFERNANO
During this school, the NANOWIR²ES project is used as an example for assessing the potential nanotoxicity of engineered nanomaterials and the use of collaboration between materials scientists,

[3] Include link and details for any mentioned publication, report, patent or communication.

geoscientists and toxicologists to re-design materials to reduce toxicity during use and at the end of a product life.

Dissemination of results:

253rd ACS - Annual spring meeting, San Francisco 2-6 April 2017

We organized a dedicated session that included talks that described the NANOWIR^{2}ES project and recent results to an international audience.

Session: Chemical Principles of Environmental, Cellular & Organismal Nanotoxicology.
Organisers : C. Celle, L. Charlet, B. Gilbert, S. Lehman, J. Simonato, C. Vulpe.

Orals
Devrah Arndt, Toybou, D, Simonato, JP, Celle, C, Gilbert, B, Charlet, L, Vulpe, C, Lehmann, S. Impact of silver nanowire length and diameter on rainbow trout RTgillW1 and RTgutGC cell lines.
Sylvia Lehmann, Gilbert B, Viau M, Toybou D, Simonato JP, Cell C, Maffeis, T, Charlet L. Toward safer silver nanowires by design: Modulation of characteristics and evaluation of dermal toxicity.
Caroline Celle, Thomas Sannicolo, Djadidi Toybou, Anthony Cabos, Jean-Pierre Simonato. Flexible transparent film heaters based on random networks of silver nanowires: synthesis, characterization and integration into devices.

Colloquium, Department of Physics, U W-Madison, Jan 26th 2018
Benjamin Gilbert et al. Nanowire technology and toxicity *Oral*

E-MRS Fall Meeting Warsaw, Poland, September 18-21 2017
Djadidi Toybou, Caroline Celle, Laurent Charlet, Jean-Pierre Simonato. A Suitable method to integrate silver nanowires on common optoelectronic applications. *Oral*

Regional Southeastern SETAC Meeting, Brunswick, Georgia, USA. September 28-30, 2017.
Devrah Arndt, Djadidi Toybou, Benjamin Gilbert, Sylvia Lehmann, Laurent Charlet, Brenda Omana, Annette Hofmann, Christopher Vulpe. Altering silver nanowire dimensions to reduce toxicity and achieve safety by design. *Oral.*

ISMET General Meeting Lisbon, Portugal. October 3-6, 2017
Brenda Omana Sanz, Hofmann, A., Lesven, L., Sobanska, S., Gilbert, B., Charlet, L. Electrochemical silver removal and recovery from nanowires in fuel cell. *Poster*

National SETAC Meeting, Minneapolis, Minnesota, USA. November 12-16, 2017.
Devrah Arndt, Sylvia Lehmann, Djadidi Toybou, Caroline Cell, Benjamin Gilbert, Christopher Vulpe. Differential toxicity of silver nanowires with different dimensions: Achieving safety by design. *Poster.*

Quarterly Reports submitted to the Consumer Product Safety Commission (US funding agency)
Devrah Arndt, Christopher Vulpe. Updates on the progress of the work.
Submitted March 26th, 2017; Submitted June 26th, 2017; Submitted September 26th, 2017;Submitted December 26th, 2017.

3 Transnational collaboration and project management

Management and communication
The NANOWIR^{2}ES project uses a range of communication methods to coordinate the research. The full group meets quarterly using internet video communication (Zoom). Topical subgroups and collaborations communicate more frequently to coordinate sample and data exchange and other issues.

In addition, we convene special sessions at international conferences as an opportunity for face-to-face meetings and to disseminate the work to the broader nanosafety and related communities. In April 2017, we organized a session at the American Chemical Society meeting in San Francisco, USA. In October 2018 we will organize 2 sessions at the Reunion des Science de la Terre meeting in Lille, France. We seek to have all project partners join these sessions and have offered financial support for attendance from LEITAT, Spain (Partner 2).

To improve communication around the project, a **website** has been built, presenting the consortium, main project objectives and research. It is used by all consortium members for communication about the project.
http://nanowir2es.univ-lille.fr/

Added value of transnational collaboration
The consortium brings together scientists of very **different disciplines**, in particular material scientists, biologists, ecotoxicologists and geochemists. The integrated investigation of Ag NW undertaken in this project, combining synthesis, design, risk and recyclability, is bringing forward **ecodesign strategies for nanomaterials and** will benefit industrial applications of new generation products.

Grenoble partner with CEA group LITEN plays a central role in the project as they provide all consortium members with Ag NW materials, synthesized specifically to meet the specific needs of the various experiments. The whole project is built on this very **tight national and transnational collaboration** between Grenoble and all partners.
Added value of collaboration between partners is found at all levels of the project.
Research on Ag NW toxicity conducted by US partner 4 and by Grenoble partner 3 is complementary with respect to the techniques employed, targeted cells, and fields investigated (environmental versus human health).The transnational collaboration of the two research groups of renown international level is most beneficial to the advancement of the project and to strong **research impact**.
In the field of material science and recycling, the collaboration between geochemists of the Lille group and Grenoble/CEA is very effective. Tools are being developed to **link** NW thin film design and the possibilty of Ag+ release. Barcelona partner 2 (LEITAT) is now provided with a commercially available AgNW product and will be able to conduct the Ag leaching study in simulated landfill. Their expertise in environmental release is **favorably linking** to the degradation and recycling research conducted by Lille partner 1.

4 Deviations to the original project plan

Preceding the start of the project, several changes to the initial proposal were made, described in a letter of statement sent to the ERA-NET SIINN call office in September 2015 and a modification to proposal joined to the consortium agreement and signed in March 2016.
Major changes concerned the **synthesis of Ag nanowires** which was taken over by Dr. Simonato's group at CEA LITEN in Grenoble, collaborating with Professor Charlet, and providing NW at no charge to the NanoWIR2ES participants. Dr. Caroline Celle in Simonato's group is implicated in all aspects of synthesis.

Objectives in **WP3** concerning the **leaching of NW enabled thin films and devices** is conducted by **Leitat**, at the discretion of their financial capabilities. We have now identified one commercially available product containing a Conductive Transparent Network with silver (CTN). This product has been acquiered by the Lille partner and was shipped to Leitat. The leaching studies are planned for year 3.

Work packages WP4 and WP5 in the NanoWIR2ES project, conducted by **Partner 3 UGA,** were limited to 2 years in the initial proposal because the funding for Sylvia Lehmann only extended until February 2018. Partner 3 has reached the principal objectives of their project which have been summarized in a scientific paper that is about to be submitted. The contract of Benjamin Gilbert as an invited researcher in the SERENADE network has been requested for another 2 years, until 2020. Partner 3 will remain an active member in the NanoWIR2ES consortium until the end of the project in 2019.

Partner 4 UFG is facing a number of analytical challenges. The Seahorse XF Bioinstrument, allowing the observation of parameters of cell metabolism recently stopped working and has been sent to the manufacturer for repair (delay of WP6.2). The single particle ICPMS technique was proposed for the quantification of cellular uptake in WP7 (T7.1) and for determining tissue distribution (T7.2). The application of this technique is limited because the AgNWs in our study are too large (lengths of 10-25 µm) for homogeneous transport by the nebulizer in the instrument. Partner 4 is uncertain wether this issue can be resolved within time and money constraints. It was previously determined that AgNWs could be easily imaged using darkfield microscopy. Unfortunately, the confocal microscope available at UFG is not equipped with darkfield capabilities. Also, silver nanowires are not functionalized, so it is not possible to attach a fluorescent probe to the nanowires for visualization. Partner 4 is exploring additional resources to accomplish the goals of WP7. WP8 is delayed because results from toxicity assays in WP6 and uptake studies in WP7 are expected to inform the design of the exposures for RNAseq analysis (previous results from toxicity and uptake studies will indicate the optimal time course and exposure concentrations for gene expression analysis).

The research actions by **Partner 1 ULille** are focusing on the development of an electrochemical cell for the oxydative extraction of the Ag from CTN. Here the thickness of the protective coating of AgNW CTNs is a crucial factor conditionning the extend of recovery of silver. It appears that even thick coatings cannot inhibit Ag^+ release completely. This finding motivates partner 1 to consider the technique for silver recovery also as a tool for describing the risk of Ag^+ release during environmental chemical degradation of a device containing AgNW CTNs. In year 3, this aspect will be explored in parallel to the principal goal of recovering Ag^+ from CTNs.

Use of resources
Resources have been used as intended initially. Partners 1 and 4 are the only partners that received funding for the NanoWIR2ES project. The post-doctoral researchers hired at the start of the project in 2016 (Brenda Oman a-Sanz, Devrah Arndt) have been maintained in their positions. Both partners have spent resources for attending conferences in San Francisco-USA (April 2017), Lisbon-Portugal (October 2017), and Minneapolis, MN, USA (November 2017).

Partner 1 has allocated resources for consumables for the experimental work, for electron-microscopy machine time and for chemical analyses. Resources for partner 4 have been allocated to cell culture maintenance, cytotoxicity assays, RNAseq analysis software, and aquarium set up and maintenance for rainbow trout.

5 Final comments

Objectives for the coming year are following the key goals initially defined. Main objectives are summarized below:

Intrinsic toxicity of silver nanowires (Ag NW) comprising studies on cellular toxicity, biological effects of exposure, environmental toxicity:

Cytotoxicity assays using AgNWs of different lengths and diameters are completed. The following objectives will be addressed in the following year:

1. Toxicity of AgNWs to whole organisms (WP6.3) will be completed. AgNW toxicity will be explored in chorionated and de-chorionated embryos and in rainbow trout fry with static renewal exposure designs.

2. Time course exposures for uptake (WP7.1) and bioaccumulation (WP7.2) of AgNWs in cells and tissues will be completed using confocal darkfield microscopy. Access to confocal darkfield microscopy is currently being explored and may require travel to an enabled facility.

3. RNA extraction and library creation for RNAseq experiments and analysis (WP8).

Release & recovery studies of silver from conducting films

Standard condition have been defined for the recovery of silver in AgNW CTNs. In the coming year following goals will be reached :

1. Extend of Ag release from AgNW CTN will be quantified as a function of thickness of coating, electrochemical cell potential and pH (D.9.1), leading to a tool to measure AgNW weatherability in CTN's.

2. Sustainable materials for Ag+ sorption have been identified. Adsorption isotherms will be determined at several pHes and the best suited material will be retained for integration in the silver release reactor.

3. Findings in the above will be summarised in an international research journal.

4. The differential anodic stripping voltametry method for the semi-quantitative measurement of Ag^+ concentrations, set-up in year 2, will be improved.

Outreach for commercially relevant nanowire enabled devices:

One commercial item has been purchased during year 2. The effort to find other products will be continued.

Silver release in simulated landfill conditions

Data will be acquired for commercial AgNW containing products (D 3.2). At minimum one device, already indentified, will be investigated.

Progress report n° 1

Please, note:

- The same font and style should be used for the whole report (Times New Roman, 11pt, single spaced).
- Adhere to the given page limits.
- All of the following sections have to be filled in.

This report must be submitted to the SIINN Call Office (siinn2014@fct.pt) within 60 days of the due date.

Project Acronym:

NanoWIR2ES

Project Full Title:

NanoWire Intelligent Re-design and Recycling for Environmental Safety

Runtime of the project:

☐ 1st year ☒ 2nd year ☐ 3rd year

Period covered:

From 01/04/2016 to 31/03/2017

Contact Data (Project Coordinator)

Name	Annette Hofmann
Organisation	Université Lille 1 Sciences et Technologies
Country	France
Address	Sciences de la Terre bâtiment SN5, Av. Paul Langevin, 59655Villeneuve d'Ascq
Phone	+33 320 43 41 49
E-mail	annette.hofmann@univ-lille1.fr

Partners

Number	Country	Organisation	Principal Investigator[1]	Other personnel[2]
1 Coordinator	France	Université Lille 1 Sciences et Technologies (ULille)	Annette Hofmann	Brenda Omana-Sanz Sophie Sobanska Ludovic Lesven Tech. personnel
2	Spain	Acondicionamiento Tarrasense (LEITAT)	Vincent Jamier	Tech. personnel
3	France	Université Grenoble Alpes (UGA)	Laurent Charlet	Sylvia Lehmann Muriel Viau (till 20/11/16) Benjamin Gilbert Djadidi Toybou Caroline Celle Jean-Pierre Simonato
4	US	University of Florida, Gainsville (UFG)	Chris Vulpe	Devrah Arndt Tech. personnel

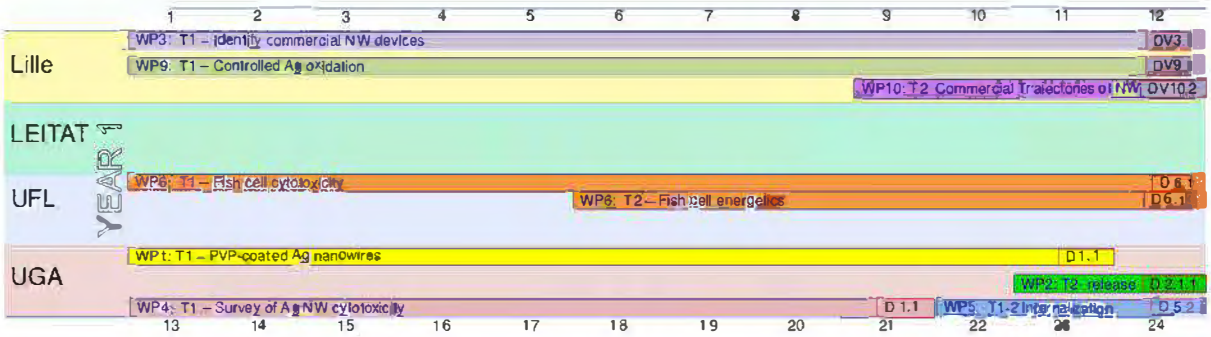
[1] The Principal Investigator (PI) is the point of contact of the partner for the corresponding Funding Organisation
[2] Name of other personnel participating in the project

1 Project objectives and work progress

Describe the project objectives and deliverables for the period covered. List the main achievements and outputs of your collaborative project. Explain the progress of the work in line with the work plan described in the joint proposal. Illustrate the progress of the work to date (e.g. using a Gantt chart or equivalent). Outline the work carried out over the past year for each partner.
(max. 3 pages)

The project covers several aspects of safety and safety improvement of Ag-nanowire in view of designing products with minimized potential exposure and health risks for consumers. The fields addressed in year 1 are in line with the work plan described in the joined proposal. Following fields were covered:
- materials design to minimize the health risks for consumers,
- intrinsic toxicity of silver nanowires (Ag NW) comprising studies on cellular toxicity, dermal toxicity, biological effects of exposure, environmental toxicity,
- release & recovery studies of silver from conducting films,
- outreach for commercially relevant nanowire enabled devices.

Research conducted during year 1 is outlined in the Gantt chart below.



Institutional abbreviations: Lille is the University of Lille (A. Hofmann); LEITAT is the Spanish Technological Center specialized in production technologies in Barcelona (V. Jamier); UFL is the University of Florida – Gainsville (C. Vulpe); UGA is the University of Grenoble Alps (L. Charlet).
***D** = Deliverable, **WP** = Work Package, **T** = Talk*

Outline of work carried out over the past year by each partner

Materials design (WP1) (contributors: Djadidi Toybou, Caroline Celle, Jean-Pierre Simonato, Laurent Charlet, Benjamin Gilbert)

The synthesis of Ag NW with well-controlled physical and chemical properties is needed for accurate studies of cytotoxicity and ecotoxicology. In year 1, special emphasis was placed on **Ag NW dimensions**. By adjusting and fine-tuning the synthesis protocol for Ag-NW, a complete set of NW with different aspect ratios was produced, in response to the factors that may influence cell and organismal toxicity, while maintaining the physical characteristics needed for technological application.
Synthesis studies are part of PhD research by Djadidi Toybou, they are conducted at CEA, Liten, Grenoble in tight collaboration with Partner 3-UGA.
Based on polyol process, nanowire dimensions were tuned by adding co-nucleant, by modifying process or length of capping agent (PVP). Indeed, this parametrical study has allowed a really good control of both silver nanowire diameters ranging from 20 to 120nm and lengths from 3 to 60µm exhibiting aspect ratio from 100 to 1000. This group plays the role of **material hub**: the different

syntheses are made available to all project partners for cellular and organismal toxicity studies as well as recycling and release studies.
Deliverables from year 1 comprise PVP-coated Ag NW systematically varying in dimensions (D1.1).

Dermal cytotoxicity of Ag NW (WP 4) (contributors: Sylvia Lehmann, Muriel Viau, Benjamin Gilbert, Laurent Charlet)

Keratocytes and fibrobasts, the major cells of the epidermis and dermis layers of skin, have been used as model skin cells in Ag NW exposure experiments. We have established laboratory protocols using assays (MTT, RNU, ATP measurements) to quantify toxicity and dark-field optical microscopy to quantify internalization. We have designed toxicity tests as a function of Ag NW dimensions that were synthesized and made available by CEA-Grenoble partner WP1. We have worked with CEA Grenoble to establish Ag NW suspension purity and concentrations to ensure reproducible toxicity results within these assays as well as between this WP and the ecotoxicity assays of WP 6-8.
Several processes are being investigated, in particular the factors favoring **NW internalization**, the relation between **internalization and cell viability**, and the **toxicity** of Ag ions compared to nanowires. Experiments conducted in year 1 showed that Ag NW are internalized by both cell types and that the exposure to NW has a negative effect on cell viability. Compared to the toxicity of ionic silver (Ag+), NW toxicity was less. Fibroblasts show higher sensitivity to Ag NW than keratinocytes. Keratinocyctes were sensitive to NW number concentration as function of cell surface while the length of NW did not play a significant role.
In addition, we acquired cell viability data on murine fibroblasts for synchrotron X-ray microscopy and spectromicroscopy described in WP 5.
Deliverables from year 1 experiments comprise (a) verified and reproducible Ag NW and protocols for toxicity assays (b) the finding that Ag NW are less cytotoxic than equivalent concentration of silver ion (D4.1), (c) the finding that no significant difference is observed between short and long Ag NW (D4.1)

Optical and X-ray microscopy studies of Ag NW uptake and intracellular localization (WP 5) (contributors: Sylvia Lehmann, Benjamin Gilbert, Laurent Charlet)

We studied mouse fibroblast cells grown on a silicon nitride membrane and exposed to a low dose of silver nanowires (Ag NWs) for 24 hours. We studied Ag NW of similar mean length (9 μm) and two diameters (30 nm and 90 nm) to test for differences in internalization and fate. The cells were washed, rapidly frozen and studied under cryogenic conditions. We performed X-ray fluorescence mapping at 33 keV of exposed and control cells to identify individual cells containing NWs. We acquired phase contrast microscope images at two incident angles to construct stereogram images of cells containing Ag NW to verify that the NWs had been internalized. We acquired a full tomographic tilt series for a single cell.
All internalized Ag NW were morphologically altered relative to their shape in water or cell culture medium. The 90-nm NW were bent while the 30-nm NW were completely crumpled and looped. These observations show that the NWs experience significant forces during internalization that we will seek to estimate.
None of the Ag NW penetrated the cell nucleus. Thus, although we observed single- and double-strand DNA damage, the mechanism must be through an indirect chemical process.
Deliverables from year 1 experiments comprise (a) the finding that Ag NW internalization causes time-dependent sulfidization (b) the finding that thinner nanowires are mechanically crumpled during the cellular internalization process.

Cellular, organismal and environmental toxicity (WP 6, 7, 8) (contributors: Devrah Arndt, Chris Vulpe, Benjamin Gilbert)

Rainbow trout gill and intestinal cells were selected (RTgill-W1, RTgutCC) for experimentation because they are a highly sensitive indicator species. To discriminate between possible mechanisms of

action of Ag NWs, **multi-parameter and high content screening assays** were used. In year one, observations were conducted on ATP activity (CellTiter-Glo Luminescent Cell Viability Assay), plasma membrane integrity (LDH Cytotoxicity Assay Kit), mitochondrial membrane potential (MMP) (MitoTracker Orange CMTMRos, Cy5 filter), and lysosome pH and mass (Lysosensor Green DND-189, GFP filter). Changes in cellular bioenergetics were measured with a Seahorse XF96 instrument, although further technical adaptations are needed for reliable fish cell studies. All experiments were conducted with standard growth medium (L15 Medium + 10% FBS).
Partner 3 CEA Grenoble (WP1) synthesized Ag NW of different dimensions for these studies. Results show that Ag NWs reduced ATP production, increased lysosome pH, and elevated MMP in a dose dependent manner (concentration as mass/volume). Nanowire dimensions influenced lysosomal pH and MMP. Differential toxicity was also observed by CellTiter-Glo when concentration was adjusted to particle number/volume. No differential toxicity relative to Ag NW length was observed.
Deliverables of year 1 comprise (a) cellular toxicity of Ag NWs in standard cell culture media was confirmed (D6.1), (b) preliminary studies on the bioenergetics of Ag NW exposure were performed (D6.1.3).

Release & recovery studies of silver from conducting films (WP9) (contributors: Brenda Omana, Annette Hofmann, Ludovic Lesven, Sophie Sobanska, Benjamin Gilbert)

Production of nano silver reached 450 t in 2010 worldwide of which more than 50 % are estimated to end up as waste in soil, water and air compartments of the environment (Keller et al. 2013). Silver nanowires are an emerging material with wide application in numerous fields of electronics, optics and textile industry. Here we develop an electrochemical **method for demetallizing Ag NW thin-films in transparent electrode technology** as well as Ag fiber equipped textiles. The aim is to eliminate the risk of **toxicologically critical Ag compounds** entering an e-Waste stream, and to develop ways to recover and reuse nano Ag, using sustainable methodology.
The Ag/Ag^+ standard potential predicts that silver is readily oxidized to Ag^+ when oxygen is reduced. Year 1 was dedicated to the construction of an **electrochemical cell** equipped with an air cathode for O_2 reduction, and an anode made of Ag NW film, where silver is oxidized. The films are provided by CEA Grenoble partner (WP1) and consist of NW of different densities deposited on transparent PEN substrate. A potentiostat has been bought to measure and control the electrochemical conditions in the cell. The cell potential proves to be positive over a wide pH range, indicating **favorable conditions for spontaneous Ag oxidation**. However the reaction does not proceed at a measurable speed, which is due to inhibiting processes.
Although we still do have to overcome the initial inhibition to current flow, the system with artificially boosted current allowed us to evaluate the rate of Ag oxidation and production of Ag+. The calculated values show that a minimum current of 0,2 mA is needed to reach reasonable treatment times in the order of minutes for Ag removal from NW-films of 10's of cm^2.
Deliverables of year 1 comprise (a) an electrochemical cell for Ag NW oxidation was built (D9.1), (b) Ag dissolution is obtained when boosting the natural cell potential with a moderate external potential (D9.1).

Industrial contacts and procurement of nanowir2es enabled devices (WP3, WP10) (collaborators: Sylvia Lehmann, Brenda Omana, Annette Hofmann, Christopher Vulpe, Benjamin Gilbert, Laurent Charlet, Caroline Celle, Jean-Pierre Simonato)

The European, North American and worldwide market for nanowire production and application has been scanned. The field of **application of Ag NW** is fairly wide, comprising conducting inks and pastes as basic products, antibacterial films in medical use, transparent flexible conducting films for touch sensor products, OLED and photovoltaic surfaces, and connected textiles and plastics for thermal control. Companies have been identified that are susceptible to use Ag NW in their products. We have also identified the market of Ag coated textile fibers. While inks and pastes, as well as textiles containing Ag coated fibers are easy to procure, electronic and optic devices equipped with transparent conducting NW films are difficult to access. Our effort of getting in contact with the most adequate manufacturing companies is continuing.

2 Impact[3]

State any contribution made to the development of a consolidated framework to address nano-related risks and the management of these risks for humans and the environment by the academic and/or industrial partners. Outline what data have been supplied to the SIINN section of the NANOhub database[4] and other relevant databases for nanorisk and nanosafety. Explain the actions taken for the dissemination of results (e.g. by means of publications, workshops, conferences or other events) and exploitation of results (e.g. by means of patents or spin-offs). List publications, events attended and invited to speak at, patents, spin-offs, over the past year.
(max. 3 pages)

Over year 1, the project partners have built a **framework around Ag NW** exposure assessment, toxicity mechanisms and effects on human health and environment, which is expected to have a strong **impact on NM safety issues, safety by design development and eco-friendly high end novel products**.
Ag NW environmental and cellular exposure studies have been conducted in direct link with NW material design studies, and the definition of metrics that can act as toxicity indicator parameters. Important results obtained are the linkage between NW diameter and length with cell internalization and cell viability (WP4, D4.1, WP6 D6.1.1). The effort of linking toxicity studies to material design will be continued in year 2. In particular the relation between NW solubility, NW geometry and toxicity, as well as the effects of NW coatings will be addressed. We are working towards a consolidated framework coupling **NW risks and NW design**, and developing metrics to identify **safest design for minimum risk solutions**.
This frame precedes a second one, where the above aspects on NW risk and design will be investigated at the level of the thin film of conductive NW network. This frame will span the gap between **toxicity of the NW network, design and recyclability of the material**. End of life recycling of NW films (WP9) and the need of thinking **"design for recycling"** will be included in this second frame.

Initial results are in the course of being acquired. No data have yet been supplied to NANOhub or e-nanoMapper databases. First inputs will be provided during year 2.

Dissemination of results:
Fifth Nanosafe International Conference 7-10 November 2016 Grenoble France
Talks
Devrah Arndt, Toybou, D., Gilbert , B., Lehmann, S., Charlet, L., Omana, B., Hofmann, A., Vulpe, C. Impact of silver nanowire length, diameter, and surface chemistry on rainbow trout RtgillW1 and RtgutGC cell lines, *Oral*
Sylvia Lehmann, Gilbert, B., Maffeis, T., Rachidi, W., Seve, M., Charlet, L. Evaluation of the potential health risk of silver nanowires via dermal exposure. *Poster*
Muriel Viau, Toybou, D., Lehmann, S., Simonato, J.-P., Celle, C., Gilbert, B., Charlet, L. The effect of silver nanowire diameter on cytoxicity to human fibroblasts. *Poster*
Brenda Omana Sanz, Hofmann, A., Lesven, L., Sobanska, S., Gilbert, B., Charlet, L. Metallic nanowaste: towards sustainable recycling solutions. *Poster*
Djadidi Toybou, Celle, C., Viau, M., Lehmann, S., Charlet, L., Simonato, J.-P., Gilbert, B. Morphology od silver nanowires : between performances and toxicity. *Oral*

Session: Safer by design nanomaterials and process
Organizer: Benjamin Gilbert,
Keynote speaker: Benjamin Gilbert

[3] Include link and details for any mentioned publication, report, patent or communication.
[4] Link to the NANOhub database: http://www.napira.eu/

ACS - annual meeting, San Francisco 2-6 April 2017
Talks
Devrah Arndt, Toybou, D, Simonato, JP, Celle, C, Gilbert, B, Charlet, L, Vulpe, C, Lehmann, S. Impact of silver nanowire length and diameter on rainbow trout RTgillW1 and RTgutGC cell lines. *Oral*
Sylvia Lehmann, Gilbert B, Viau M, Toybou D, Simonato JP, Cell C, Maffeis, T, Charlet L. Toward safer silver nanowires by design: Modulation of characteristics and evaluation of dermal toxicity. *Oral*

Session: Chemical Principles of Environmental, Cellular & Organismal Nanotoxicology.
Organisers : C. Celle, L. Charlet, B. Gilbert, S. Lehman, J. Simonato, C. Vulpe.

Other talks:
Arndt D, Vulpe C. Silver nanowire toxicity to cells and organisms: A silver bullet to ecosystem health? University of Lille, France. May 23, 2016.
Arndt D, Tagmount A, Vulpe, C. Impact of silver nanowire length and diameter on rainbow trout RTgillW1 cells. Southeast Society of Environmental Toxicology and Chemistry Meeting. Gainesville, FL. September 22, 2016.
Hofmann A, Omana B. L'argent - métal miracle de nos sociétés modernes ? Présentation du projet NanoWIR2ES, University of Lille, LOG seminar, 5 January 2017

3 Transnational collaboration and project management

Describe the added value and synergies in the collaboration, any obstacles to the transnational collaboration, and the proposed solution (if necessary). Outline what economic and/or societal benefits the research activities have had on Europe. Indicate connections to the Nanosafety Cluster activities and other international initiatives. Present the processes and tools used for management and communication. List consortium meetings over the past year.
(max. 1 page)

The consortium brings together scientists of very **different disciplines**, in particular material scientists, biologists, ecotoxicologists and geochemists. The diversity of scientific expertise is highly beneficial for the project progression.
Grenoble partner with CEA group LITEN plays a central role in the project as they provide all consortium members with Ag NW materials, synthesized specifically to meet the specific needs of the various experiments. The whole project is built on this very **tight national and transnational collaboration** between Grenoble and all partners.
Added value of collaboration between partners is to be found at all levels of the project. For instance, research on Ag NW toxicity is conducted by the US partner 4 and by Grenoble partner 3. Environmental cellular toxicity on the one side of the Atlantic, human cytotoxicity on the other side, are investigated using some common and some very different techniques. The differences in scientific "culture" are leading to lively discussions and a profound reflection on the right choice of toxicity assays, testing conditions and comparability of results. Here the transnational collaboration of two research groups of renown international level is most beneficial to the advancement of the project and to strong **research impact**.
In the field of material science and recycling, the collaboration between geochemists of the Lille group and Grenoble/CEA will allow to build synergies between NW thin film design and most appropriate parameters for recycling.

The holistic approach of Ag NW development taken in this project, combining synthesis, design, risk and recycling will benefit industrial applications of new generation eco-friendly products. The European Union strongly supports the development of nanosafety databases (nanoHUB, e-NanoMapper). Europe is ahead in the development of geographical NM inventories (Pati et al. 2016). The integrated investigation of Ag NW taken in this project is pioneering the **application of ecodesign strategies to nanomaterials**.

The project is managed according to the scheme given in the project proposal. A monthly group meeting is held with all participants via video link. Progress reports and presentations are given by junior scientists and discussed by all. Material and technical issues, miscellaneous issues are discussed during these meetings. Email is used for ongoing discussions and video link is again used for quarterly meetings of the Project Management Council. No particular problem has emerged so far needing action to be taken.
During year 1, the project members organized two full sessions at international conferences, at Nanosafe International Conference Grenoble (November 2016) "Safer by design nanomaterial and process" and at ACS annual conference San Francisco (April 2017) "Chemical Principles of Environmental, Cellular & Organismal Nanotoxicology". A key note talk was given by Benjamin Gilbert at Nanosafe Grenoble, were he was a voice of the NanoWIR2ES group. At both occasions, a 1 day workshop type extended group-meeting was held at the conference location.
The mode of functioning has been satisfactory for all members. We intend to continue communicating in this manner.

4 Deviations to the original project plan

Explain any changes to the project that could affect the completion of tasks/objectives, and of any difficulties/problems encountered with the scientific work, staff or resources. Specify any changes to team composition among the collaborative partners over the past year. Give a brief explanation of use of resources[5], including possible deviations from joint proposal. Summarise your action plan to solve these issues. Detail the key goals and any deviations from the original workplan (if any) for the coming year.
(max. 1 page)

Preceding the start of the project, we learned that one of the consortium partners, Leitat, Spain, could not be supported by the Spanish funding agency. This implied several changes to the initial proposal, described in a letter of statement sent to the ERA-NET SIINN call office in September 2015 and a modification to proposal joined to the consortium agreement and signed in March 2016.
Major changes concern the **synthesis of Ag nanowires** which was taken over by Dr. Simonato's group at CEA LITEN in Grenoble, collaborating with Professor Charlet, and providing NW at no charge to the NanoWIR2ES participants. Dr. Caroline Celle in Simonato's group is implicated in all aspects of synthesis. **Objectives in WP3 concerning the degradation of NW enabled thin films and devices is conducted by Leitat**, but at the discretion of their financial capabilities and depending on the accessibility of commercially available NW enabled products. Progression in this field does not impede advancement of all other parts of the project.

Partner 4 UFL (US) has received **funding with a delay of 5 months** due to a transfer of funding competence from National Science Foundation to Consumer Protection Safety Commission. Despite this delay, partner 4 is catching up with the project time table quickly.

There are no deviations to the project goals as defined initially (1/4/2016). Some modifications occur in the timing and in the techniques applied. For instance the proteomics study in WP4 (partner 3 UGA/CEA) are postponed due to high costs and replaced by less costly assays. The study on degradation of NW enabled thin films (WP3) is not associated to a precise slot.

Team composition
1. In the Grenoble group, Muriel Viau, post-doctoral collaborator, has quit her position. Her tasks within SERENADE and NanoWIR2ES were taken over by Sylvia Lehmann, already part of the group.
2. In the Lille group, team member Sophie Sobanska has moved to Bordeaux, Institut des Sciences Moléculaires (ISM) - UMR CNRS 5255, Université de Bordeaux. She maintains her membership in the group.

Use of resources
Resources have been used as intended initially. Partners 1 and 4, the only partners that received funding for the NanoWIR2ES project have each hired one post-doctoral researcher (Brenda Omana-Sanz, Devrah Arndt). Both partners have spent resources for attending conferences in Lille, Grenoble and San Francisco.
Partner 1 has acquired a potentiostat, a major piece of equipment for the project and materials for electrochemical work.
Resources for partner 4 have been allocated to cell culture maintenance, various cytotoxicity assays, and the purchase of a water chiller and aquaria for the aquarium set up that is required for whole organism assays.

[5] Justification of costs is performed at the national level.

5 Final comments

Objectives for the coming year are following the key goals initially defined. Main objectives are summarized below:

Intrinsic toxicity of silver nanowires (Ag NW) comprising studies on cellular toxicity, dermal toxicity, biological effects of exposure, environmental toxicity:

1. The important influence of NW diameter on skin cell viability evidenced in year 1 will be explored by investigating physical (flexibility), chemical (solubility) and biological (uptake) factors. The role of NW in provoking oxidative stress is another aspect that will be developed. These studies will advance the understanding of the **mechanisms of Ag NW toxicity** (D2.2, D4.2).
2. The interaction between **growth media** and Ag NW is suspected to interfere with Ag NWs by binding with the Ag+ ion and masking the toxicity of Ag NWs. Solubility of NW, Ag+ reactivity in the different growth media used will be investigated and considered in terms of toxicity effects (D2.2, D 6.1.2).
3. For **environmental exposure**, the rainbow trout is considered. However, being a less traditional model organism, and some assay antibodies are expensive and not readily available. Alternative methods to investigate H2AX DNA damage and cytochrome C endpoints will be explored (D6 1.1 and D6 1.2).
4. Single particle ICPMS, now available in the Vulpe laboratory, is an innovating technique for investigating **Ag NW cellular uptake** and transport studies on rainbow trout RTgill W1 and RTgutGC cells (D7.1).
5. **Whole organism studies** using rainbow trout embryos and larvae are planned (D6 2.2).
6. **Mechanisms of Ag NW environmental toxicity** will be studied by applying genomic techniques (D 8.1) including RNA sequencing, building libraries, and computational techniques.

Release & recovery studies of silver from conducting films

A number of optimizing steps are needed before the prototype electrochemical cell for Ag NW film removal can work efficiently. An **induced current of at least 0,2 mA** in aimed for, which will insure sufficiently fast removal of Ag from the Ag-NW films.
1. Solutions will be investigated to increase the electric potential of the cathode (air-cathode) by adding oxidants stronger than oxygen (D9.1).
2. Removal of Ag from Ag NW films currently results in **Ag-oxide formation** which passivates the NW and hinders further treatment. A technique for recovery of Ag+ is to be developed that removes the ion from the vicinity of the NWfilm and sequesters it for further use. Several options will be considered (D9.2).
3. The potentiostat acquired specifically by the Lille laboratory to develop the technique of silver recovery from Ag NW-film, will also be used to develop a square wave potential scan method for the **semi-quantitative measurement of Ag+** concentrations in the electrochemical cell during treatment (D9.1).

Outreach for commercially relevant nanowire enabled devices: This effort will be continued.

References cited in the document:

Keller et al. 2013 J Nanopart Res 15:1692
Pati et al. 2016 Environ. Sci. Nano, 3:1133

Replies to Progress report – Scientific Rapporteur

Project Acronym:

NanoWIR2ES

Project Full Title:

NanoWire Intelligent Re-design and Recycling for Environmental Safety

Runtime of the project:

☐ 1st year ☒ 2nd year ☐ 3rd year

Period covered:

From 01/04/2017 to 31/03/2018

Project Coordinator :

Annette Hofmann

Projet Rapporteur :

Evangelia Sarantopoulou

Replies

1. Project objectives and work progress

WP3 « Degradation of NW-enabled thin-films and devices »

It has proven extremely difficult to procure Ag-NW-enabled thin-films from consumer-goods and industrial parts. Industry and industrial suppliers are very protective and for patenting reasons do not give access or information on these compounds. We have identified one device, a heatable skiing-goggle, which has been purchuased and sent to partner 2 (Spain) to conduct degradation studies and Ag release studies in simulated landfill setting (WP3 T3.2) (Progress report 2, page 8-9). Although only one NW-equiped item is available, the full leaching study, as initially planned, will be conducted.

WP2-4-5 Issues in these work packages have been addressed thoroughly and the main outcomes are summarized on pages 3-4 of the Progress report. Additional figures will be available in a manuscript in prep. presenting observations and mechanisms of acute cell toxicity of Ag-nanowires.
In particular, the rate and extent of silver ion release over time periods relevant to toxicity studies have been investigated, electron microscopy and synchrotron X-ray images have been obtained, 3D reconstructions have been realized and elemental 2D maps have been measured.
Several items in WP2, in particular studies on sufidation of Ag-NW (T2.3) are now being conducted in the frame-work of a specific SERENADE post-doctoral project parallel to NanoWIR2ES. This was necessary for financial reasons since partner 2 is not financed through the ERA-NET SIINN NanoWIR2ES project.

4 Deviations to the original project plan

Partner 4 (Florida, US) is experiencing deviations from WP7 and WP8 due to equipment failure and/or limitations, but these problems are not without resolution.

The Seahorse XF instrument had a mechanical failure that has been repaired since the submission of the original progress update in May 2018, and metabolic experiments have since been completed.

Single particle ICPMS (spICPMS) was proposed to measure uptake and distribution of nanowires in cells and fish embryos, but the splCPMS technique has upper particle size detection limits (5 µm) [1] that are lower than the length of our nanowires (10 µm). Because the length of our nanowires exceeds the upper detection limit, the particles won't be homogenously transported by the nebulizer, and many of the larger nanowires are likely to be omitted from the analysis altogether. One possible solution to this problem is to have our collaboration produce nanowires that are below the upper detection limit of the technique but still long enough to produce a wire with a sufficient high aspect ratio (length of 5 µM). We plan to discuss the possibility of making nanowires with lengths of 5 µm during our next conference call. We have also contacted a colleague, Manuel Montano at the University of Vienna, who specializes in analytical chemistry and splCPMS to inquire about additional ways to resolve the problem regarding spICPMS upper detection limits. We also consider digesting AgNW cell and tissue samples to measure silver concentrations and quantify uptake and distribution, but we won't be able to extract any particle-specific information using this method. Additional methods that could accomplish the deliverables outlined in WP7 and WP8 include TEM- or SEM-EDX. Our laboratory also has access to a Nanosight instrument, which has been previously noted for its use in real-time tracking of metal-based nanoparticles.

Partner 4 considers using confocal darkfield microscopy to study uptake (WP7.1) and bioaccumulation (WP7.2) of AgNWs in cells and tissues as has been mentionned in the Progress Report on page 10. Indeed it was previously shown that AgNWs could be easily imaged using darkfield microscopy. The confocal microscope available at the University of Florida is not equipped with darkfield capabilities. However this problem can be resolved by gaining access to a confocal microscope that does have darkfield capabilities, and it has been determined that the University of California – Berkeley has this instrument.

Reference 1. Laborda, F., E. Bolea, and J. Jimenez-Lamana, *Single particle inductively coupled plasma mass spectrometry: a powerful tool for nanoanalysis.* Anal Chem, 2014. **86**(5): p. 2270-8.

WP3 Leaching studies conducted by partner 2 (Barcelona, Spain) were shifted from year 2 to year 3. Investigations have currently started.

The position of Benjamin Gilbert as an invited researcher has been funded for another two years, until 2020.

5 Final comments – Recommendations

We are confident that the project objectives will be met. Equipment limitations encountered in WP 7-8 have been addressed, solutions have been identified and are currently developed. We have a very limited number of Ag-NW equiped devices for degradation studies but we will nonetheless conduct experiments and finetune the leaching methods to ajust to the NW field specifically.

An electrochemical method to rapidly assess the environmental risk of silver release from nanowire transparent conductive films

Brenda Omaña-Sanz[a*], Djadidi Toybou[b,c], Ludovic Lesven[d], Valerie Gaucher[e], Alexandre Fadel[e], Ahmed Addad[e], Philippe Recourt[a], Delphine Yeghicheyan[f], Devrah Arndt[g], Caroline Celle[c], Jean-Pierre Simonato[c], Christopher Vulpe[g], Laurent Charlet[b], Sophie Sobanska[h], Benjamin Gilbert[i], Annette Hofmann[a*]

[a] Univ. Lille, CNRS, Univ. Littoral Côte d'Opale, UMR 8187, LOG, Laboratoire d'Océanologie et de Géosciences, F 59000 Lille, France
[b] Institut des Sciences de la Terre, Université de Grenoble–Alpes, CNRS, F-38000 Grenoble Cedex 9, France;
[c] Laboratoire d'Innovation pour les Technologies des Energies Nouvelles et les Nanomatériaux, Département des Technologies des Nouveaux Matériaux, Université de Grenoble–Alpes, Commissariat à l'Énergie Atomique et aux Énergies Alternatives, F-38054 Grenoble Cedex 9, France;
[d] Lasir CNRS UMR 8516, University of Lille, 59 655 Villeneuve d'Ascq Cedex, France ;
[e] Univ. Lille, CNRS, INRA, ENSCL, UMR 8207 - UMET - Unité Matériaux et Transformations, F-59000 Lille, France;
[f] Service d'Analyse des Roches et des Minéraux (SARM) CRPG, CNRS UMR 7358, Université de Lorraine, BP20, 54501 Vandoeuvre-lès-Nancy, France
[g] University of Florida Center for Environmental and Human Toxicology, 2187 Mowry Road, Gainesville, Florida, 32611, USA
[h] Institut des Sciences Moléculaires, UMR CNRS 5255, Université de Bordeaux, 33405 Talence, France;
[i] Energy Geoscience Division, Lawrence Berkeley National Laboratory, Berkeley, CA 94720

Keywords

Silver nanowire networks – polysiloxane coatings – electrochemical corrosion – accelerated aging – silver exposure

ABSTRACT

Silver nanowires (AgNW) are new nanomaterials designed to be incorporated into transparent conductive films in electronics, microelectrodes, heated surfaces and others. Although in these films, the AgNW are generally protected by a coating material, a risk for release of silver at all stages of the nanoproduct life cycle does exist due to corrodibility of the metal. Since ionic and nanoparticulate Ag represent a toxicological risk for a large number of living cells, there is a need for quantifying the potential Ag release from these product components. We developed an electrochemical method to evaluate possible corrosion activity of silver in AgNW transparent conductive films (TCFs) and concomitant Ag^+ release. A polysiloxane polymer was used as protective coating of AgNW TCFs. A consistent correlation is observed between the degree of corrosion and the coatings' characteristics, in particular the thicknesses. A major advantage of the new approach, compared to classical aging studies, is the short experimentation time: 20 min are sufficient for a diagnostic result. The method is an accelerated corrosion and release test. It is environmentally sound methodology with use of very low electric power and with no harmful reagents. A particularly attractive application could be in the field of environmental risk assessment of metals from portable electronics and biosensors.

*Corresponding authors.

E-mail addresses: brenda.omana-sanz@univ-lille.fr (B. Omaña-Sanz), annette.hofmann@univ-lille.fr (A. Hofmann).

1. Introduction

Silver nanowires (AgNWs) are one of the most promising alternative engineered nanomaterials (ENMs) to replace indium tin oxide (ITO) in transparent electrodes (Sannicolo et al., 2016). More so, AgNWs enter a wide range of technological developpments. Random networks of AgNWs are being used for transparent conductive films (TCFs) for applications ranging from electronics, optoelectronics to heatable films and biomedical devices (Doganay et al., 2016; He and Ye, 2015; Sun et al., 2019; Toybou et al., 2019; Zhang and Engholm, 2018). The technical specificity of metallic nanowires, e.g. AgNWs, lies in that interconnection creates electric conductive paths allowing electrical current flow. The high transparency, high electrical conductivity and mechanical flexibility of the AgNW implemented electrodes, as well as their lower synthesis cost explain the recent technology trend.

It is known that metallic silver is corroded when exposed to atmospheric conditions (Graedel, 1992; Molleman and Hiemstra, 2017); similarly silver nanowires are also easily corroded, thereby causing failures in conductive networks. To counter this problem, application of a nano to micrometer thick layer of a protective coating material over the conductive NW network, before its integration into a device, is often performed in order to stop the diffusion of water vapor and gases, thus to avoid corrosion. Other external damages like scratches and bends are also avoided by this protection.

Different materials have been considered for creation of a protective coating, including reduced graphene oxide (Ahn et al., 2012; Duan et al., 2019), metal oxides and carbonates (Jeong et al., 2019; Khan et al., 2018), optical adhesive (Miller et al., 2013), chitosan (Jin et al., 2017), and polymers (Chen et al., 2014; Kim et al., 2015; Miao et al., 2019; Moreno et al., 2013; Xu and Zhu, 2012). Although the coatings could delay corrosion, 100 % of effectiveness on the long-term still remains an issue (Deignan and Goldthorpe, 2017; Jiu et

al., 2015). A noticeable factor is that most of the protective coating materials in use, especially polymers have some degree of permeability at the molecular level.

Besides the permeability aspect, wear and tear of the NW containing products throughout their life cycle, i.e. from their active use to end-of-life disposal, may degrade the mechanical and chemical quality of the conductive film and enhance the corrosion of the nanowires. Corrosion is responsible for the release of ionic silver and the formation of nanoscopic wire debris. These forms of silver are toxic at low levels to aquatic organisms and microorganisms, and to certain types of human cells (Lehmann et al., 2018; Liao et al., 2019). Corrosion of AgNW TCFs may lead to critical silver exposure of the device user and/or to pollution of the receiving environment.

According to the inventory of available databases e.g. the Nanodatabase, R-Nano and StatNano (R-Nano.fr, https://www.r-nano.fr; STATNANO, https://statnano.com/; The Nanodatabase, http://nanodb.dk/), the number of household products and biomedical devices incorporating nano-silver is rapidly expanding (Bodycap, http://www.bodycap-medical.com/fr/; Naidu et al., 2015; Rajski et al., 2019; Yao et al., 2017). Despite this fact, studies concerning silver release have mainly focused on textiles (Part et al., 2018). Very few predictive models of Ag-ENM fate in the environment have been conducted with data from provenances other than textiles (Giese et al., 2018; Sun et al., 2016). Concerning silver nanowires, various studies have investigated the toxicological effect of AgNWs on biological systems (Lehmann et al., 2019; Schinwald and Donaldson 2012; Stoehr et al 2011; Scanlan et al., 2013; Theodorou et al., 2017). Studies on silver nanowires for transparent conductive films (TCFs) have been mainly dedicated to technical quality control of the AgNW transparent electrodes, in particular on their lifetime stability (Deignan and Goldthorpe, 2017; Elechiguerra et al., 2005; Jiu et al., 2015; Khaligh and Goldthorpe, 2013; Lin et al., 2018; Mayousse et al., 2015). To our knowledge, this is the first investigation in which the capacity

of corrosion of AgNW in TCFs (hereafter referred to as corrodibility) is evaluated with the aim to assess the risk of metal release to users and the environment. In this work, we developed an electrochemical method where corrosive conditions are imposed on AgNW TCF under aqueous conditions, to evaluate the potential for formation of ionic silver and tiny debris of nanowires and their transport from inside to outside the conducting film. An accelerated corrosion test is developed, completed within 20 min. Moreover, it can be considered environmentally sound methodology as only very low electric power is needed and no chemical reagents other than tiny amounts of standard electrolyte salt are used.

The paper reports corrosion experiments conducted with AgNW films, either uncoated or coated with a protective layer of anti-scratch varnish AE12® of different thicknesses. Some AgNW films underwent natural alteration prior to experiments. These samples were considered as "pre-aged" and representative of nanoproducts during their life cycle or at end of life. Two commercial products were also investigated.

2. Materials and Methods

2.1. AgNW film samples

AgNW networks were deposited on large dimension transparent flexible substrate (10 x 10 cm) of polyethylene naphthalate (PEN), called hereafter "AgNW large plate". These large plates were used to prepare the "AgNW film" samples used in the corrosion experiments. The synthesis of AgNWs was conducted following the polyol method, the preparation of the large plates is described in Mayousse et al. (2013). The initial sheet resistances of AgNW large plates varied from 8 to 26 Ω/□. The nanowires used had diameters in the range 50-90 nm and lengths between 6-26 µm (SI appendix, Table S1) according to control measurements conducted with ImageJ software on FESEM images.

132 The AgNW large plates, when received, had an age of several months to more than one year
133 and thus showed different degrees of aging. A thorough electron microscopic investigation
134 was conducted on each plate before use, in order to discard damaged specimens not suitable
135 for the experiments, or amenable to misleading interpretations.
136 To prepare the AgNW film samples, the AgNW large plates described above were cut into
137 2.5 x 3.5 cm^2 pieces, hereafter referred to as AgNW TCF or AgNW film. At least 3 samples
138 were prepared from each AgNW large plate in order to assure reproducibility of results. Part
139 of the samples were coated with a polysiloxane polymer (anti-scratch varnish AE12®, from
140 ISOCHEM) used as a non-conductive coating material to protect the AgNW films used in the
141 experiments (see details in SI appendix). Coating thicknesses of 500 and 700 nm (mean
142 values) were achieved by varying the spin coating parameters. Polysiloxane is one of the
143 materials studied as potential candidate for TCF coatings. According the findings by Toybou
144 (2018), this coating effectively preserves the AgNW TCF under ambient degradation
145 conditions and seems to improve initial conduction performances of the samples.
146 In addition, AgNW films of 2.5 x 3.5 cm^2 size, readily coated (by dip coating) with a
147 protective layer of 5µm thickness (mean value), were prepared from new materials that had
148 not undergone any aging prior to the experiments in this study.
149 Two commercial products were also tested with the method developed here. A silver
150 antibacterial textile (silver-coated fibers) and heatable glasses from a skiing goggle containing
151 silver ENM. Both products were purchased specifically for the experiments. The skiing
152 goggle glasses were composed of a layer containing the ENMs and protected with a very thick
153 over-coating layer of a polymer called polycarbonite by the manufacturer. For the
154 experiments, both materials were cut into pieces of 2.5 x 3.5 cm^2.
155
156

157 *2.2. Sample characterization*

158 The effect of the corrosion experiments on the AgNW films were evaluated by Field
159 Emission Scanning Electron Microscopy (FESEM) using a JEOL JSM-7800F LV equipped
160 with EDS/EBSD system, and a FEI Quanta 200 environmental scanning electron microscope
161 (ESEM). The observations by FESEM were performed at 5 kV on coated AgNW films and at
162 15 kV on uncoated AgNW films. The observations by ESEM were conducted at 20 kV. Both
163 secondary electron (SE) and electron backscattering (BSE) modes of imaging were used. To
164 avoid surface charging, samples were chromium coated (70 Å) by electro-sputting under
165 vacuum prior to FESEM observations.

166 Atomic force microscopy (AFM) topographic observations and roughness measurements
167 were carried out on a Dimension 3100 apparatus from Digital Instruments operated in
168 Tapping Mode, using a set-point amplitude ratio close to 0.9 in order to reduce indentation
169 effects and thereby optimize the topographic contrast. The Nanoworld silicon SPM sensors
170 had a tip radius less than 10 nm, the nominal spring constant and resonance frequency of the
171 cantilever being around respectively 50 N m^{-1} and 250 kHz.

172 *2.3. Corrosion cell*

173 The corrosion experiments were carried out in an electrochemical cell in a three electrode
174 configuration (Figure 1). The AgNW film of 2.5 x 3.5 cm^2 was used as the anode, a platinum
175 grid of similar size as the cathode. A mercury/mercurous sulfate electrode was used as
176 reference. The electrochemical cell, specially designed for the experiments, made of PTFE,
177 consists of a rectangular chamber of 10 mL volume, with two facing walls of the size of the
178 electrodes. In the chamber top, slots are foreseen for positioning cathode and anode, and a
179 hole for insertion of the reference electrode. The inter-electrode distance between anode and
180 cathode is 0.5 cm. The reference electrode (RE-2CP, ALS Co.) is located outside the anode-

cathode path. The electrolyte is introduced into the chamber through another small hole in the chamber top. An automatic titrator (Titroline 7000, SI Analytics) controls solution addition.

All experiments were performed in a Faraday cage. A potentiostat (VSP, Bio-Logic) served to set the cell potential and to record the current generated.

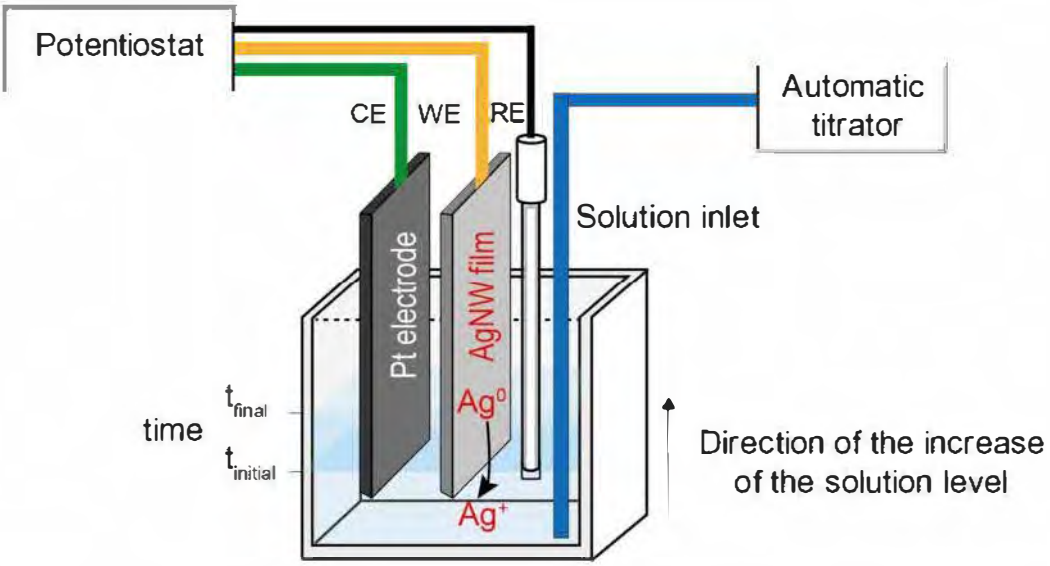


Figure 1. Schematic of the electrochemical corrosion cell used in the corrosion tests. A three-electrode configuration was used: the AgNW film was used as anode (working electrode WE), a platinum grid as cathode (counter electrode CE), a mercury/mercurous sulfate electrode as reference electrode (RE). The automatic titrator allowed constant addition of solution over the duration of the experiment ($t_{final} - t_{initial}$ = 20 min). The arrow shows the direction of increase of the solution level, thus the progression of the provoked corrosion.

2.4. Experimental conditions

$NaNO_3$ at 0.01 M was used as electrolyte solution (SI appendix, Figure S1). The 0.01 M $NaNO_3$ salt solution established naturally at a pH of 5.0 ± 0.2. This pH was retained as the working pH because it was well within the range where polysiloxane is chemically stable (SI appendix). Ionic strength and pH of the experimental solutions are representative of environmental conditions of continental surface and subsurface waters (Stumm and Morgan,

200 1996). However, the chemical composition of this electrolyte solution, a pure $NaNO_3$
201 solution, can not be directly related to normal ion composition in natural waters.

202 We determined the free corrosion potential (E_{corr}) of a 2 mm silver rod (Advent Research
203 Materials) and of commercial AgNW (2.23% wt/wt dispersion, Nanogap) dispersed onto a
204 carbon paste electrode. The corrosion potential of these silver materials was 0.45 V, detailed
205 results can be found in Lehmann et al. (2019). To study corrosion on the AgNW film
206 electrodes, we applied an overpotential of 0.8 V vs. SHE. At this voltage, the nanowires were
207 not subject to any Joule heating problem (Khaligh et al., 2017) neither in the aqueous nor in
208 the aerial part of the electrode. Also the water electrolysis reaction ($E°(pH5) = 0.925$ V) is
209 negligibly small at a potential of 0.8 V, thus not interfering with the Ag redox reaction or the
210 pH of the solution.

211 The $NaNO_3$ electrolyte solutions and the K_2SO_4 0.6 M reference electrode solution were
212 prepared with ultrapure water and analytical grade reagents.

213 *2.5. Experimental procedure*

214 The corrosion experiments were conducted in the electrochemical cell under constant
215 addition of electrolyte solution. This allowed to continuously renewing the solution-air
216 contact line on the film surface. The continuous shifting of this "active zone" allowed
217 maintaining high corrosion conditions throughout the experiment. Some tests were also
218 conducted under fixed electrolyte volume conditions (SI appendix, Figure S2 A-B).

219 An initial volume of around 2 mL of electrolyte solution was placed in the reactor chamber,
220 just enough to allow the solution surface to touch the lower edge of the AgNW film. The
221 electric potential (set to 0.8V vs. SHE) and the automatic titrator were switched on
222 simultaneously, thus insuring constant addition of solution over the duration of the experiment
223 and starting at time $t_{initial}$ (Figure 1). A total volume of 2 mL of electrolyte solution was added
224 at a flow rate of 100 $\mu L.min^{-1}$ (20 min). This flow rate corresponds to a displacement of the

225 solution/air interface inside the reactor chamber of 0.04 cm/min. Preliminary experiments
226 showed that the chosen rate was enough to ensure a constant shift of the interfacial line
227 between solution and air on the AgNW film. Electron microscopic controls showed that the
228 movement is rapid enough to contain the capillary ascent of solution along the nanowires and
229 along the borders of the film. The solution inside the cell was not stirred but the continuous
230 addition of solution induced some degree of turbulent mixing. The pH of the solution, initially
231 at 5, was controlled before and after the experiment. After 20 min, the experiment was
232 stopped, the electrodes were removed carefully and an aliquot of the electrolyte solution was
233 taken for Ag^+ analysis. The AgNW film sample was removed from the reactor, the area that
234 had been immersed was cut off, carefully rinsed and placed in a desiccator for later surface
235 analysis. The remaining part of the film, which had not been immersed in the electrolyte
236 solution, was used for a subsequent experiment on the same sample.
237 All the solution samples were filtered through 0.2 µm pore size polycarbonate filters (PCTE
238 Sartorius, Fisher Scientific), and several samples were additionally filtered through 10 kDa
239 (equivalent to 4 nm) ultrafiltration membranes (Ultracel, Amicon 25 mm, Merck) using a
240 stirred ultrafiltration cell system (Amicon, model 8010, 10 mL, Merck) in order to separate
241 Ag nanoparticles from the truly dissolved fraction. All filtrates were acidified with HNO_3 2%
242 trace analysis/ultrapure quality. Analyses of silver concentrations were conducted by
243 Inductively-Coupled Plasma Mass Spectrometry (ICP-MS Agilent 7700x).
244
245 **3. Results and discussion**
246 In the electrochemical cell (Figure 1), the oxidation of silver metal is promoted by using
247 the AgNW TCF as the anode and applying an electric potential higher than the corrosion
248 potential. The silver metal is oxidized and goes into solution in the form of ionic silver (eq. 1).
249 *Reaction at the anode (WE):* $Ag_{(s)} \leftrightarrow Ag^+_{(aq)} + e^-$ (eq. 1)

In this study, we investigated the corrodibility of polysiloxane coated AgNW films with coating thicknesses varying from thin (500 nm), medium (700 nm) to thick coated (5 µm). Uncoated AgNW films were also studied. Each selected film was submitted to the electrochemical corrosion test. The current response to the applied voltage was recorded via the potenstiostat. The raw and qualitative data was then transformed by integrating the traces over the experiment time, to obtain Q the quantity of charge, i.e. a single number representative of a given experiment (calculation details in SI appendix).

3.1. Features of corrosion after experiments

As mentioned above, the AgNW large plates available for the preparation of the samples, i.e. the "AgNW films" were carefully studied by Field Emission Scanning Electron Microscopy (FESEM) before use to discard damaged specimens not suitable for the experiments. On the plates kept for the study, the signatures of natural pre-aging processes or of synthesis related defects were identified (SI appendix, Figures S3, S4) so that the effects of our corrosion experiment could be clearly recognized.

The characteristic signature of nanowire degradation by electrochemical corrosion (our experiments) appears mainly in the active zone, i.e. along a triple junction line between solution, air and the partly immersed sample. The active zone was continuously renewed by a constant addition of electrolyte solution, thereby maintaining high electrical conduction thus continuous corrosion conditions. Electrochemical corrosion signature was fragmentation of the nanowires into an alignment of nanoparticles and/or nanorods. This feature appeared similarly for uncoated (Figure 2 A-D) and thin-coated AgNW films (Figure 2 E-H). Fragmentation creates discontinuities leading to electrical failure. It has been shown previously that the degradation of an AgNW network is dependent on several network characteristics (Elechiguerra et al., 2005; Mayousse et al., 2015). Our results show that dense

nanowire networks (films with low initial sheet resistances) produced higher quantity of charge than sparser networks (SI appendix, Figure S5.A and S5.B). In other words, corrosion rate (relative to surface area of the film) is enhanced in dense nanowire networks (calculation details in SI appendix). Concerning nanowire diameter, in a given sample, nanowires with smaller diameters were corroded preferentially to thicker nanowires. This has been previously reported in the literature (Khaligh and Goldthorpe 2013; Deignan and Goldthorpe 2017; Toybou, 2018).

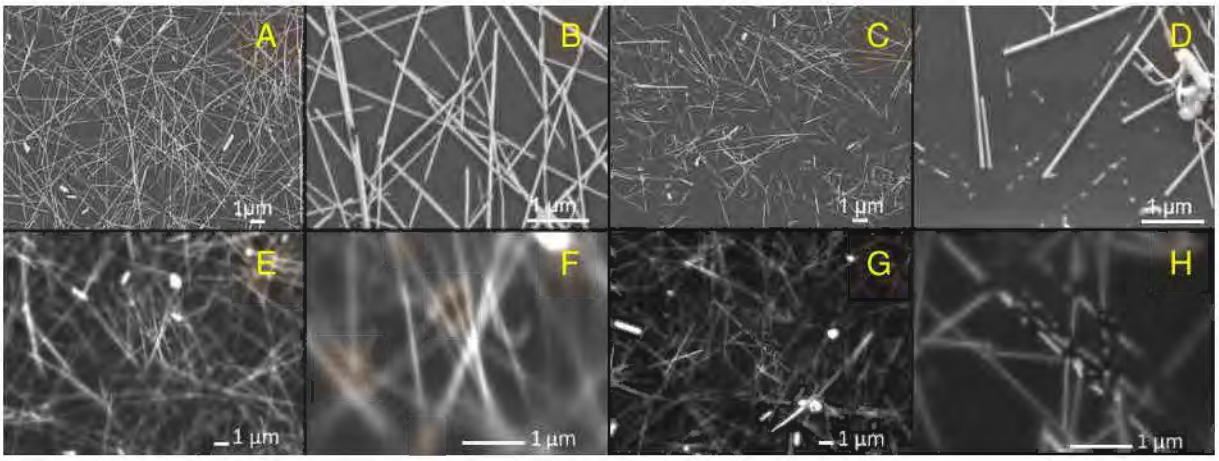


Figure 2. SEM images (BSE) of AgNW films from plate 5 (NW diameter 57 ± 10 nm, NW length 7 ± 2 µm) unexposed and exposed to the corrosion test. Each pair of images (e.g. A-B; C-D, etc) are images of the same film at different magnifications. A-B) Pristine nanowires in an uncoated film, some larger silver particles are seen, an evidence that natural corrosion had taken place prior to experiments. C-D) Fragmentation, nanowire discontinuities and formation of nanoparticles as a result of 20 min of corrosion test. E-F) Pristine nanowires in a film protected with a thin layer of AE12 coating. G-H) Same film after 20 min of corrosion test; fragmented nanowires with dissolution gaps appearing dark. Another example of electrochemical corrosion signatures on AgNW films (from plate 9: NW diameter 86 ± 13 nm nm, NW length ± 4 µm) can be found in SI appendix, Figure S13.

In some spots of the AgNW film, roughening of the nanowire surface was observed together with a decrease in NW brightness. Although this suggested mineral deposition, evidence of silver oxide, hydroxide or sulfide on the wire surface was only detected on very

few occasions by EDS (SI appendix, Figure S4). As discussed previously by other authors (Lin et al., 2018; Toybou, 2018), it is not excluded that low amounts of these products, particularly of sulfide, do occur, but at concentrations below the detection limits of the technique used (SEM-EDS). It is most probable that the observed roughening was due to overgrowths made essentially of elemental silver (Lin et al., 2018), i.e. an effect of Ostwald ripening. No sign of melting was observed at NW junctions or at NW tips, discarding the hypothesis of Joule heating (Khaligh et al., 2017) to contribute to wire failure under our experimental conditions.

In AgNW films with medium coating thickness, similar pattern were observed as described above for thin coated and uncoated films, i.e. highly fragmented networks in the active zone and numerous patches of darkened nanowire surfaces (Figure 2 G-H) (Mayousse et al., 2015). Films coated with a thick layer of polymer (5 ± 1µm) could not be investigated by SEM because of insufficient electron-transparency of that thick layer.

Tiny cubic nanoparticles were observed in AgNW films from plates 1 and 9. These nanocubes, precipitates of Ag and Cl, appeared on the surface of nanowires or the surface of the polymer coating in case of coated films. The presence of traces of chlorine in our experimental set-up is attributed to Cl^- impurities contained in AgNW, inherited from the reagents used for NW synthesis and then released upon NW dissolution. Details of this unexpected occurrence of Ag-Cl nanocubes and speciation calculations are discussed in SI appendix, Figures S6-S8.

3.2. Corrosion of AgNWs as a function of coating layer thickness

The presence of a protective layer on AgNW films and the thickness of this layer play a crucial role in limiting corrosion and failure of AgNW films.

Corrosion current responses are presented for four categories of AgNW films (Figure 3.a), from uncoated films to films with a thick coating layer of AE12 (see details in SI appendix Table S1). The results are consistent with expectations, i.e. the developed quantity of charge was higher in experiments with AgNW films with a thin layer of polysiloxane and decreased as the thickness of the protective layer increased (Figure 3.a). For uncoated AgNW films, the quantity of charge developed was lower than for thin coated films, which may reflect loss of NWs during the experiment, due to insufficient adhesion of NWs to the PEN substrate in the unprotected films. It is interesting to highlight that a corrosion current was measured in all cases. Even when the AgNW film was overcoated with a thick layer (5 µm) of protective AE12, an electrical current could still be measured. Collectively, these results on corrodibility of coated AgNW films indicate that polysiloxane is permeable to water molecules and Ag^{+} ions.

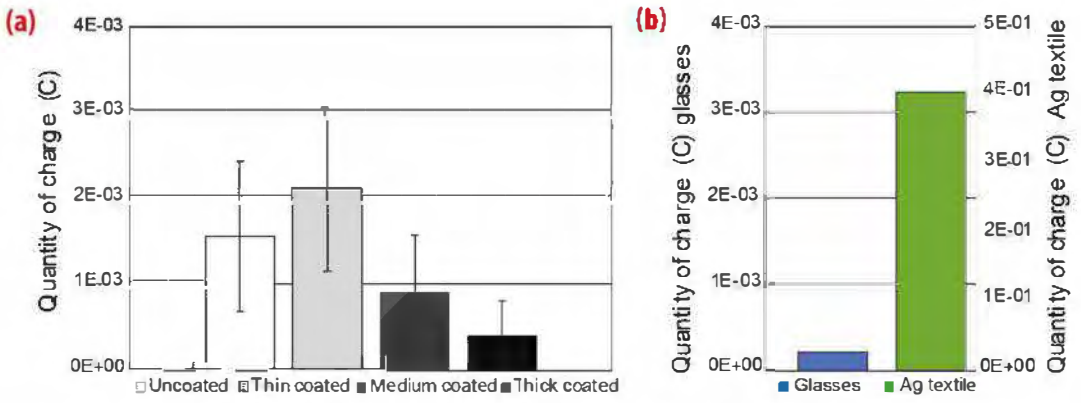


Figure 3. a) Comparison between quantity of charge (mean values) generated in AgNW films with varying thicknesses of polysiloxane coating: uncoated, thin (500 nm), medium (700 nm) and thick (around 5 µm). The high standard deviation values (n= 5) observed in each bar are related to the variable qualities of the different plates used (see SI appendix, Figure S12). b) Quantity of charge for the commercial products tested: heatable glasses containing silver ENM (blue bar) and silver antibacterial textile (green bar). All the results correspond to experiments at 0.01 M ionic strength, an imposed potential of 0.8 V vs. SHE and a pH 5.

Polymers such as PEDOT:PSS, PVP, PVA, that have often been used as coating materials (Jiu et al., 2015), are hydrophilic polymers with a plurality of polar groups which tend to sorb water. Similarly, AE12 polysiloxane used in the present work is also hydrophilic according to

351 fabricant communication and corroborating results from water contact angle measurements
352 (SI appendix, Figure S9). Celle et al. (2018) reported a water vapor transmission rate of 80
353 $mg.m^{-2}.d^{-1}$ for a polysiloxane layer of 1.5 µm thickness. The polarity of these polymers
354 confers them some degree of permeability, which explains why ion diffusion is possible
355 through the protective layer, therefore why a corrosion current is measured by the
356 potentiostat.

357 To control that the coating remained intact throughout the experiment, we conducted AFM
358 surface roughness measurements on the surface of an AgNW thin coated film, on treated and
359 untreated zones. Results (SI appendix, Figure S10) show no significant difference between
360 roughness values in both zones, indicating that the polymeric material exhibits no textural
361 alteration at a scale of tens of nanometers.

362 Electrochemical corrosion results of the two commercial products containing silver, a silver
363 antibacterial textile and a skiing goggle with heatable glasses containing silver ENM (Figure
364 3.b) showed evidence of corrosion in both materials. The skiing goggle glasses (blue bar)
365 generated low corrosion current compared to the quantity of charge measured in the AgNW
366 films of the present study. This result may be related to the very thick layer of protective
367 coating covering the conductive layer. In the case of silver antibacterial textile (green bar), the
368 values were 2 orders of magnitude higher than for the AgNW films.

369 In the next section, the release of ionic silver during the corrosion test will be investigated
370 quantitatively.

371

372 *3.3. Quantification of dissolved silver (Ag^+)*

373 Silver released from AgNWs during the corrosion experiments was quantified by ICP-MS.
374 To distinguish between nanoparticulate Ag and truly dissolved Ag^+, the filtration procedure
375 included micro-filtration through a 0.2 µm pore size membrane and ultra-filtration through a

376 10 kDa membrane. Potential sources of silver contamination as well as interactions between
377 Ag^+ and filter materials (i.e. sorption and desorption) were controlled but proved to be
378 neglegible under experimental conditions in this study (SI appendix, p.14).

379 Silver concentration was measured after each filtration step. Total silver was not quantified
380 systematically due to the limitation of sample volume. Results showed no significant
381 difference in concentration between nanoparticulate-dissolved (0.2μm) and truly dissolved
382 (10 kDa) fractions (SI appendix, Figure S11). This strongly suggests that the main transfer
383 path of silver from the AgNW film to the solution was via diffusion of Ag^+ through the
384 polymer coating, or via direct diffusion in the case of uncoated AgNW film. In consequence,
385 0.2 um polycarbonate filtrated solutions were considered as true solutions.

386 In Figure 4.a, concentrations of dissolved silver (originating from AgNW films) are
387 represented as a function of the thickness of the protective coating layer. Clearly, the amount
388 of dissolved silver decreases as the thickness of the protection layer increases. For uncoated
389 films, release of dissolved silver was not significantly higher than for coated films,
390 corroborating the fact that corrosion is limited by detachment of silver nanowires, which
391 thereby modulates the formation of ionic silver. While Figure 4.a shows mean values, single
392 results specific to two given plates are presented in SI appendix, Figure S12.

393 To find out if a direct relation can be established between the amount of Ag^+ detected in
394 solution (by ICP-MS) and the electrochemical results, we converted the parameters quantity
395 of charge, resulting from the corrosion current measured by the potentiostat, and the amount
396 of ionic silver released from AgNW films to comparable units (calculation details in SI
397 appendix, p. 15).

398 As seen in Figure 4.b, the data sets originating from coated films (blue diamonds) show a
399 same general trend. A linear correlation can be drawn with a slope of almost 1, suggesting
400 that silver corrosion feeds the electric current with almost no electron loss. Although data are

somewhat scattered around the correlation line (R^2 = 0.83), due to the heterogeneity in the quality of the samples studied, this result supports the initial hypothesis that the amount of charge determined for an experiment is representative of the amount of silver released to solution. In contrast, the data sets for uncoated films (red dots) result in very scattered data, among which two sets are out of range with an Ag^+/electron ratio close to 2:1. Here the relatively high value for the Ag^+ release may be attributed to loss of nanowire debris to solution, and these debris may have been included in the quantification of silver. Over all the results suggest that the measure of quantity of charge obtained in the corrosion experiment is a number representative of the amount of corrosion of silver in AgNW films. For coated films, it is directly proportional.

Concerning the quantification of results of the commercial products containing silver, the skiing goggle glasses released small amounts of dissolved silver (40.88 µg/L), comparable to

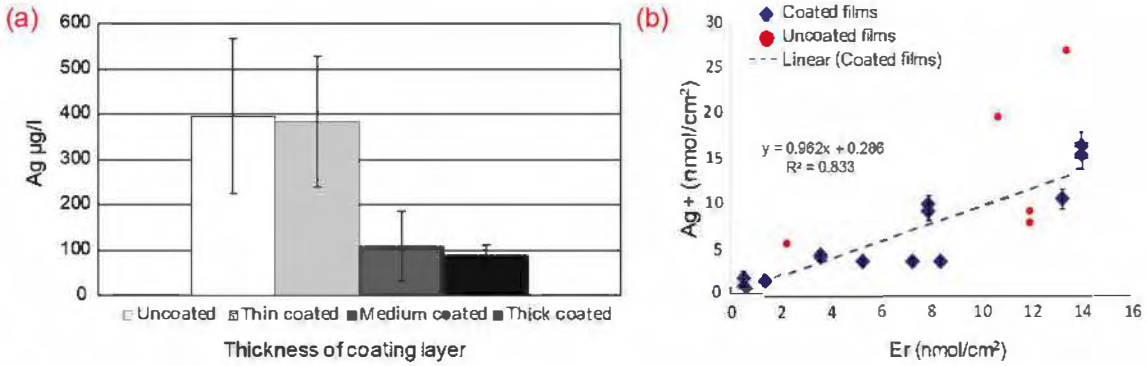


Figure 4. a) Silver concentrations (mean values) in the reactor solutions of the experiments presented in Fig.3.a. The high standard deviation values (n= 5) are related to the variable qualities of the different plates used (see SI appendix, Figure S12). b) Correlation between data of individual samples: dissolved silver (Ag^+) from AgNW films detected in solution by ICP-MS versus quantity of electrons released (Er) derived from current measurements by potentiostat. Coated films (blue diamonds) show good linear relation between both variables with a slope of almost 1, while uncoated films (red dots) gave rise to more scattered data. Standard deviation of silver concentration measurements are given. Detailed calculations of both parameters in SI appendix.

424 the levels obtained in the thick coated AgNW films of the present study. Interestingly, indium
425 was also released to solution (7.28 µg/L). EDS analyses suggest the presence of indium oxide
426 in the skiing goggle glasses.
427 In the case of silver antibacterial textile, the concentration of dissolved silver reached 4.57
428 x10^4 µg/L. The high specific surface area of the silver used as a coating of the textile fibers
429 may explain this very high release.
430 These first trials on commercial Ag containing household products demonstrate that the
431 corrosion method developed here allows obtaining a rapid estimate on the corrodibility of a
432 silver containing material and on the potential metal release. The application of the method to
433 the antibacterial textile shows that it can be used as well for macro-scale corrodible silver
434 containing compounds.
435
436 *3.4. Anthropogenic silver release to the environment and potential impacts*
437 When considering all categories of AgNW conductive transparent films investigated in this
438 study, the range of silver concentrations released from the AgNWs films ranged from
439 approximately 90 µg/L to 400 µg/L (0.83 µmol/L to 3.7 µmol/L). These concentrations can be
440 confronted to toxicity data. Ionic silver has been shown to bioaccumulate in various aquatic
441 organisms and to be highly toxic to bacteria, phytoplankton, and aquatic invertebrates and
442 vertebrates with toxicity threshold values typically around 0.2 µg/L (Croteau et al., 2011;
443 Davies, 1978; Farkas et al., 2010; US EPA, https://www.epa.gov/wqc/ambient-water-quality-
444 criteria-silver). These organisms have important roles in the trophic ecosystem as primary
445 producers and consumers, and adverse impacts of silver to these organisms could have
446 detrimental consequences to overall ecosystem health (Glibert, 2012; Vaughn, 2010).
447 The toxicity of ionic silver to humans depends on the type of cells that are exposed, the
448 liver cells being the most sensitive. According to the American Conference of Governmental

449 Industrial Hygienists (ACGIH, https://www.acgih.org/), the occupational exposure limit for
450 dissolved silver to prevent argyria is 0.01 mg/m^3 (0.01 µg/L).

451 Overall these limits demonstrate that the silver released during the corrosion test of the
452 AgNW films is orders of magnitude above the levels acceptable for the natural environment
453 and living species, even in the case of a thick protective coating (90 µg/L), thereby they point
454 to the relevancy of this test.

455 Concerning anthropogenic input of silver to aquatic systems, Zhang et al. (2008) reported
456 concentrations of dissolved silver in the surface water of Tokyo Bay ranging from $6.5x10^{-4}$
457 µg/L to $1.7 x10^{-3}$ µg/L, a level also obtained for the surface waters of the Japan Sea. Deycard
458 et al. (2017) found average dissolved concentrations of $1x10^{-3}$ µg/L in river water of the "La
459 Réole site" in France. They also reported that the daily silver concentration in an urban
460 wastewater treatment plant located on the fluvial part of the Gironde estuary, ranged from
461 0.14 to 0.55 µg/L depending on weekday and meteorological conditions.

462 Estimations of nanosilver environmental release are primarily based on modelling
463 approaches. Giese et al. (2018) estimated environmental concentrations of Ag-ENM arising
464 from medical applications to have reached $3.8x10^{-4}$ µg/L in surface fresh waters in Germany
465 in 2017 and predicted an increase to $8.9x10^{-4}$ µg/L for 2030. In sewage treatment effluents,
466 modelling calculations gave $1.89x10^{-2}$ µg/L for 2017 and $3.06x10^{-2}$ µg/L for 2030
467 respectively.

468 Silver released from AgNW films during the corrosion test may be put into the perspective
469 of current background levels of Ag in anthropogenically contaminated waters. Indeed the 5-10
470 mL of solution in the electrochemical cell would have to be diluted by an average factor of
471 2000 before "disappearing" in the general background. The diversification of (nano)silver
472 containing products and the increase in their production will irrevocably increase the Ag
473 background concentrations in natural systems and affect water quality.

474 The human body may come in direct contact with silver from anthropogenic source.
475 Deycard et al. (2017) reported that Ag concentrations in a typical household personal care
476 product (deodorant) labeled as containing Ag, range from 0.2 to 0.4 mg/kg of solution (200
477 µg/L to 400 µg/L). These concentrations are within the interval of values generated during our
478 corrosion test. The comparison is appealing because AgNW TCF are predestined to be in
479 contact with skin. Thus, silver released from AgNW TCF may be significant compared to
480 current silver levels in contact with the body.
481
482
483
484 *3.5. Relevance of the developed corrosion test*
485 The experimental results obtained in this work on silver corrosion in AgNW films allow us
486 to estimate an average silver corrosion rate of about 3.7×10^{-10} g/s (Calculation details in SI
487 appendix, Table S3), which is a factor 30 larger than the rate measured for the natural
488 dissolution of a noble silver foil in water saturated with air (Graedel, 1992). This comparison
489 highlights the strength of the method developed here, where a corrosion potential stress,
490 slightly enhanced compared to natural conditions, leads to a fast and easily measurable
491 response of the investigated sample. This accelerated corrosion method is therefore well
492 adapted to assess the risk of metal release from everyday consumer products to their
493 immediate environment (skin, corporal fluids, effluent waters, aquatic systems).
494 The potential application fields of the accelerated release test are wide, however the field of
495 biosensors appears as a very promising one because the use of wearable electronic sensors
496 containing metallic and in particular nanowire silver is growing and because in these devices,
497 the film electrodes are often located in humid environment and in direct contact with the skin.

498 Moreover biocompatible capping agents e.g. citrate are often used in antibacterial Ag-ENMs,
499 which can enhance silver solubility (Liao et al., 2019).
500 An important aspect also is that the method can be applied at any time in the life cycle of
501 the AgNW film. Nowack and Mitrano (2018) discuss the importance of performing aging-
502 release tests in ENM-nanoproducts during use rather than in pristine materials, as consumers
503 and the environment are mostly exposed to aged and transformed nanomaterials (Baun et al.,
504 2017). The method may play a role in predicting the silver flux from household products,
505 biosensor products and personal electronics containing nano-silver (Steinhäuser and Sayre,
506 2017) during life-time and at end of life. In the case of these products come in contact with
507 the environmental water compartment, the method will again provide valuable data for flux
508 estimates.
509 While experiments presented in this work on AgNW coated films have been conducted with
510 a polysiloxane coating (anti-scratch varnish AE12®), more research needs be performed with
511 AgNW films with other matrices, other coatings, in particular hydrophobic polymers like
512 epoxy or new materials such as optical adhesive or reduced graphene oxide layers, is
513 necessary to work out the method limitations. Reference AgNW TCF materials would also
514 need to be developed with known toxicological characteristics.
515 The current lack of standard methods to quantify the risk of exposure of ENM enabled
516 devices to the environment, underline the importance of developing the accelerated corrosion
517 method into a standard method for quantifying the releasability of metals from conductive
518 thin films. The way forward will be to constitute a data base for reference conductive films to
519 which new materials could be compared.
520

521 **4. Conclusions**

In this work, we developed an electrochemical method to evaluate the potential corrosion activity of silver in nanowire transparent conductive films (AgNW TCFs), with or without coating (polysiloxane varnish AE12 ®), and the possible release of ionic silver. We have demonstrated that there is a consistent link between the corrosion current response obtained from coated AgNW TCFs, Ag^+ release and the coatings' characteristics. The corrosion current in each experiment is transformed into quantity of charge, a single number representative of the corrodibility of a material. This corrosion indicator is established quickly, 20 minutes are sufficient for a diagnostic result. The method is thus seen as an accelerated corrosion test, with speed being a significant advantage compared to classical aging studies. Additionally, this method is free from harmful reagents and is low power consumption.

Previous works mainly focused on technical quality control of AgNW transparent electrodes, in particular on lifetime stability of the devices. To our knowledge, this is the first investigation of the corrodibility of a conductive nanomaterial under aqueous conditions, in order to assess the risk of metal release to users and the environment.

A significant result obtained with the accelerated corrosion test is the fact that protective coatings of AgNW films do not systematically provide full protection against corrosion of silver and the release of ionic silver to outside the material. Our results demonstrated that even a thick 5 µm layer of protective polysiloxane coating could not stop AgNW corrosion completely, still leaving a risk of silver (Ag^+) release.

The accelerated corrosion method is promising because it tests the degree of isolation of a material via the possibility of electron flow. The measure of corrodibility depends not only on the presence of (nano) silver but also on textural parameters, in particular the degree of dispersion of the particles in the material and the characteristics of protective coatings. We suggest that the accelerated corrosion method could be elevated to the level of a standard

546 material quality control method to quantify the risk of silver release by corrosion from
547 (nano)silver containing products.
548 The method is not strictly limited to the study of AgNW TCFs, and can be adapted to
549 products containing other oxidisable conductive nano-materials, in particular those with
550 oxidation potential below that of water. Several technology-critical elements (TCE),
551 increasingly applied as ENMs, with application fields where toxicology limits are even less
552 well explored, could be targeted.
553

554 **Abbreviations**

555 AgNW: silver nanowires
556 Argyria: is a condition caused by excessive exposure to chemical compounds of the
557 element silver, or to silver dust.
558 ENMs: enginered nanomaterials
559 ITO: indium tin oxide
560 Nanoproduct: product containing nanomaterial
561 PEDOT:PSS : poly(3,4-Ethylenedioxythiophene):poly(StyreneSulfonate)
562 PEN: polyethylene naphtalate
563 PTFE: polytetrafluoroethylene
564 PVA: polyvinyl alcohol
565 PVP: poly-vinylpyrrolidone
566 SHE: standard hydrogen electrode
567 TCF: transparent conductive film
568

569 **Acknowledgements**

570 This study was conducted in the framework of the project NanoWIR2ES (Nanowire
571 Intelligent Re-Design and Recycling for Environmental Safety) supported by the European
572 Union ERANET-SIINN program (European Research Area Network Safe Implementation of
573 Innovative Nanoscience and Nanotechnology) and was funded by the « Region Hauts de
574 France ». Many thanks to Guillaume Bai (University of Lille) who conducted water contact
575 angle measurements on polysiloxane resin, and Sandra Ventalon (University of Lille) for her
576 help in the spectroscopic observations. We greatly appreciated working with Christophe
577 Vermander (Ecole Centrale de Lille) who realized the electrochemical reactor and Jean
578 Claude Bruyere who helped elucidating the chemical properties of polysiloxane AE12.

579

580 **Appendix . Supporting information**

581 Supplementary data contain complementary information regarding methods and results:
582 table of large plates used for preparation of AgNW films, description of coating material and
583 sample preparation, effect of ionic strength on current generation, details on calculation of
584 quantity of charge, experiment results under fixed electrolyte volume, images showing NW
585 displacement on uncoated films, images highlighting characteristic features of natural
586 alteration (artifacts) on NW films, effects of NW-network density and NW diameter on
587 current, description of silver-chloride nanocubes and EDS spectra, table of stability constants
588 for silver species, model calculations for aqueous silver speciation, water contact angle
589 measurement on control samples, AFM roughness measurements on coated AgNW films,
590 dissolved silver after micro and ultrafiltration, comparison between unaltered and altered
591 large plates, conversion of Ag+ concentrations and electric current generated to a common
592 unit ($nmol.cm^{-2}$), details for calculations of corrosion rates, supplementary images illustrating
593 corrosion signatures on AgNWs after corrosion test.

References

Ahn, Y., Jeong, Y., Lee, Y., 2012. Improved Thermal Oxidation Stability of Solution-Processable Silver Nanowire Transparent Electrode by Reduced Graphene Oxide. ACS Applied Materials & Interfaces 4, 6410–6414. https://doi.org/10.1021/am301913w

Baker, D.R., Lundgren, C.A., 2017. Electrochemical determination of the gallium-nitride photocorrosion potential in acidic media. J. Mater. Chem. A 5, 20978–20984. https://doi.org/10.1039/C7TA04545J

Baun, A., Sayre, P., Steinhäuser, K.G., Rose, J., 2017. Regulatory relevant and reliable methods and data for determining the environmental fate of manufactured nanomaterials. NanoImpact 8, 1–10. https://doi.org/10.1016/j.impact.2017.06.004

Bodycap, le compagnon santé connectée. URL http://www.bodycap-medical.com/fr/ (accessed 7.19.19).

Celle, C., Cabos, A., Fontecave, T., Laguitton, B., Benayad, A., Guettaz, L., Pélissier, N., Nguyen, V.H., Bellet, D., Muñoz-Rojas, D., Simonato, J.-P., 2018. Oxidation of copper nanowire based transparent electrodes in ambient conditions and their stabilization by encapsulation: application to transparent film heaters. Nanotechnology 29, 085701. https://doi.org/10.1088/1361-6528/aaa48e

Chen, S., Song, L., Tao, Z., Shao, X., Huang, Y., Cui, Q., Guo, X., 2014. Neutral-pH PEDOT:PSS as over-coating layer for stable silver nanowire flexible transparent conductive films. Organic Electronics 15, 3654–3659. https://doi.org/10.1016/j.orgel.2014.09.047

Croteau, M.-N., Misra, S.K., Luoma, S.N., Valsami-Jones, E., 2011. Silver Bioaccumulation Dynamics in a Freshwater Invertebrate after Aqueous and Dietary Exposures to Nanosized and Ionic Ag. Environmental Science & Technology 45, 6600–6607. https://doi.org/10.1021/es200880c

Davies, P., 1978. Toxicity of silver to rainbow trout (Salmo gairdneri). Water Research 12, 113–117. https://doi.org/10.1016/0043-1354(78)90014-3

Deignan, G., Goldthorpe, I.A., 2017. The dependence of silver nanowire stability on network composition and processing parameters. RSC Advances 7, 35590–35597. https://doi.org/10.1039/C7RA06524H

Deycard, V.N., Schäfer, J., Petit, J.C.J., Coynel, A., Lanceleur, L., Dutruch, L., Bossy, C., Ventura, A., Blanc, G., 2017. Inputs, dynamics and potential impacts of silver (Ag)

636 from urban wastewater to a highly turbid estuary (SW France). Chemosphere 167,
637 501–511. https://doi.org/10.1016/j.chemosphere.2016.09.154
638
639 Doganay, D., Coskun, S., Genlik, S.P., Unalan, H.E., 2016. Silver nanowire decorated
640 heatable textiles. Nanotechnology 27, 435201. https://doi.org/10.1088/0957-
641 4484/27/43/435201
642
643 Duan, F., Li, W., Wang, G., Weng, C., Jin, H., Zhang, H., Zhang, Z., 2019. Can insulating
644 graphene oxide contribute the enhanced conductivity and durability of silver nanowire
645 coating? Nano Research 12, 1571–1577. https://doi.org/10.1007/s12274-019-2394-8
646
647 Elechiguerra, J.L., Larios-Lopez, L., Liu, C., Garcia-Gutierrez, D., Camacho-Bragado, A.,
648 Yacaman, M.J., 2005. Corrosion at the Nanoscale: The Case of Silver Nanowires and
649 Nanoparticles. Chemistry of Materials 17, 6042–6052.
650 https://doi.org/10.1021/cm051532n
651
652 Farkas, J., Christian, P., Urrea, J.A.G., Roos, N., Hassellöv, M., Tollefsen, K.E., Thomas,
653 K.V., 2010. Effects of silver and gold nanoparticles on rainbow trout (Oncorhynchus
654 mykiss) hepatocytes. Aquatic Toxicology 96, 44–52.
655 https://doi.org/10.1016/j.aquatox.2009.09.016
656
657 Giese, B., Klaessig, F., Park, B., Kaegi, R., Steinfeldt, M., Wigger, H., von Gleich, A.,
658 Gottschalk, F., 2018. Risks, Release and Concentrations of Engineered Nanomaterial
659 in the Environment. Scientific Reports 8. https://doi.org/10.1038/s41598-018-19275-4
660
661 Glibert, P.M., 2012. Ecological stoichiometry and its implications for aquatic ecosystem
662 sustainability. Current Opinion in Environmental Sustainability 4, 272–277.
663 https://doi.org/10.1016/j.cosust.2012.05.009
664
665 Graedel, T.E., 1992. Corrosion mechanisms for silver exposed to the atmosphere. Journal of
666 the Electrochemical Society 139, 1963–1970.
667
668 He, W., Ye, C., 2015. Flexible Transparent Conductive Films on the Basis of Ag Nanowires:
669 Design and Applications: A Review. Journal of Materials Science & Technology, A
670 Special Issue on 1D NanomaterialsSynthesis, Properties, and Applications 31, 581–
671 588. https://doi.org/10.1016/j.jmst.2014.11.020
672
673 Jeong, Y.-C., Nam, J., Kim, J., Kim, C., Jo, S., 2019. Enhancing Thermal Oxidation Stability
674 of Silver Nanowire Transparent Electrodes by Using a Cesium Carbonate-
675 Incorporated Overcoating Layer. Materials 12, 1140.
676 https://doi.org/10.3390/ma12071140
677
678 Jin, Y., Wang, K., Cheng, Y., Pei, Q., Xu, Y., Xiao, F., 2017. Removable Large-Area
679 Ultrasmooth Silver Nanowire Transparent Composite Electrode. ACS Applied
680 Materials & Interfaces 9, 4733–4741. https://doi.org/10.1021/acsami.6b15025

Jiu, J., Wang, J., Sugahara, T., Nagao, S., Nogi, M., Koga, H., Suganuma, K., Hara, M., Nakazawa, E., Uchida, H., 2015. The effect of light and humidity on the stability of silver nanowire transparent electrodes. RSC Advances 5, 27657–27664. https://doi.org/10.1039/C5RA02722E

Khaligh, H.H., Goldthorpe, I.A., 2013. Failure of silver nanowire transparent electrodes under current flow. Nanoscale research letters 8, 1–6.

Khaligh, H.H., Xu, L., Khosropour, A., Madeira, A., Romano, M., Pradére, C., Tréguer-Delapierre, M., Servant, L., Pope, M.A., Goldthorpe, I.A., 2017. The Joule heating problem in silver nanowire transparent electrodes. Nanotechnology 28, 425703.

Khan, A., Nguyen, V.H., Muñoz-Rojas, D., Aghazadehchors, S., Jiménez, C., Nguyen, N.D., Bellet, D., 2018. Stability Enhancement of Silver Nanowire Networks with Conformal ZnO Coatings Deposited by Atmospheric Pressure Spatial Atomic Layer Deposition. ACS Applied Materials & Interfaces 10, 19208–19217. https://doi.org/10.1021/acsami.8b03079

Kim, S., Kim, S.Y., Chung, M.H., Kim, J., Kim, J.H., 2015. A one-step roll-to-roll process of stable AgNW/PEDOT:PSS solution using imidazole as a mild base for highly conductive and transparent films: optimizations and mechanisms. J. Mater. Chem. C 3, 5859–5868. https://doi.org/10.1039/C5TC00801H

Lehmann, S., Gilbert, B., Maffeis, T., Grichine, A., Pignot-Paintrand, I., Clavaguera, S., Rachidi, W., Seve, M., Charlet, L., 2018. In Vitro Dermal Safety Assessment of Silver Nanowires after Acute Exposure: Tissue vs. Cell Models. Nanomaterials 8, 232. https://doi.org/10.3390/nano8040232

Lehmann, S.G., Toybou, D., Pradas del Real, A.-E., Arndt, D., Tagmount, A., Viau, M., Safi, M., Pacureanu, A., Cloetens, P., Bohic, S., Salomé, M., Castillo-Michel, H., Omaña-Sanz, B., Hofmann, A., Vulpe, C., Simonato, J.-P., Celle, C., Charlet, L., Gilbert, B., 2019. Crumpling of silver nanowires by endolysosomes strongly reduces toxicity. Proceedings of the National Academy of Sciences 201820041. https://doi.org/10.1073/pnas.1820041116

Liao, C., Li, Y., Tjong, S.C., 2019. Bactericidal and Cytotoxic Properties of Silver Nanoparticles. Int J Mol Sci 20. https://doi.org/10.3390/ijms20020449

Lin, C.-C., Lin, D.-X., Jhan, J.-T., 2018. Effects of Environmental Factors on the Stability of Silver Nanowire Transparent Electrodes, in: 2018 IEEE 13th Nanotechnology Materials and Devices Conference (NMDC). Presented at the 2018 IEEE 13th Nanotechnology Materials and Devices Conference (NMDC), IEEE, Portland, OR, pp. 1–4. https://doi.org/10.1109/NMDC.2018.8605897

726 Mayousse, C., Celle, C., Fraczkiewicz, A., Simonato, J.-P., 2015. Stability of silver nanowire
727 based electrodes under environmental and electrical stresses. Nanoscale 7, 2107–2115.
728 https://doi.org/10.1039/C4NR06783E
729
730 Miao, L., Liu, G., McEleney, K., Wang, J., 2019. Epoxy-embedded silver nanowire meshes
731 for transparent flexible electrodes. Journal of Materials Science 54, 10355–10370.
732 https://doi.org/10.1007/s10853-019-03507-7
733
734 Miller, M.S., O'Kane, J.C., Niec, A., Carmichael, R.S., Carmichael, T.B., 2013. Silver
735 Nanowire/Optical Adhesive Coatings as Transparent Electrodes for Flexible
736 Electronics. ACS Applied Materials & Interfaces 5, 10165–10172.
737 https://doi.org/10.1021/am402847y
738
739 Molleman, B., Hiemstra, T., 2017. Time, pH, and size dependency of silver nanoparticle
740 dissolution: the road to equilibrium. Environmental Science: Nano 4, 1314–1327.
741 https://doi.org/10.1039/C6EN00564K
742
743 Moreno, I., Navascues, N., Arruebo, M., Irusta, S., Santamaria, J., 2013. Facile preparation of
744 transparent and conductive polymer films based on silver nanowire/polycarbonate
745 nanocomposites. Nanotechnology 24, 275603. https://doi.org/10.1088/0957-
746 4484/24/27/275603
747
748 Naidu, K.S.B., Govender, P., Adam, J.K., 2015. Nano Silver Particles in Biomedical and
749 Clinical Applications : Review 10.
750
751 Nowack, B., Mitrano, D.M., 2018. Procedures for the production and use of synthetically
752 aged and product released nanomaterials for further environmental and ecotoxicity
753 testing. NanoImpact 10, 70–80. https://doi.org/10.1016/j.impact.2017.12.001
754
755 Part, F., Berge, N., Baran, P., Stringfellow, A., Sun, W., Bartelt-Hunt, S., Mitrano, D., Li, L.,
756 Hennebert, P., Quicker, P., Bolyard, S.C., Huber-Humer, M., 2018. A review of the
757 fate of engineered nanomaterials in municipal solid waste streams. Waste
758 Management 75, 427–449. https://doi.org/10.1016/j.wasman.2018.02.012
759
760 Rajski, L., Juda, M., Los, A., Witun, E., Malm, A., 2019. Medical textiles with
761 silver/nanosilver and their potential application for the prevention and control of
762 healthcare-associated infections – mini-review. Current Issues in Pharmacy and
763 Medical Sciences 32, 104–107. https://doi.org/10.2478/cipms-2019-0020
764
765 R-Nano.fr. URL https://www.r-nano.fr/ (accessed 7.18.19).
766
767 Sannicolo, T., Lagrange, M., Cabos, A., Celle, C., Simonato, J.-P., Bellet, D., 2016. Metallic
768 Nanowire-Based Transparent Electrodes for Next Generation Flexible Devices: a
769 Review. Small 12, 6052–6075. https://doi.org/10.1002/smll.201602581
770

771 Scanlan, L. D., Reed, R. B., Loguinov, A. V., Antczak, P., Tagmount, A., Aloni, S., ... &
772 Pham, D., 2013. Silver nanowire exposure results in internalization and toxicity to Daphnia
773 magna. Acs Nano, 7,12, 10681-10694. https://pubs.acs.org/doi/10.1021/nn4034103
774
775 Schinwald, A., & Donaldson, K., 2012. Use of back-scatter electron signals to visualise
776 cell/nanowires interactions in vitro and in vivo; frustrated phagocytosis of long fibres in
777 macrophages and compartmentalisation in mesothelial cells in vivo. Particle and fibre
778 toxicology, 9, 34. https://doi.org/10.1186/1743-8977-9-34
779 STATNANO : Nano Science, Technology and Industry Information. URL
780 https://statnano.com/ (accessed 7.18.19).
781
782 Steinhäuser, K.G., Sayre, P.G., 2017. Reliability of methods and data for regulatory
783 assessment of nanomaterial risks. NanoImpact 7, 66–74.
784 https://doi.org/10.1016/j.impact.2017.06.001
785
786 Stoehr, L. C., Gonzalez, E., Stampfl, A., Casals, E., Duschl, A., Puntes, V., & Oostingh, G. J.,
787 201). Shape matters: effects of silver nanospheres and wires on human alveolar epithelial
788 cells. Particle and fibre toxicology, 8, 36. https://doi.org/10.1186/1743-8977-8-36
789
790 Stumm, W., Morgan, J.J., 1996. Aquatic Chemistry-chemical equilibria and rates in natural
791 waters. Wiley, 3rd edition.
792
793 Sun, T.Y., Bornhöft, N.A., Hungerbühler, K., Nowack, B., 2016. Dynamic Probabilistic
794 Modeling of Environmental Emissions of Engineered Nanomaterials. Environmental
795 Science & Technology 50, 4701–4711. https://doi.org/10.1021/acs.est.5b05828
796
797 Sun, Y., Chang, M., Meng, L. et al., 2019. Flexible organic photovoltaics based on water-
798 processed silver nanowire electrodes. Nat Electron 2, 513–520. https://doi-org.
799 /10.1038/s41928-019-0315-1
800
801 Theodorou, I. G., Müller, K. H., Chen, S., Goode, A. E., Yufit, V., Ryan, M. P., & Porter, A.
802 E., 2017. Silver nanowire particle reactivity with human monocyte-derived macrophage cells:
803 intracellular availability of silver governs their cytotoxicity. ACS Biomaterials Science &
804 Engineering, 3,10, 2336-2347. https://doi.org/10.1021/acsbiomaterials.7b00479
805
806 Toybou, D., 2018. Nanofils d'argent à dimensions maîtrisées: synthèse, toxicité et fabrication
807 d'électrodes transparentes Ph.D. Dissertation, Université de Grenoble-Alpes, FR. 301.
808
809 Toybou, D., Celle, C., Aude-Garcia, C., Rabilloud, T., Simonato, J.-P., 2019. A toxicology-
810 informed, safer by design approach for the fabrication of transparent electrodes based

on silver nanowires. Environmental Science: Nano. https://doi.org/10.1039/C8EN00890F

US EPA. (1980). Ambient Water Quality Criteria for Silver. URL https://www.epa.gov/sites/production/files/2019-03/documents/ambient-wqc-silver-1980.pdf (accessed 9.27.19).

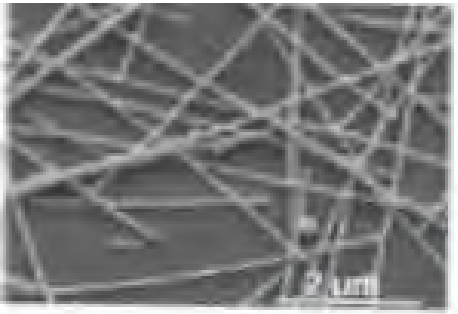
Vaughn, C.C., 2010. Biodiversity Losses and Ecosystem Function in Freshwaters: Emerging Conclusions and Research Directions. BioScience 60, 25–35. https://doi.org/10.1525/bio.2010.60.1.7

Welcome to The Nanodatabase. URL http://nanodb.dk/ (accessed 7.18.19).

Xu, F., Zhu, Y., 2012. Highly Conductive and Stretchable Silver Nanowire Conductors. Advanced Materials 24, 5117–5122. https://doi.org/10.1002/adma.201201886

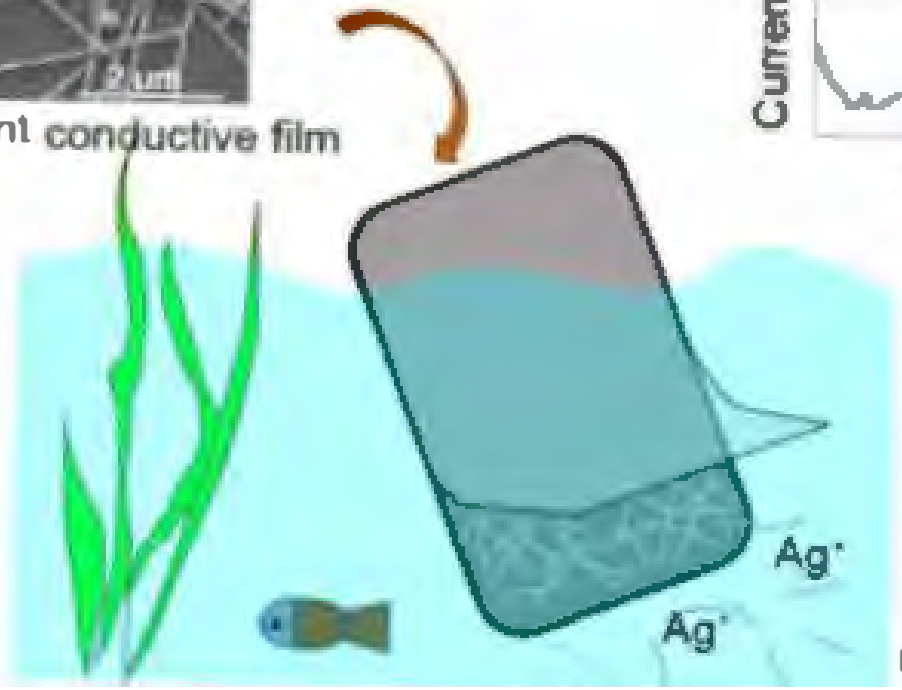
Yao, S., Myers, A., Malhotra, A., Lin, F., Bozkurt, A., Muth, J.F., Zhu, Y., 2017. A Wearable Hydration Sensor with Conformal Nanowire Electrodes. Advanced Healthcare Materials 6, 1601159. https://doi.org/10.1002/adhm.201601159

Zhang, R., Engholm, M., 2018. Recent Progress on the Fabrication and Properties of Silver Nanowire-Based Transparent Electrodes. Nanomaterials 8, 628. https://doi.org/10.3390/nano8080628

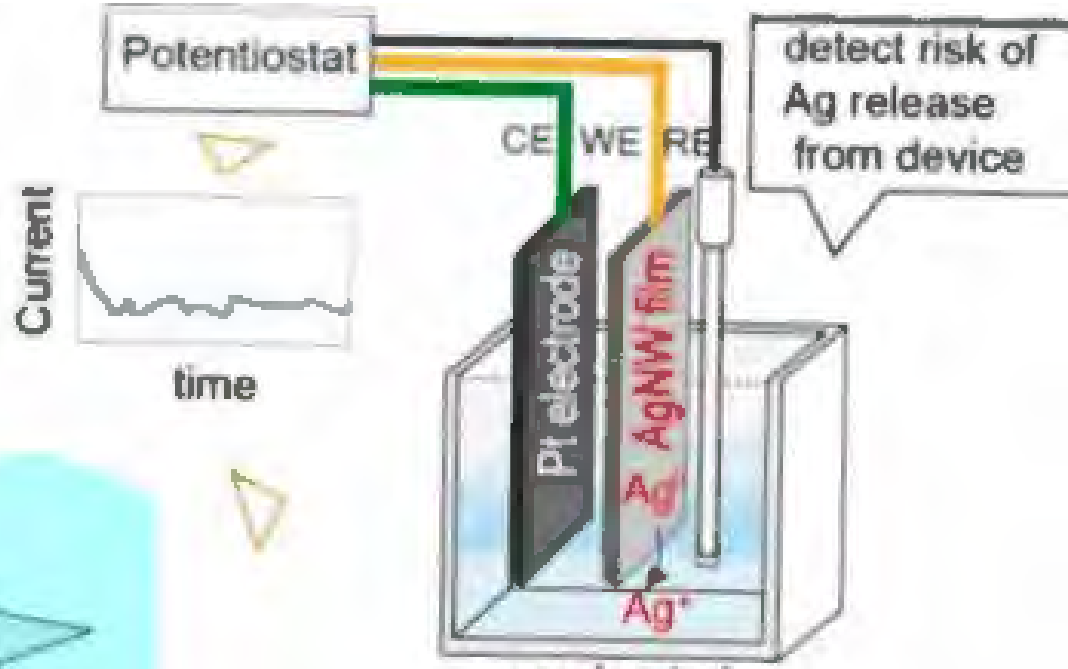


silver nanowire

transparent conductive film



release & pollution



accelerated corrosion test

Crumpling of silver nanowires by endolysosomes strongly reduces toxicity

Sylvia G. Lehmann[a], Djadidi Toybou[a,b], Ana-Elena Pradas del Real[c], Devrah Arndt[d], Abderrahmane Tagmount[d], Muriel Viau[a], Malak Safi[a], Alexandra Pacureanu[c], Peter Cloetens[c], Sylvain Bohic[c,e], Murielle Salomé[c], Hiram Castillo-Michel[c], Brenda Omaña-Sanz[f,g,h], Annette Hofmann[f,g,h], Christopher Vulpe[d], Jean-Pierre Simonato[b], Caroline Celle[b], Laurent Charlet[a], and Benjamin Gilbert[a,i,1]

[a]Institut des Sciences de la Terre, Université de Grenoble–Alpes, CNRS, F-38000 Grenoble Cedex 9, France; [b]Laboratoire d'Innovation pour les Technologies des Energies Nouvelles et les Nanomatériaux, Département des Technologies des Nouveaux Matériaux, Université de Grenoble–Alpes, Commissariat à l'Énergie Atomique et aux Énergies Alternatives, F-38054 Grenoble Cedex 9, France; [c]European Synchrotron Radiation Facility, Course Spéciale (CS) 40220, 38043 Grenoble Cedex 9, France; [d]Center for Environmental and Human Toxicology, Department of Physiological Sciences, College of Veterinary Medicine, University of Florida, Gainesville, FL 32611; [e]Synchrotron Radiation for Biomedicine, CS 40220, Institut National de la Santé et de la Recherche Médicale, 38043 Grenoble Cedex 9, France; [f]Laboratoire d'Océanologie et de Géosciences (LOG), UMR 8187, Université Lille, F 59000 Lille, France; [g]UMR 8187, CNRS, F 59000 Lille, France; [h]UMR 8187, Université Littoral Côte d'Opale, F 62930 Wimereux, France; and [i]Energy Geoscience Division, Lawrence Berkeley National Laboratory, Berkeley, CA 94720

Edited by Howard A. Stone, Princeton University, Princeton, NJ, and approved June 12, 2019 (received for review November 23, 2018)

Fibrous particles interact with cells and organisms in complex ways that can lead to cellular dysfunction, cell death, inflammation, and disease. The development of conductive transparent networks (CTNs) composed of metallic silver nanowires (AgNWs) for flexible touchscreen displays raises new possibilities for the intimate contact between novel fibers and human skin. Here, we report that a material property, nanowire-bending stiffness that is a function of diameter, controls the cytotoxicity of AgNWs to nonimmune cells from humans, mice, and fish without deterioration of critical CTN performance parameters: electrical conductivity and optical transparency. Both 30- and 90-nm-diameter AgNWs are readily internalized by cells, but thinner NWs are mechanically crumpled by the forces imposed during or after endocytosis, while thicker nanowires puncture the enclosing membrane and release silver ions and lysosomal contents to the cytoplasm, thereby initiating oxidative stress. This finding extends the fiber pathology paradigm and will enable the manufacture of safer products incorporating AgNWs.

nanotechnology | endocytosis | fiber toxicity

In the 20th century, the widespread use of high-aspect-ratio asbestos fibers led to adverse health consequences, including pleural mesothelioma, among exposed individuals. Painstaking epidemiology studies led to the establishment of the fiber pathology paradigm (FPP) that defines the distinctive injurious properties of high-aspect-ratio inorganic particles (1). Critical characteristics include biological persistence, mobility, redox reactivity, and proinflammatory capabilities. The replacement of asbestos by vitreous silica fibers eventually led to a decline in asbestos-related diseases in the developed world (2), but the asbestos experience holds considerable relevance for the manufacture and distribution of novel fibrous materials such as metallic nanowires (3).

Silver nanowires (AgNWs) are of considerable utility for consumer electronics because they provide economically and technically competitive approaches for fabricating flexible, optically transparent, and electrically conductive films (4, 5). AgNWs can be deposited from solution onto substrates to form conductive transparent networks (CTNs) that do not degrade by repeated bending. Flexible AgNW-based devices will likely replace existing technology based on doped tin oxide layers on brittle glass sheets for displays and touch screens (6). All AgNW applications balance a range of competing performance and cost characteristics (4) that typically do not include consideration of potential physiological interactions and toxicity.

Because of their morphology and redox reactivity, we (7–9) and other groups (10–13) suspected that AgNWs may exhibit fiberlike toxicity. Indeed, AgNWs exhibit a length threshold for phagocytosis in macrophages (14), induce toxicity in alveolar epithelial cells (15) and provoke numerous indicators of pulmonary toxicity in mice and rats (8, 11). Prior studies, however, have been relevant to pulmonary (i.e., inhalation) exposures, while the anticipated consumer products for AgNWs, including portable electronic devices, suggest the need to evaluate dermal exposure and toxicity. The few studies of nonphagotic cells observed ready internalization of AgNWs through an unidentified endocytosis pathway (9, 15, 16). Although the dermis of healthy human skin provides a barrier to short-term NW exposure (9), we have also observed that AgNWs can translocate through epithelial barriers (7).

The goal of this research collaboration was to establish whether AgNWs could be designed to minimize their intrinsic cellular toxicity while retaining necessary technical properties for CTNs. The link between fiber length and cytotoxicity is well established. The FPP identifies a threshold around 10 μm, above which macrophages cannot successfully internalize nanowires (1) or other nanofibers (17)—this phenomenon called frustrated phagocytosis leads to apoptosis and inflammation. We reasoned that AgNW diameter could be an important control on the extent of internalization via endocytosis. At least 3 endocytotic pathways have been identified that initiate the engulfment of an

Significance

Silver nanowires are a type of novel fiber that are likely to have wide application in consumer electronics but that can also carry risk for cell internalization and toxicity. Here, we show that silver nanowire toxicity can be greatly diminished by reducing nanowire diameter without affecting device performance. X-ray microscopy and supporting studies revealed that endolysosome membranes can mechanically crumple thin silver nanowires, the likely mechanism for diminishing toxicity.

Author contributions: S.G.L., D.A., A.H., C.V., J.-P.S., C.C., L.C., and B.G. designed research; S.G.L., D.T., A.-E.P.d.R., D.A., A.T., M.V., M. Safi, A.P., P.C., S.B., M. Salomé, H.C.-M., B.O.-S., and B.G. performed research; D.T., A.T., and P.C. contributed new reagents/analytic tools; S.G.L., A.-E.P.d.R., D.A., A.T., M.V., A.P., P.C., B.O.-S., A.H., C.V., L.C., and B.G. analyzed data; and S.G.L., C.V., J.-P.S., C.C., L.C., and B.G. wrote the paper.

The authors declare no conflict of interest.

This article is a PNAS Direct Submission.

[1]To whom correspondence may be addressed. Email: bgilbert@lbl.gov.

This article contains supporting information online at www.pnas.org/lookup/suppl/doi:10.1073/pnas.1820041116/-/DCSupplemental.

external object by the outer membrane (18) and a common driving force is the free energy of interaction between the object and the outer membrane. Imaging (9) and simulation (19) studies indicate that the internalization of rod-shaped particles occurs via tip–membrane interaction and entry. Smaller diameter objects lower the interaction free energy and raise the energy penalty for membrane curvature. As a consequence, the endocytosis efficiency of nanoparticles and nanorods drops significantly below diameters of 50 to 60 nm, a size at which virus capsids can initiate cell entry without specific molecular interactions that recruit membrane proteins (20). Thus, we sought to establish the effect of AgNW diameter on internalization and toxicity.

Commercial products cover the range of AgNW dimensions required for this study (e.g., refs. 21 and 22) but AgNWs are not commercially available with independent control of length and diameter that in standard syntheses are highly correlated (23). Hence, we performed a multiparameter study of the polyol synthesis method (24, 25) to identify nucleation, growth and purification procedures and conditions to independently vary AgNW diameter and length. We synthesized polyvinylpyrrolidone (PVP)-coated AgNWs with very similar length distributions, around 9 μm and 25 μm, but with mean diameters ranging from 30 to 90 nm (*SI Appendix*, Table S1 and Fig. 1*A*). We assayed the acute cytotoxicity of the AgNWs in three cell lines: murine fibroblasts (Fig. 1*C*), rainbow trout gut cells (Fig. 1*D*) and human primary fibroblasts (*SI Appendix*, Fig. S4) finding in all cases a dose-dependent reduction in cell viability. Following the consensus for nanomaterial dose reporting, we report AgNW dose as number of particles per volume that was calculated from total silver concentration and verified by direct counting. The trends are preserved for doses reported in silver mass concentration (*SI Appendix*, Fig. S2). For ionic silver, a positive control (*SI Appendix*, Fig. S2), the concentration for a 50% reduction in viability (LC_{50}) is in good agreement with prior studies (26).

In accordance with our project hypothesis, we found that AgNW toxicity could be lowered by reducing the diameter of ~10-μm-long AgNWs. For murine fibroblasts, LC_{50} is almost 2 orders of magnitude higher for 30-nm than for 90-nm-diameter nanowires (Fig. 1*C*). For rainbow trout gut cells the dose–response curves show ~1 order of magnitude reduction in toxicity (Fig. 1*D*). In accordance with the FPP, 25-μm-long AgNWs are significantly more cytotoxic than ~10-μm-long NWs, confirming that a "frustrated endocytosis" mechanism applies to fiber exposure in nonphagocytic cells. Counter to our hypothesis, however, reducing the diameter of ~25-μm-long AgNWs had only a small reduction in toxicity, suggesting diameter did not primarily affect internalization. We used dark-field visible light microscopy to compare the uptake of 30- versus 90-nm-diameter, ~10-μm-long AgNWs by murine fibroblasts (*SI Appendix*, Fig. S3). In each case, close to 100% of the administered AgNWs were internalized within 24 h. We further checked for differences in the release of ionic silver in the cell culture media finding it to be around 0.4 μg/mL for the smallest and 0.3 μg/mL for the largest-diameter AgNWs (*SI Appendix*, Fig. S1), values that are significantly below the 50% lethal dose (LD_{50}) for silver nitrate and run counter to the toxicity trends. Based on all these findings, the differential toxicity must be caused by physiological interactions following internalization.

We used a combination of synchrotron X-ray imaging and microchemical analysis methods at beamlines ID-16A and ID-21 at the European Synchrotron Radiation Facility (ESRF) to study the intracellular location, morphology and chemistry of 33- or 93-nm diameter, 9-μm long, AgNWs administered to murine fibroblasts cultured on X-ray transparent Si_3N_4 membranes, cryofixed immediately before analysis and analyzed at 120 K to minimize X-ray radiation damage. Holographic X-ray nano-imaging generated 2D projection images (phase contrast maps) and stereographic images with ~40-nm resolution. Full 3D reconstructions with ~80-nm resolution were obtained from holo-tomography. Nanofocus X-ray fluorescence (XRF) of the same specimens provided 2D elemental maps with ~40-nm resolution. Microfocus XRF of duplicate samples provided 2D elemental maps that could be complemented by Ag L_{III}-edge X-ray absorption spectra to determine silver speciation. Key findings from these data are summarized in Fig. 2.

The X-ray images revealed a striking difference in the intracellular fate of AgNWs with different diameters. Intracellular 93-nm AgNWs were slightly bent with respect to nanowires after synthesis and suspension in cell culture media but nevertheless retained the elongate nanowire morphology (Fig. 2*A*).

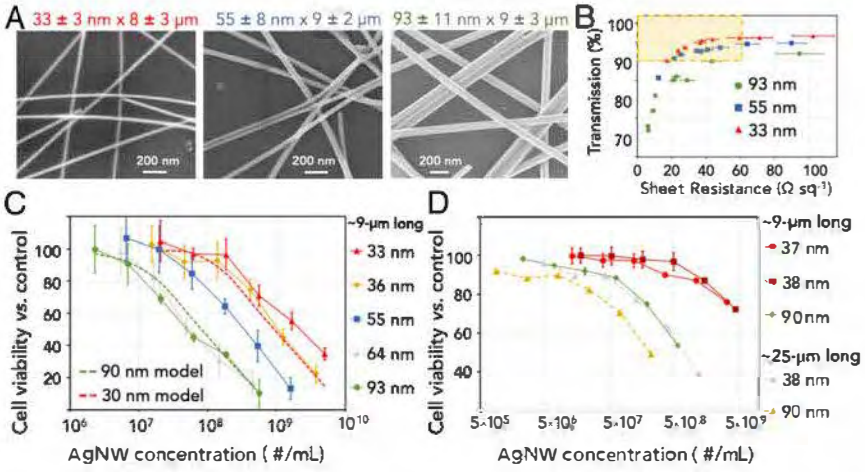


Fig. 1. Diameter-dependent performance and cytotoxicity of AgNWs. (*A*) Scanning-electron microscopy images of 8- to 9-μm long AgNW with 3 mean diameters. (*B*) Relationship between optical transparency and sheet resistance for CTNs fabricated by ~9-μm-long AgNWs with three different diameters. Each data point represents a spray-coated network at a different AgNW density. Yellow-shaded region represents the technological target for commercial CTNs. (*C*) Viability of murine fibroblasts cells following 24-h exposure with AgNWs of different diameter, as determined by the MTT assay. The dashed lines are model predictions as described in the text. (*D*) Viability of rainbow trout gut cells following 24-h exposure with AgNWs of different diameter determined by CellTiter-Glo assay. Cell-viability data are presented as a percentages relative to the nontreated control cells. Error bars on triplicate measurements are 1 SD.

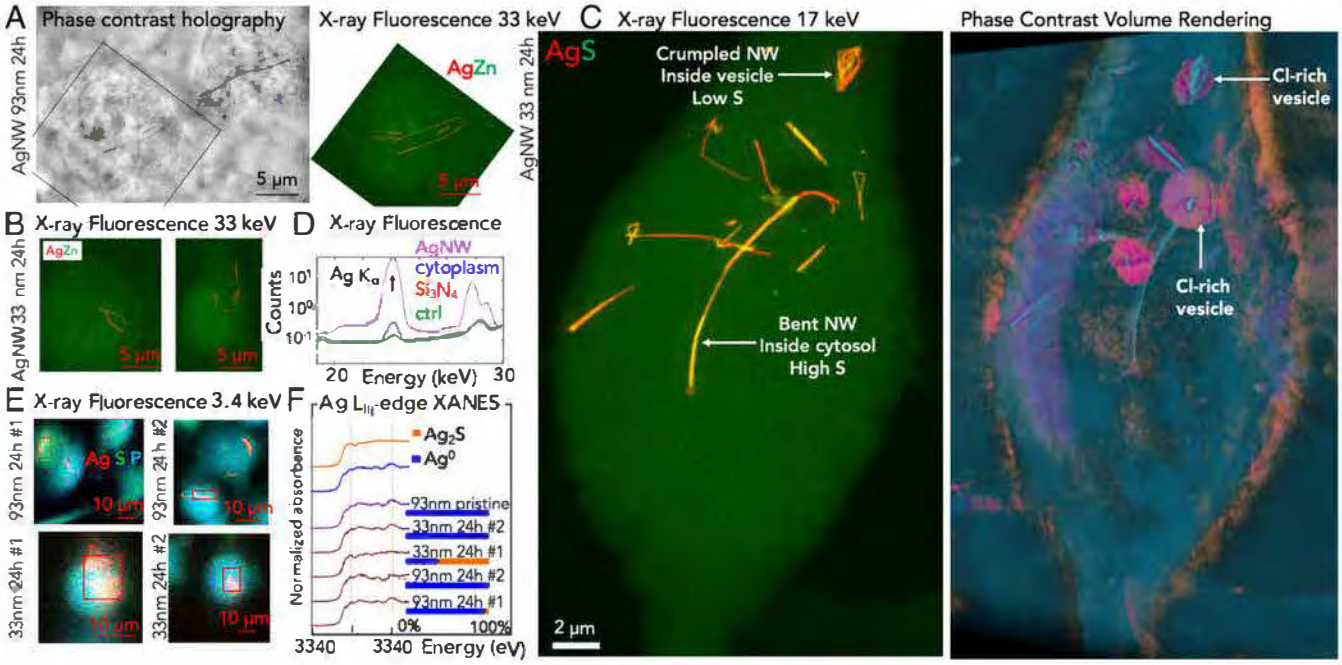


Fig. 2. X-ray analyses of murine fibroblasts exposed to 33- or 93-nm diameter and ~9-µm-long AgNWs. (*A*) Phase-contrast image (*Left*) and nanofocus Zn and Ag elemental map (*Right*) of two cells containing 93-nm AgNW acquired at 33 keV. (*B*) Elemental maps acquired at 33 keV of cells exposed to 33-nm AgNW. (*C*) Elemental map (*Left*) and phase-contrast image (*Right*) acquired at 17 keV of a fibroblast cell exposed to 33-nm AgNW. Yellow regions of the left image indicate colocalization of Ag and S. An animated 3D tomogram is given in Movie S1. (*D*) X-ray fluorescence spectra from internalized AgNWs and cytoplasm of exposed cell, control cell ("ctrl") and X-ray transparent substrate (Si_3N_4). (*E*) Examples of microfocus elemental maps of cells exposed to 33- and 93-nm-diameter AgNW. (*F*) Selected area Ag L_{III}-edge XANES spectra from reference materials and AgNWs incubated in cell culture medium ("pristine") or exposed to cells for 24 h. Names of the spectra correspond to images given in *E*. Dashed lines are linear combination fits of reference spectra to the data and the bars represent the proportion of Ag_2S and Ag^0 determined by the fitting.

By contrast, the majority of intracellular 33-nm AgNWs were completely deformed and exhibited crumpled, collapsed, and even circular shapes (Fig. 2*B*).

A combined XRF and tomographic analysis at 17 keV of a single fibroblast cell exposed to 30-nm-diameter AgNWs for 24 h provided more insight into the internalization pathway and AgNW fate (Fig. 2*C* and Movie S1). All AgNWs were associated with chlorine-rich intracellular vesicles we infer to be late endosomes or lysosomes (18). One crumpled AgNW is completely contained within a vesicle and has a low association of sulfur with silver. One bent but elongate AgNW has only the tip clearly contained. That tip has low sulfur, but in contrast region that extends away from the vesicle is extensively associated with sulfur. Ag L_{III}-edge X-ray absorption near-edge structure (XANES) showed that 33- and 93-nm-diameter AgNWs that were internalized underwent varying degrees of sulfidation to form Ag_2S, a reaction that did not occur over 7 d in cell-free culture medium (Fig. 2*F*). Silver organosulfur species and silver chloride were not detectable. Integrating the Ag K_α fluorescence from selected areas provided evidence of an above-background signal from diffuse silver inside the cytoplasm of a cell containing 93-nm AgNWs but not neighboring cells without AgNWs or cells exposed to 33-nm AgNWs.

Cellular injury caused by nanomaterial exposure typically falls within an oxidative-stress hierarchy in which the generation of reactive oxygen species (ROS) affects cellular physiology, causes proinflammatory responses and ultimately cell death (27, 28). Murine fibroblast cells were exposed to 10^6 to 10^8 NW/mL of 36- or 93-nm-diameter AgNWs for 24 h, and we used fluorescent probes and epifluorescence microscopy to assess selected cellular responses. Using a fluorescent probe for general ROS (CM-H2DCFDA), we determined dose–response for the 2 AgNW diameters, finding no intracellular ROS production at the lowest dose but an increasing dose–response for the 93-nm AgNWs (Fig. 3 *A* and *D*). Using a probe for mitochondrial membrane potential, ψ_m, (JC-1) we find, at equivalent doses, that 93-nm but not 36-nm-diameter AgNWs cause substantial reduction of ψ_m (Fig. 3 *B* and *D*). Intracellular calcium release from storage sites such as the endoplasmic reticulum is a major oxidative stress response associated with mitochondrial perturbation and cell death. Using a probe for Ca^{2+} (Fluo-4 AM) we find, at equivalent doses, that 93-nm but not 36-nm AgNWs provoke substantial calcium release (Fig. 3 *C* and *D*). Apoptotic cells loose membrane integrity which can be assessed by the uptake of propidium iodide (PI). Only cultures exposed to 93-nm AgNWs showed PI permeant cells (Fig. 3 *C* and *D*). Thus, the fluorescence assays demonstrate that 93-nm AgNWs provoke a significantly stronger cytotoxic response.

These findings demonstrate that the cytotoxicity of AgNWs shorter than ~10 µm is determined by the ability to puncture the enclosing endocytotic membranes during internalization and processing. Endolysosome puncturing could initiate an oxidative stress response by 2 pathways. First, we find that exposure to the cytoplasm initiates or accelerates oxidative sulfidation of silver. Although precipitation as a sulfide strongly reduces the activity of ionic silver (15), sulfidation is a complex redox reaction that can generate radicals (29) and deplete H_2S, a signaling molecule with antioxidant properties (30). Second, membrane puncturing could release lysosomal contents including acids and proteases that can initiate stress responses (31). Indeed, AgNWs are more effective than Ag nanoparticles at the induction of NLRP3 inflammasome activation and IL-1β production in phagocytes but until now a mechanism for membrane damage has not been clearly revealed (32, 33).

Further evidence that AgNWs can puncture lipid bilayer membranes is provided by studies of AgNW phagocytosis by rat

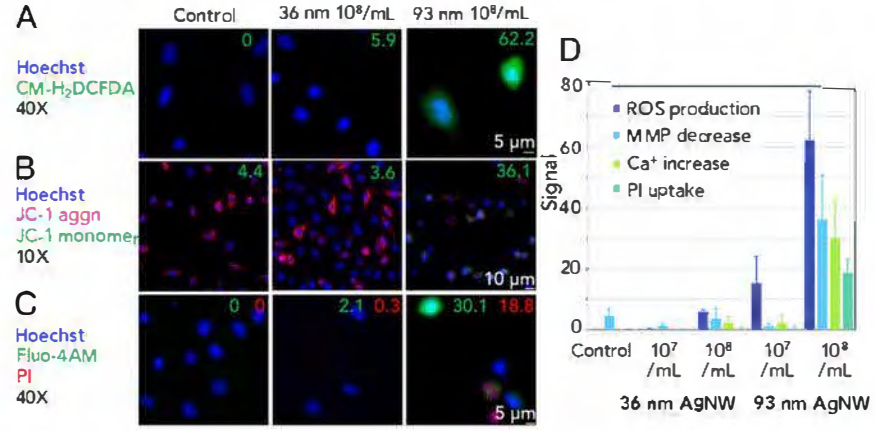


Fig. 3. Fluorescence assays of diameter dependent cellular toxicity. (*A*–*C*) Merged epifluorescence images of murine fibroblast cells exposed to 10^8 NW/mL of 36- or 93-nm-diameter AgNWs for 24 h with the fluorescence channels set to the indicated probe molecules and using the microscope objectives indicated. Colored numbers show the integrated probe fluorescence intensity. (*D*) Summary of intracellular fluorescent probe quantitation. Error bars report 1 SD on triplicate measurements.

alveolar cells. We covalently attached a pH-sensitive fluorescent dye to commercial silica-coated AgNWs and used time-lapse fluorescence microscopy to observe internalization and intracellular fate. In growth medium, these AgNWs are nonfluorescent, but within about 30 min of exposure they developed a strong green fluorescence indicating uptake into acidifying vesicles (*SI Appendix*, Fig. S2 and Movie S2). We observed multiple examples of punctured phagolysosomes and acid release into the cytoplasm following AgNW internalization (*SI Appendix*, Fig. S8 and Movie S3). We further sought to quantify the extent of endolysosome puncturing in nonimmune cells using the same PVP-coated AgNWs developed for this study. We labeled 60-nm-diameter AgNWs with a histidine-terminated fluorescent protein, mCherry, and coadministered them with established fluorescent probes for endosomes and lysosomes. Following AgNW internalization, the AgNW-mCherry fluorescence only partly colocalizes with endolysosome markers (*SI Appendix*, Fig. S9), providing further evidence of puncturing.

Prior experimental and theoretical studies have investigation the deformation of rod-like vesicle contents including microtubules and multiwalled carbon nanotubes (MWCNTs) that could be caused by lipid bilayer membrane forces (34, 35). Most recently, Zhu et al. (36) used molecular and continuum simulation methods to estimate that MWCNTs could be subjected to forces of up to ~20 pN during endocytosis and predicted that MWCNTs with a buckling threshold below this value would have a low risk for membrane penetration and permeation. In support of this prediction, studies of the internalization of stiff and flexible MWCNTs reported different intracellular fates and toxicity (36, 37) but these studies could not provide quantitative tests of mechanical forces because the flexible MWCNTs were significantly longer and formed tangled balls. Ji et al. (38) determined a length threshold around 200 nm for proinflammatory response of sub-10-nm-diameter CeO_2 nanorods but did not consider the mechanical interactions or investigate a diameter effect. The present study of AgNWs with the same mean lengths but different mean diameters will allow more quantitative assessment of the hypothesis that membrane forces exerted on internalized fibers can cause deformation (*SI Appendix*). For 10-μm-long AgNWs, the predicted compression forces due to membrane bending elasticity could reach 100 pN, while the linear rod buckling force requires ~200 pN. However, the presence of initial curvature, lattice defects and strain or the action of intracellular degradation processes could weaken the AgNWs.

A conceptual model for stiffness dependent AgNW toxicity is given in Fig. 4, and an accompanying quantitative model for the dose-dependence of the cellular cytotoxicity of AgNWs is described in *SI Appendix* and plotted in Fig. 1. Following calibration of the model at low AgNW dose, good agreement with the cell viability data at higher doses supports the concept that cell mortality is determined by the fraction of AgNWs that puncture endolysosome membranes and subsequently undergo surface chemical reactions in the cytosol.

Having established that both diameter and length thresholds affect toxicity, we evaluated key goals for CTN displays: a sheet resistance less than 50 $\Omega\ sq^{-1}$ with an optical transparency greater than 90%. Fig. 1*B* shows the relationship between total transparency and sheet resistance for ~9-μm-long AgNWs with 3 diameters. Typical AgNWs used for prototype and commercial CTNs have lengths greatly exceeding the threshold for frustrated internalization (5), but the data shown here illustrate that high-conductivity networks can be fabricated with shorter and less hazardous AgNWs. Moreover, at the targeted resistivity, the 3-fold decrease in diameter increases transmission from 92 to 96%. Thus, reducing AgNW diameter is an effective nanomaterial design strategy for lowering cellular injury that can be achieved without a deterioration in target technology performance. Other strategies to lower the hazard from other nanomaterials have been proposed that have not been experimentally proven and that do not consider links to technical performance (39, 40).

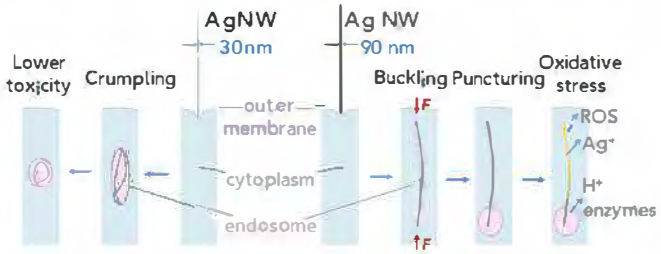


Fig. 4. Conceptual model for a biomechanical threshold causing differential toxicity of AgNW. Both thinner and thicker AgNWs are internalized by endocytosis. (*Left*) Membrane forces during or following endocytosis exceed the yield threshold for thinner AgNW, causing crumpling, containment within the endolysosome and lower toxicity. (*Right*) Membrane forces cannot crumple the thicker AgNW, leading to membrane puncturing and release of endolysosome contents to the cytoplasm and cell toxicity. An associated quantitative model is described in *SI Appendix*.

Our group also seeks to reclaim silver from CNTs using electrochemical methods, and we find no adverse consequences of diameter reduction (*SI Appendix*, Fig. S14). In summary, our findings establish and quantify a biomechanical component to the FPP and suggest that rigidity could be harnessed for nanomedicine approaches to deliver drugs to the cytoplasm (16).

Materials and Methods

Silver Nanowires Synthesis. AgNWs with 3 distinct dimensions were prepared and purified by variations of an established protocol (25). Different quantities of NaCl and poly vinylpyrrolidone (molecular weight ≈ 40,000 g mol^{-1}) were dissolved in ethylene glycol, and slowly added into a stirred solution of $AgNO_3$ in ethylene glycol at room temperature (*SI Appendix*). The mixture was heated to 150 or 160 °C for 60 min and cooled down to room temperature. Reaction temperature and KCl addition was used to control dimensions. Purification by 2 sequential decantations removed by-products and organics. The diameter and length distributions for each batch was determined by SEM imaging. CNTs were fabricated by spray coating AgNW suspensions (Sonotek ExactACoaT). Optical transmittance was measured by UV-visible spectroscopy (Varian Cary 5000) and sheet resistance by a 4-pin probe (Loresta EP MCP-T360).

Cell Culturing and Toxicity Assays. Mouse fibroblast cells (L929) were cultivated in DMEM supplemented with 10% fetal bovine serum, 50 units/mL penicillin, and 50 μg/mL streptomycin (Gibco). Cytotoxicity in L929 cells was assessed using the 3-[4,5-dimethylthiazol-2-yl]-2,5-diphenyl tetrazolium bromide (MTT) assay (Sigma-Aldrich). Briefly, 8,000 cells/well were seeded into a 96-well plate (Nunc) and incubated 37 °C. After 24 h, cells were treated with 100 μL of suspensions containing concentrations between 0.16 to 120 μg/mL of AgNWs or $AgNO_3$ (a positive control for silver toxicity). After a further 24 h, the cells were washed 2 times with phosphate-buffered saline (PBS) and the wells replenished with fresh culture medium containing 0.5 mg/mL of MTT predissolved in PBS. After 3 h of incubation, cells were washed two times with PBS and 100 μL of dimethyl sulfoxide was added to dissolve the formazan crystals. Aliquots were transferred into new well plates to quantify formazan production from the absorbance at 560 nm using a microplate reader (FLUOstar OMEGA, BMG Labtech). Initial trials using cell-free controls and a centrifugation step found that the AgNWs were not transferred in the quantitation step and did not interfere with the assay. The dose–response data for $AgNO_3$ gave excellent agreement with prior studies (26). Cell viability was expressed as percentage relatively to negative control (nontreated cells) using alternative dose metrics: silver mass concentration in *SI Appendix*, Fig. S2 and number of particles per volume in Fig. 1 *C* and *D* and *SI Appendix*, Fig. S4. Viability assays for other cell lines are described in *SI Appendix* and included similar controls.

The impact of AgNW exposure to L929 cells on oxidative stress production, mitochondrial membrane potential, intracellular calcium level and membrane integrity was assessed using fluorescent probes as described in *SI Appendix*. A combination of dark-field and epifluorescent microscopy was used to count the number of internalized AgNWs in L929 cultures exposed to either 30- or 90-nm mean diameter nanowires and to classify the proportion of rod-like or crumpled nanowires (*SI Appendix*, Fig. S3). These data were used to calibrate a statistical model for the dose-dependent viability of murine cells exposed to either 30- or 90-nm mean diameter AgNWs described in *SI Appendix*.

X-Ray Imaging and Chemical Analysis. L929 cells were grown on silicon nitride membrane (Silson) previously coated with poly L-lysine 0.01% (Sigma) in 24-well plate for 24 h before treatment with 33- or 93-nm-diameter, ~10-μm-long, AgNW at a concentration of 10^6 NW/mL for 24 h. After 24 h of treatment, some cell samples were washed with PBS and allow to grow for further 48 h. At the end of the incubation, samples were rinsed with ammonium formate, cryofixed with a Leica EM GP Automatic Plunge Freezer (ESRF) and stored in liquid nitrogen until the analysis.

The distribution of silver and physiological elements was studied by nano-XRF at ID16a at the ESRF (41) under cryogenic conditions. The beam was focused to 26 × 42 nm^2 (vertical × horizontal) using a pair of Kirkpatrick-Baez mirrors. The fluorescence signal emitted from each sample pixel was recorded by a multielement silicon drift detector (SGX Sensortech Ltd.) and fitted with the PyMCA software to generate elemental maps (42). On the same beamline, 2D phase contrast holographic images (43) and 3D tomographic data sets were acquired on selected murine fibroblast cells exposed to 33- or 90-nm-diameter AgNWs. The samples were placed at four distances downstream of the beam focus and the projections recorded at a FReLoN charge-coupled device detector are used to generate phase maps through a phase retrieval algorithm. To obtain 3D data, phase maps at 2,000 rotational angles were used for tomographic reconstruction through filtered back-projection (PyHST). To correct for the wavefront inhomogeneities, random lateral displacements in a given range were applied during the rotation (44). The resulting volume represents the 3D electron density distribution inside the sample, with a voxel size of 15 × 15 × 15 nm.

The chemical speciation of silver in cells exposed to AgNW was studied at ID-21 at the ESRF under cryogenic conditions. The beam was focused to 0.6 × 0.8 $μm^2$ (vertical × horizontal) using a pair of Kirkpatrick-Baez mirrors. Detectors included a Si_3N_7 diode for I_0 and a silicon drift detector (X-flash Bruker) for the emitted X-ray fluorescence. From rectangular regions of interest, Ag L_{III} edge spectra were generated for each pixel by stacking 2D images acquired at energy steps across the absorption edge. The XANES were aligned using the simpleElastix image registration library (45) and extracted using PyMCA. Bulk XANES spectra of reference compounds and pristine and aged AgNWs were recorded in the unfocused mode under the same conditions and fitted to the experimental data using a least-squares algorithm.

ACKNOWLEDGMENTS. This work was a collaboration between the Charlet group of the Safe(r) Ecodesign Research and Education Applied to Nanomaterial Development (SERENADE) LabEx (S.G.L., D.T., A.-E.P.d.R., M.V., M. Safi, J.-P.S., C.C., L.C., B.G.) and the Nanowire Intelligent Re-Design and Recycling for Environmental Safety (NANOWIR2ES) project (S.G.L., B.O.-S., D.A., C.V., B.G., L.C., A.H.). The SERENADE LabEx project received funding from Excellence Initiative of Aix-Marseille University, a French "Investissements d'Avenir" program. The NANOWIR2ES project received funding from the European Union European Research Area Network Safe Implementation of Innovative Nanoscience and Nanotechnology program and the US Consumer Product Safety Commission. The University of Grenoble-Alpes cofunded (with SERENADE) the Institut des Sciences de la Terre biology lab and an Invited Professor position for B.G. Chemical studies in the B.G. laboratory at Lawrence Berkeley National Laboratory (LBNL) was supported by US Department of Energy Chemical Sciences, Geosciences, and Biosciences Division under Contract DE-AC02-05CH11231. Synchrotron experiments were performed on beamlines ID16A and ID21 at the ESRF, Grenoble, France, under User Proposal LS-2549. We thank Dr. Alejandro Quieruga (LBNL) for discussions on wire mechanics and Dr. Ludovic Lesven (Université de Lille) for advice with electrochemical characterization of AgNWs. We are grateful for the careful review of this manuscript by two anonymous referees.

1. K. Donaldson, F. A. Murphy, R. Duffin, C. A. Poland, Asbestos, carbon nanotubes and the pleural mesothelium: A review of the hypothesis regarding the role of long fibre retention in the parietal pleura, inflammation and mesothelioma. *Part. Fibre Toxicol.* **7**, 5 (2010).
2. K. Donaldson, C. L. Tran, An introduction to the short-term toxicology of respirable industrial fibres. *Mutat. Res.* **553**, 5–9 (2004).
3. A. B. Kane, R. H. Hurt, Nanotoxicology: The asbestos analogy revisited. *Nat. Nanotechnol.* **3**, 378–379 (2008).
4. D. Langley *et al.*, Flexible transparent conductive materials based on silver nanowire networks: A review. *Nanotechnology* **24**, 452001 (2013).
5. T. Sannicolo *et al.*, Metallic nanowire-based transparent electrodes for next generation flexible devices: A review. *Small* **12**, 6052–6075 (2016).
6. M. Cann *et al.*, High performance transparent multi-touch sensors based on silver nanowire. *Mater. Today Commun.* **7**, 42–50 (2016).
7. L. D. Scanlan *et al.*, Silver nanowire exposure results in internalization and toxicity to *Daphnia magna*. *ACS Nano* **7**, 10681–10694 (2013).
8. R. M. Silva *et al.*, Short versus long silver nanowires: A comparison of in vivo pulmonary effects post instillation. *Part. Fibre Toxicol.* **11**, 52 (2014).
9. S. G. Lehmann *et al.*, In vitro dermal safety assessment of silver nanowires after acute exposure: Tissue vs. cell models. *Nanomaterials (Basel)* **8**, 232 (2018).
10. L. C. Stoehr *et al.*, Shape matters: Effects of silver nanospheres and wires on human alveolar epithelial cells. *Part. Fibre Toxicol.* **8**, 36 (2011).
11. A. Schinwald, K. Donaldson, Use of back-scatter electron signals to visualise cell/nanowires interactions in vitro and in vivo; frustrated phagocytosis of long fibres in macrophages and compartmentalisation in mesothelial cells in vivo. *Part. Fibre Toxicol.* **9**, 34 (2012).
12. N. K. Verma *et al.*, Autophagy induction by silver nanowires: A new aspect in the biocompatibility assessment of nanocomposite thin films. *Toxicol. Appl. Pharmacol.* **264**, 451–461 (2012).
13. I. G. Theodorou *et al.*, Silver nanowire particle reactivity with human monocyte-derived macrophage cells: Intracellular availability of silver governs their cytotoxicity. *ACS Biomater. Sci. Eng.* **3**, 2336–2347 (2017).
14. A. Schinwald, T. Chernova, K. Donaldson, Use of silver nanowires to determine thresholds for fibre length-dependent pulmonary inflammation and inhibition of macrophage migration in vitro. *Part. Fibre Toxicol.* **9**, 47 (2012).
15. S. Chen *et al.*, Sulfidation of silver nanowires inside human alveolar epithelial cells: A potential detoxification mechanism. *Nanoscale* **5**, 9839–9847 (2013).

16. M. Singh, D. Movia, O. K. Mahfoud, Y. Volkov, A. Prina-Mello, Silver nanowires as prospective carriers for drug delivery in cancer treatment: An in vitro biocompatibility study on lung adenocarcinoma cells and fibroblasts. *Eur. J. Nanomed.* **5**, 195–204 (2013).
17. R. F. Hamilton, Jr *et al.*, Particle length-dependent titanium dioxide nanomaterials toxicity and bioactivity. *Part. Fibre Toxicol.* **6**, 35 (2009).
18. L. Y. T. Chou, K. Ming, W. C. W. Chan, Strategies for the intracellular delivery of nanoparticles. *Chem. Soc. Rev.* **40**, 233–245 (2011).
19. X. Shi, A. von dem Bussche, R. H. Hurt, A. B. Kane, H. Gao, Cell entry of one-dimensional nanomaterials occurs by tip recognition and rotation. *Nat. Nanotechnol.* **6**, 714–719 (2011).
20. H. Ewers *et al.*, GM1 structure determines SV40-induced membrane invagination and infection. *Nat. Cell Biol.* **12**, 11–18 (2010).
21. R. Y. Chen *et al.*, Co-percolating graphene-wrapped silver nanowire network for high performance, highly stable, transparent conducting electrodes. *Adv. Funct. Mater.* **23**, 5150–5158 (2013).
22. S. Sorel, P. E. Lyons, S. De, J. C. Dickerson, J. N. Coleman, The dependence of the optoelectrical properties of silver nanowire networks on nanowire length and diameter. *Nanotechnology* **23**, 185201 (2012).
23. S. M. Bergin *et al.*, The effect of nanowire length and diameter on the properties of transparent, conducting nanowire films. *Nanoscale* **4**, 1996–2004 (2012).
24. Y. G. Sun, B. Gates, B. Mayers, Y. N. Xia, Crystalline silver nanowires by soft solution processing. *Nano Lett.* **2**, 165–168 (2002).
25. C. Mayousse *et al.*, Improvements in purification of silver nanowires by decantation and fabrication of flexible transparent electrodes. Application to capacitive touch sensors. *Nanotechnology* **24**, 215501 (2013).
26. J. Jiravova *et al.*, The effect of silver nanoparticles and silver ions on mammalian and plant cells in vitro. *Food Chem. Toxicol.* **96**, 50–61 (2016).
27. A. Manke, L. Wang, Y. Rojanasakul, Mechanisms of nanoparticle-induced oxidative stress and toxicity. *BioMed Res. Int.* **2013**, 942916 (2013).
28. A. Nel, T. Xia, L. Mädler, N. Li, Toxic potential of materials at the nanolevel. *Science* **311**, 622–627 (2006).
29. C. Levard, E. M. Hotze, G. V. Lowry, G. E. Brown, Jr, Environmental transformations of silver nanoparticles: Impact on stability and toxicity. *Environ. Sci. Technol.* **46**, 6900–6914 (2012).
30. U. Shefa, M. S. Kim, N. Y. Jeong, J. Jung, Antioxidant and cell-signaling functions of hydrogen sulfide in the central nervous system. *Oxid. Med. Cell. Longev.* **2018**, 1873961 (2018).
31. M. Bunderson-Schelvan, A. Holian, R. F. Hamilton, Jr, Engineered nanomaterial-induced lysosomal membrane permeabilization and anti-cathepsin agents. *J. Toxicol. Environ. Health B Crit. Rev.* **20**, 230–248 (2017).
32. H. J. Jung *et al.*, Silver wire amplifies the signaling mechanism for IL-1beta production more than silver submicroparticles in human monocytic THP-1 cells. *PLoS One* **9**, e112256 (2014).
33. B. Sun *et al.*, NADPH oxidase-dependent NLRP3 inflammasome activation and its important role in lung fibrosis by multiwalled carbon nanotubes. *Small* **11**, 2087–2097 (2015).
34. D. K. Fygenson, J. F. Marko, A. Libchaber, Mechanics of microtubule-based membrane extension. *Phys. Rev. Lett.* **79**, 4497–4500 (1997).
35. A. E. Cohen, L. Mahadevan, Kinks, rings, and rackets in filamentous structures. *Proc. Natl. Acad. Sci. U.S.A.* **100**, 12141–12146 (2003).
36. W. Zhu *et al.*, Nanomechanical mechanism for lipid bilayer damage induced by carbon nanotubes confined in intracellular vesicles. *Proc. Natl. Acad. Sci. U.S.A.* **113**, 12374–12379 (2016).
37. H. Nagai *et al.*, Diameter and rigidity of multiwalled carbon nanotubes are critical factors in mesothelial injury and carcinogenesis. *Proc. Natl. Acad. Sci. U.S.A.* **108**, E1330–E1338 (2011).
38. Z. Ji *et al.*, Designed synthesis of CeO_2 nanorods and nanowires for studying toxicological effects of high aspect ratio nanomaterials. *ACS Nano* **6**, 5366–5380 (2012).
39. E. K. Jensen, S. Y. Larsen, U. C. Nygaard, C. D. Marioara, T. Syversen, Early combination of material characteristics and toxicology is useful in the design of low toxicity carbon nanofiber. *Materials (Basel)* **5**, 1560–1580 (2012).
40. R. Li *et al.*, Surface interactions with compartmentalized cellular phosphates explain rare earth oxide nanoparticle hazard and provide opportunities for safer design. *ACS Nano* **8**, 1771–1783 (2014).
41. J. C. Da Silva, *et al.*, Efficient concentration of high-energy x-rays for diffraction-limited imaging resolution. *Optica* **4**, 492–495 (2017).
42. V. A. Solé, E. Papillon, M. Cotte, P. Walter, J. Susini, A multiplatform code for the analysis of energy-dispersive X-ray fluorescence spectra. *Spectrochim. Acta B Atom. Spectrosc.* **62**, 63–68 (2007).
43. P. Cloetens *et al.*, Holotomography: Quantitative phase tomography with micrometer resolution using hard synchrotron radiation x rays. *Appl. Phys. Lett.* **75**, 2912–2914 (1999).
44. M. Hubert *et al.*, Efficient correction of wavefront inhomogeneities in X-ray holographic nanotomography by random sample displacement. *Appl. Phys. Lett.* **112**, 203704 (2018).
45. K. Marstal, F. Berendsen, M. Staring, S. Klein, SimpleElastix: A user friendly, multi-lingual library for medical image registration. *IEEE Conference on Computer Vision and Pattern Recognition Workshops*, 10.1109/CVPRW.2016.78 (2016).

SOLICITATION/CONTRACT/ORDER FOR COMMERCIAL ITEMS
OFFEROR TO COMPLETE BLOCKS 12, 17, 23, 24, & 30

1. REQUISITION NUMBER	PAGE	OF
REQ-4000-16-0008	1	29

2. CONTRACT NO	3. AWARD/ EFFECTIVE DATE	4. ORDER NUMBER	5. SOLICITATION NUMBER	6. SOLICITATION ISSUE DATE
CPSC-S-16-0060	09/14/2016			

7. FOR SOLICITATION INFORMATION CALL:	a. NAME	b. TELEPHONE NUMBER (No collect calls)	8. OFFER DUE DATE/LOCAL TIME
	Cassandra Sterba	301-504-7837	

9. ISSUED BY	CODE	10. THIS ACQUISITION IS
CONSUMER PRODUCT SAFETY COMMISSION 4330 EAST WEST HIGHWAY BETHESDA MD 20814	CPSC	☒ UNRESTRICTED OR ☐ SET ASIDE % FOR: ☐ SMALL BUSINESS ☐ HUBZONE SMALL BUSINESS ☐ SERVICE-DISABLED VETERAN-OWNED SMALL BUSINESS ☐ WOMEN-OWNED SMALL BUSINESS (WOSB) ELIGIBLE UNDER THE WOMEN-OWNED SMALL BUSINESS PROGRAM ☐ EDWOSB ☐ 8(A) NAICS: SIZE STANDARD:

11. DELIVERY FOR FOB DESTINATION UNLESS BLOCK IS MARKED	12. DISCOUNT TERMS	13a. THIS CONTRACT IS A RATED ORDER UNDER DPAS (15 CFR 700)	13b. RATING
☐ SEE SCHEDULE	Net 30	☐	14. METHOD OF SOLICITATION ☐ RFQ ☐ IFB ☐ RFP

15. DELIVER TO	CODE	16. ADMINISTERED BY	CODE
CONSUMER PRODUCT SAFETY COMMISSION OFFICE OF HAZARD ID & REDUCTION 5 Research Place Rockville MD 20850	EXHR	CONSUMER PRODUCT SAFETY COMMISSION DIV OF PROCUREMENT SERVICES 4330 EAST WEST HWY ROOM 523 BETHESDA MD 20814	EMPS

17a. CONTRACTOR/ OFFEROR	CODE	FACILITY CODE	18a. PAYMENT WILL BE MADE BY	CODE
UNIVERSITY OF FLORIDA DIVISION OF SPONSORED RESEARCH 207 GRINTER HALL GAINESVILLE FL 326110001	969663814		CPSC Accounts Payable Branch AMZ 160 P. O. Box 25710 Oklahoma City OK 73125	FMFS

TELEPHONE NO.

☐ 17b. CHECK IF REMITTANCE IS DIFFERENT AND PUT SUCH ADDRESS IN OFFER

18b. SUBMIT INVOICES TO ADDRESS SHOWN IN BLOCK 18a UNLESS BLOCK BELOW IS CHECKED ☐ SEE ADDENDUM

19. ITEM NO.	20. SCHEDULE OF SUPPLIES/SERVICES	21. QUANTITY	22. UNIT	23. UNIT PRICE	24. AMOUNT
	DUNS Number: 969663814 COR: Joanna Matheson Phone: 301-987-2564 Email: jmatheson@cpsc.gov The Contractor shall deliver the items listed below in accordance with the attached terms and conditions. Delivery: 08/15/2019 *(Use Reverse and/or Attach Additional Sheets as Necessary)*				

25. ACCOUNTING AND APPROPRIATION DATA
0100A16DSE-2016-2370400000-EXHR004000-255B0

26. TOTAL AWARD AMOUNT *(For Govt. Use Only)*
$444,812.00

☐ 27a. SOLICITATION INCORPORATES BY REFERENCE FAR 52.212-1, 52.212-4. FAR 52.212-3 AND 52.212-5 ARE ATTACHED. ADDENDA ☐ ARE ☐ ARE NOT ATTACHED.

☒ 27b. CONTRACT/PURCHASE ORDER INCORPORATES BY REFERENCE FAR 52.212-4. FAR 52.212-5 IS ATTACHED. ADDENDA ☒ ARE ☐ ARE NOT ATTACHED.

☐ 28. CONTRACTOR IS REQUIRED TO SIGN THIS DOCUMENT AND RETURN _____ COPIES TO ISSUING OFFICE. CONTRACTOR AGREES TO FURNISH AND DELIVER ALL ITEMS SET FORTH OR OTHERWISE IDENTIFIED ABOVE AND ON ANY ADDITIONAL SHEETS SUBJECT TO THE TERMS AND CONDITIONS SPECIFIED.

☒ 29. AWARD OF CONTRACT: _____ OFFER DATED 08/24/2016. YOUR OFFER ON SOLICITATION (BLOCK 5), INCLUDING ANY ADDITIONS OR CHANGES WHICH ARE SET FORTH HEREIN, IS ACCEPTED AS TO ITEMS:

30a. SIGNATURE OF OFFEROR/CONTRACTOR	31a. UNITED STATES OF AMERICA *(SIGNATURE OF CONTRACTING OFFICER)*
	[signature]

30b. NAME AND TITLE OF SIGNER *(Type or print)*	30c. DATE SIGNED	31b. NAME OF CONTRACTING OFFICER *(Type or print)*	31c. DATE SIGNED
		Eddie Ahmad	9/15/16

AUTHORIZED FOR LOCAL REPRODUCTION
PREVIOUS EDITION IS NOT USABLE

STANDARD FORM 1449 (REV. 2/2012)
Prescribed by GSA - FAR (48 CFR) 53.212

19. ITEM NO.	20. SCHEDULE OF SUPPLIES/SERVICES	21. QUANTITY	22. UNIT	23. UNIT PRICE	24. AMOUNT
	Period of Performance: 09/13/2016 to 08/15/2019				
0001	CPSC access to University of Florida research investigating the potential exposure to silver nanowires in touchscreen displays (e.g., cell phones, computers, watches, interactive child toys) under The European Area Research Network (ERA NET) Safe Implementation of Innovative Nanoscience & Nanotechnology (SIINN) consortium. The total amount of award: $444,812.00. The obligation for this award is shown in box 26.	1	EA	444,812.00	444,812.00

32a. QUANTITY IN COLUMN 21 HAS BEEN

☐ RECEIVED ☐ INSPECTED ☐ ACCEPTED, AND CONFORMS TO THE CONTRACT, EXCEPT AS NOTED: _____

32b. SIGNATURE OF AUTHORIZED GOVERNMENT REPRESENTATIVE | 32c. DATE | 32d. PRINTED NAME AND TITLE OF AUTHORIZED GOVERNMENT REPRESENTATIVE

32e. MAILING ADDRESS OF AUTHORIZED GOVERNMENT REPRESENTATIVE | 32f. TELEPHONE NUMBER OF AUTHORIZED GOVERNMENT REPRESENTATIVE

32g. E-MAIL OF AUTHORIZED GOVERNMENT REPRESENTATIVE

33. SHIP NUMBER	34. VOUCHER NUMBER	35. AMOUNT VERIFIED CORRECT FOR	36. PAYMENT	37. CHECK NUMBER
☐ PARTIAL ☐ FINAL			☐ COMPLETE ☐ PARTIAL ☐ FINAL	
38. S/R ACCOUNT NUMBER	39. S/R VOUCHER NUMBER	40. PAID BY		

41a. I CERTIFY THIS ACCOUNT IS CORRECT AND PROPER FOR PAYMENT

41b. SIGNATURE AND TITLE OF CERTIFYING OFFICER | 41c. DATE

42a. RECEIVED BY *(Print)*

42b. RECEIVED AT *(Location)*

42c. DATE REC'D *(YY/MM/DD)* | 42d. TOTAL CONTAINERS

Statement of Work

NanoWire Intelligent Re-design and Recycling for Environmental Safety

I. INTRODUCTION

The U.S. Consumer Product Safety Commission (CPSC) requires the University of Florida, Gainesville (University of Florida), subject to the terms and conditions herein, to conduct research to investigate the human and environmental risk of silver nanowires in touchscreen displays (e.g., cell phones, computers, watches, interactive child toys) and provide CPSC access to reports and results on the research conducted. Although there has been a substantial effort to understand the human and environmental impacts of engineered nanomaterials, there is a serious lack of knowledge about the specific hazards of silver nanowires. Silver nanowires are high aspect ratio (length to width ratio) nanomaterials, and this raises concern about their unique properties and their interactions with living systems.

The required research will investigate the exposure and toxicity of silver nanowires, with a specific focus on consumer protection. The research in this contract is an essential part of a multi-investigator international research project entitled "NanoWIR²ES: NanoWire intelligent re-design and recycling for environmental safety". The work was selected by international peer review for funding under the Safe Implementation of Innovative Nanoscience & Nanotechnology (SIINN) program. The research project will be conducted by research groups at the University of Lille and the University of Grenoble Alps, in France, the Leitat Technology Center in Barcelona, Spain and the University of Florida, who is the sole U.S. – based researcher in this international consortium. Together, the researchers in the consortium will investigate the release of silver nanowires from touchscreen displays, the toxicity of the silver nanowires to human and ecosystem health, and the mechanisms by which silver nanowires induce toxicity. A goal of this research is to understand the intrinsic characteristics that

influence silver nanowire release and their toxicity to biological systems, so that silver nanowires can be synthesized in ways that minimize harm to people and the environment.

II. BACKGROUND

Nanomaterials are used in national and international consumer products (*e.g.*, electronic computer housing, soft furnishings, and cosmetic products) because their novel reactivity can maximize market and consumer performance and minimize cost requirements for production. Despite the increasing number of nanotechnology-enabled consumer products, there are growing concerns regarding the adverse health effects associated with nanomaterial exposure during synthesis, consumption, and disposal. Limited data is available on nanoparticle release from consumer products and nanomaterial toxicity to ecosystems and human health, and it is not clear if the novel reactivity of these materials, while beneficial for many applications, could be hazardous to people and the environment.

The integration of semiconductor and metallic nanowires into thin films, fabrics, and other composite materials will facilitate the generation of a new class of consumer products, including electronic devices embedded in a limitless number of forms. The first major commercial application of nanowires will be the use of silver nanowires in transparent touchscreen displays and flexible touchscreen films. Silver nanowires are rapidly displacing the current technology, indium tin oxide, for use in touchscreen displays; a market estimated to have an annual worth of $1.5-$2 billion. However, the high aspect ratio of silver nanowires raises concern about their potential for adverse impacts on human and ecosystem health. The SIINN NanoWIR2ES consortium will evaluate silver nanowires of varying sizes, coatings, and morphologies for potential human and environmental impacts. Toxicity data will be used to identify silver nanowire properties of concern and develop methods to synthesize nanowires with inherently reduced hazard potential.

Relative to spherical nanoparticles of the same composition, nanowires show altered patterns of transport in the environment and within organisms, exhibit distinct modes of cellular uptake and injury, and pose a significant risk for asbestos-like toxicity because high aspect-ratio materials can defeat biological clearance mechanisms. These observations do not preclude the safe and successful development of nanowire-enabled consumer devices, but they strongly motivate a dedicated research effort to identify key links between material properties and biological and environmental impacts in real-world usage, release, and end-of-life scenarios. This requirement will provide CPSC with access to information on the modification of silver nanowires used in touchscreen devices to minimize adverse effects to people and ecosystems.

III. PURPOSE AND OBJECTIVES

The objectives of the research that University of Florida will conduct are to determine the key links between the material properties of silver nanowires and their potential for toxicity with respect to (1) human toxicity, (2) ecosystem toxicity, and (3) silver nanowire recycling and reuse. The University of Florida is responsible for key components of the toxicity assessment work in the consortium. The research shall provide CPSC with information on the toxicity of untransformed silver nanowires, and of secondary products generated by release and transformation of silver nanowires in biological systems. The research will also investigate nanowire properties that influence biological interactions, and ways to minimize potential hazards of silver nanowires to living systems. Additionally, the research will test concepts for minimizing toxicity through information and material exchange amongst the consortium participants. The resilience of emerging nanowire-enabled products in will be assessed in aging and nanowire-release studies.

Currently, there are few methods that have been validated for assessing the potential adverse impacts of nanomaterials to people and the environment. Therefore, a critical component of this project will entail exploratory research

efforts to establish and validate methods for testing nanomaterial release and toxicity. The research will include development of quantitative criteria of toxicant release during controlled use and aging conditions.

IV. SPECIFIC TASKS

The specific research activities that CPSC requires access to information on are:

- **Area 1: Evaluating and minimizing toxicant release nanowire-enabled products.**

  Nanowire-enabled touchscreens will be exposed to aqueous solutions, stimulated by solar radiation and mechanical stresses, and the release of ions or other potential toxicants will be measured. The researcher will develop quantitative criteria of toxicant release from commercial devices that are under controlled use or aging conditions. The experiments for the release and recycling of silver nanowires will be conducted in coordination with the human and environmental studies detailed in Areas 2 and 3, and this will maximize the ability of the research to inform silver nanowire synthesis and reduce potential hazards of silver nanowires to people and the environment.

- **Area 2: Silver nanowire dermal toxicity**

  The aim of this activity is to assess the cellular toxicity of silver nanowires with well-controlled physical and chemical properties including different lengths, coatings and morphology. Silver nanowire toxicity will be assessed in cultured human skin epithelial cells along multiple axes of materials properties: stability with respect to silver ion (Ag+) release, diameter, length, and surface chemistry. In addition, the researchers will identify silver nanowires with minimal cytotoxicity as defined by established assays for cell viability and toxicity.

- **Area 3: Silver nanowire environmental toxicity**

  Silver nanowires released from products may make their way to the environment. The potential hazard to environmentally relevant organisms will

be evaluated through cellular and whole organism silver nanowire toxicity studies, bioaccumulation and trophic transfer of silver nanowires. These studies in conjunction with Area 2 will enable selection and development of nanowires with less potential for harm to people or the environment.

University of Florida shall provide CPSC with research information and results related to the research activities identified above. The delivered information shall be provided in accordance with the remainder of the terms and conditions of this SOW.

V. UNIVERSITY OF FLORIDA FURNISHED MATERIALS/EQUIPMENT

The University of Florida shall furnish all necessary personnel, equipment, materials, services, and facilities to complete their components of the consortium objectives listed in Section IV, "Purpose and Objectives" and to complete the relevant activities listed in Section IV, "Specific Tasks".

VI. CONFIDENTIALITY REQUIREMENTS

The University of Florida staff will submit to the CPSC any report, manuscript or other document containing the results of work performed under this agreement before such document is published or otherwise disclosed to the public in order to assure compliance with Section 6(b) of the Consumer Product Safety Act (15 USC 2055(b)), Commission regulations (16 CFR Part 1101), and a Commission Directive (Order No. 1450.2). This clearance restricts disclosure of information that: (1) permits the public to identify particular consumer products, or (2) reflects on the safety of a class of consumer products. Prior submission allows the CPSC staff to ensure compliance with applicable disclosure provisions. University of Florida staff agrees to consult with CPSC staff and to provide any drafts of reports or presentation materials to CPSC staff for review.

VII. REPORTING REQUIREMENTS

Within 14 days of award, but not later than September 30, 2016, the University of Florida shall host a kickoff meeting via conference call with the CPSC to discuss the program and provide any updates on the program. University of Florida will provide interim reports in March and September in 2017, and in 2018. These interim reports shall include a brief discussion on the project objectives, the progress of the work and any deviations from the original plan. Updates on the status of the project shall be provided on a quarterly basis, providing basic information on the progress of the research, any information, reports or manuscripts delivered to CPSC in the month, and details about any issues which would impact schedule, quality or price associated with this requirement. A final report will be submitted to the project officer no later than June 30, 2019 which shall include protocols used for the activities listed in Section IV, "Specific Tasks", and should contain:

- an executive summary
- a description of the project context and objectives
- a description of the results
- a brief outline of the impact, especially its impact on Environmental Health and Safety (EHS)-related challenges
- a list of the scientific publications over the project duration (if applicable, please indicate the corresponding websites)

VIII. PERIOD OF PERFORMANCE

The period of performance shall begin on the effective date and shall not extend beyond August 15, 2019.

IX. DELIVERY OR PERFORMANCE

All deliverables required under the terms and conditions of this contract shall be provided to CPSC. The required performance standards include quality and timeliness of deliverables. And deliverable that is rejected by CPSC will be rejected in writing within 5 business days of receipt by the Contracting Officer's Representative (COR). The COR shall detail the failure of the deliverable to meet the requirements of this SOW. The University of Florida shall have 5 business days from rejection to correct any unacceptable deliverables.

The contractor shall provide the requested information in the form of a written report. The report shall be in the format of a scientific report with full citations and tables, as appropriate. The reviewer shall e-mail the report to the COR as a Microsoft Word file.

The contractor shall provide the service or deliverable listed in Table 1 per the delivery or performance listed.

Table 1. Delivery Schedule

Item(s)	Quantity	Delivery or Performance
Kickoff meeting by teleconference to discuss and initiate the contractor's project plan.	1	Within 14 calendar days of award, but not later than September 30, 2016.
Quarterly Status Reports	12	Once per quarter after project initiation.
The Contractor shall submit an interim report to the CPSC COR.	1	March 30, 2017
The Contractor shall submit an interim report to the CPSC COR.	1	September 30, 2017

The Contractor shall submit an interim report to the CPSC COR.	1	March 30, 2018
The Contractor shall submit an interim report to the CPSC COR.	1	September 30, 2018
The Contractor shall submit a draft final report to the CPSC COR.	1	No later than June 30, 2019.
The CPSC COR will provide written comments on the draft final report to the Contractor.	1	Within 14 calendar days after receipt of the draft final report.
The Contractor shall submit a final report to the CPSC COR.	1	Within 30 calendar days after receipt of comments.

X. POINTS OF CONTACT

CPSC Points of Contact

Treye A. Thomas, Ph.D.

Leader, Chemical Hazards Program

Office of Hazard Identification and Reduction

U.S. Consumer Product Safety Commission

4330 East West Highway Suite 600

Bethesda, MD 20814

Tel 301-987-2560

Email: tthomas@cpsc.gov

Joanna Matheson, PhD

Lead for Nanotechnology Interagency Agreements and Contracts

Health Sciences Directorate

U.S. Consumer Product Safety Commission

5 Research Place

Rockville, MD 20850

Tel 301-987-2564

Email: jmatheson@cpsc.gov

University of Florida Point of Contact

Chris Vulpe, PhD

Professor

Physiological Sciences, College of Veterinary Medicine

University of Florida, Gainsville

2187 Mowry Drive, Building 470, Rm 113

Gainsville, Florida 32611

Tel: (352) 294-5821

Email: cvulpe@ufl.edu

CLAUSES:

52.212-5 Contract Terms and Conditions Required To Implement Statutes or Executive Orders—Commercial Items. (JUN 2016)

(a) The Contractor shall comply with the following Federal Acquisition Regulation (FAR) clauses, which are incorporated in this contract by reference, to implement provisions of law or Executive orders applicable to acquisitions of commercial items:

(1) 52.209-10, Prohibition on Contracting with Inverted Domestic Corporations (Nov 2015)

(2) 52.233-3, Protest After Award (AUG 1996) (31 U.S.C. 3553).

(3) 52.233-4, Applicable Law for Breach of Contract Claim (OCT 2004)(Public Laws 108-77 and 108-78 (19 U.S.C. 3805 note)).

(b) The Contractor shall comply with the FAR clauses in this paragraph (b) that the Contracting Officer has indicated as being incorporated in this contract by reference to implement provisions of law or Executive orders applicable to acquisitions of commercial items:

_X_ (1) 52.203-6, Restrictions on Subcontractor Sales to the Government (Sept 2006), with Alternate I (Oct 1995) (41 U.S.C. 4704 and 10 U.S.C. 2402).

_X_ (2) 52.203-13, Contractor Code of Business Ethics and Conduct (Oct 2015) (41 U.S.C. 3509)).

__ (3) 52.203-15, Whistleblower Protections under the American Recovery and Reinvestment Act of 2009 (June 2010) (Section 1553 of Pub. L. 111-5). (Applies to contracts funded by the American Recovery and Reinvestment Act of 2009.)

_X_ (4) 52.204-10, Reporting Executive Compensation and First-Tier Subcontract Awards (Oct 2015) (Pub. L. 109-282) (31 U.S.C. 6101 note).
__ (5) [Reserved].
__ (6) 52.204-14, Service Contract Reporting Requirements (Jan 2014) (Pub. L. 111-117, section 743 of Div. C).
__ (7) 52.204-15, Service Contract Reporting Requirements for Indefinite-Delivery Contracts (Jan 2014) (Pub. L. 111-117, section 743 of Div. C).
_X_ (8) 52.209-6, Protecting the Government's Interest When Subcontracting with Contractors Debarred, Suspended, or Proposed for Debarment. (Oct 2015) (31 U.S.C. 6101 note).
__ (9) 52.209-9, Updates of Publicly Available Information Regarding RespoXnsibility Matters (Jul 2013) (41 U.S.C. 2313).
__ (10) [Reserved].
__ (11)(i) 52.219-3, Notice of HUBZone Set-Aside or Sole-Source Award (Nov 2011) (15 U.S.C. 657a).
__ (ii) Alternate I (Nov 2011) of 52.219-3.
__ (12)(i) 52.219-4, Notice of Price Evaluation Preference for HUBZone Small Business Concerns (OCT 2014) (if the offeror elects to waive the preference, it shall so indicate in its offer) (15 U.S.C. 657a).
__ (ii) Alternate I (JAN 2011) of 52.219-4.
__ (13) [Reserved]
__ (14)(i) 52.219-6, Notice of Total Small Business Set-Aside (Nov 2011) (15 U.S.C. 644).
__ (ii) Alternate I (Nov 2011).
__ (iii) Alternate II (Nov 2011).
__ (15)(i) 52.219-7, Notice of Partial Small Business Set-Aside (June 2003) (15 U.S.C. 644).
__ (ii) Alternate I (Oct 1995) of 52.219-7.
__ (iii) Alternate II (Mar 2004) of 52.219-7.
__ (16) 52.219-8, Utilization of Small Business Concerns (Oct 2014) (15 U.S.C. 637(d)(2) and (3)).
__ (17)(i) 52.219-9, Small Business Subcontracting Plan (Oct 2015) (15 U.S.C. 637(d)(4)).
__ (ii) Alternate I (Oct 2001) of 52.219-9.
__ (iii) Alternate II (Oct 2001) of 52.219-9.
__ (iv) Alternate III (Oct 2015) of 52.219-9.
__ (18) 52.219-13, Notice of Set-Aside of Orders (Nov 2011) (15 U.S.C. 644(r)).
__ (19) 52.219-14, Limitations on Subcontracting (Nov 2011) (15 U.S.C. 637(a)(14)).

__ (20) 52.219-16, Liquidated Damages—Subcon-tracting Plan (Jan 1999) (15 U.S.C. 637(d)(4)(F)(i)).
__ (21) 52.219-27, Notice of Service-Disabled Veteran-Owned Small Business Set-Aside (Nov 2011) (15 U.S.C. 657 f).
_X_ (22) 52.219-28, Post Award Small Business Program Rerepresentation (Jul 2013) (15 U.S.C. 632(a)(2)).
__ (23) 52.219-29, Notice of Set-Aside for, or Sole Source Award to, Economically Disadvantaged Women-Owned Small Business Concerns (Dec 2015) (15 U.S.C. 637(m)).
__ (24) 52.219-30, Notice of Set-Aside for, or Sole Source Award to, Women-Owned Small Business Concerns Eligible Under the Women-Owned Small Business Program (Dec 2015) (15 U.S.C. 637(m)).
_X_ (25) 52.222-3, Convict Labor (June 2003) (E.O. 11755).
__ (26) 52.222-19, Child Labor- Cooperation with Authorities and Remedies (Feb 2016) (E.O. 13126).
_X_ (27) 52.222-21, Prohibition of Segregated Facilities (Apr 2015).
_X_ (28) 52.222-26, Equal Opportunity (Apr 2015) (E.O. 11246).
_X_ (29) 52.222-35, Equal Opportunity for Veterans (Oct 2015)(38 U.S.C. 4212).
_X_ (30) 52.222-36, Equal Opportunity for Workers with Disabilities (Jul 2014) (29 U.S.C. 793).
_X_ (31) 52.222-37, Employment Reports on Veterans (FEB 2016) (38 U.S.C. 4212).
_X_ (32) 52.222-40, Notification of Employee Rights Under the National Labor Relations Act (Dec 2010) (E.O. 13496).
_X_ (33)(i) 52.222-50, Combating Trafficking in Persons (Mar 2015) (22 U.S.C. chapter 78 and E.O. 13627).
__ (ii) Alternate I (Mar 2015) of 52.222-50 (22 U.S.C. chapter 78 and E.O. 13627).
__ (34) 52.222-54, Employment Eligibility Verification (OCT 2015). (Executive Order 12989). (Not applicable to the acquisition of commercially available off-the-shelf items or certain other types of commercial items as prescribed in 22.1803.)
__ (35)(i) 52.223-9, Estimate of Percentage of Recovered Material Content for EPA–Designated Items (May 2008) (42 U.S.C. 6962(c)(3)(A)(ii)). (Not applicable to the acquisition of commercially available off-the-shelf items.)
__ (ii) Alternate I (May 2008) of 52.223-9 (42 U.S.C. 6962(i)(2)(C)). (Not applicable to the acquisition of commercially available off-the-shelf items.)
__ (36) 52.223-11, Ozone-Depleting Substances and High Global Warming Potential Hydrofluorocarbons (JUN 2016) (E.O. 13693).
__ (37) 52.223-12, Maintenance, Service, Repair, or Disposal of Refrigeration Equipment and Air Conditioners (JUN 2016) (E.O. 13693).

__ (38)(i) 52.223-13, Acquisition of EPEAT®-Registered Imaging Equipment (JUN 2014) (E.O.s 13423 and 13514).
__ (ii) Alternate I (Oct 2015) of 52.223-13.
__ (39)(i) 52.223-14, Acquisition of EPEAT®-Registered Televisions (JUN 2014) (E.O.s 13423 and 13514).
__ (ii) Alternate I (Jun 2014) of 52.223-14.
__ (40) 52.223-15, Energy Efficiency in Energy-Consuming Products (DEC 2007) (42 U.S.C. 8259b).
__ (41)(i) 52.223-16, Acquisition of EPEAT®-Registered Personal Computer Products (OCT 2015) (E.O.s 13423 and 13514).
__ (ii) Alternate I (Jun 2014) of 52.223-16.
_X_ (42) 52.223-18, Encouraging Contractor Policies to Ban Text Messaging While Driving (AUG 2011) (E.O. 13513).
__ (43) 52.223-20, Aerosols (JUN 2016) (E.O. 13693).
__ (44) 52.223-21, Foams (JUN 2016) (E.O. 13693).
__ (45) 52.225-1, Buy American—Supplies (May 2014) (41 U.S.C. chapter 83).
__ (46)(i) 52.225-3, Buy American—Free Trade Agreements—Israeli Trade Act (May 2014) (41 U.S.C. chapter 83, 19 U.S.C. 3301 note, 19 U.S.C. 2112 note, 19 U.S.C. 3805 note, 19 U.S.C. 4001 note, Pub. L. 103-182, 108-77, 108-78, 108-286, 108-302, 109-53, 109-169, 109-283, 110-138, 112-41, 112-42, and 112-43.
__ (ii) Alternate I (May 2014) of 52.225-3.
__ (iii) Alternate II (May 2014) of 52.225-3.
__ (iv) Alternate III (May 2014) of 52.225-3.
__ (47) 52.225-5, Trade Agreements (FEB 2016) (19 U.S.C. 2501, et seq., 19 U.S.C. 3301 note).
_X_ (48) 52.225-13, Restrictions on Certain Foreign Purchases (June 2008) (E.O.'s, proclamations, and statutes administered by the Office of Foreign Assets Control of the Department of the Treasury).
__ (49) 52.225-26, Contractors Performing Private Security Functions Outside the United States (Jul 2013) (Section 862, as amended, of the National Defense Authorization Act for Fiscal Year 2008; 10 U.S.C. 2302 Note).
__ (50) 52.226-4, Notice of Disaster or Emergency Area Set-Aside (Nov 2007) (42 U.S.C. 5150).
__ (51) 52.226-5, Restrictions on Subcontracting Outside Disaster or Emergency Area (Nov 2007) (42 U.S.C. 5150).
__ (52) 52.232-29, Terms for Financing of Purchases of Commercial Items (Feb 2002) (41 U.S.C. 4505, 10 U.S.C. 2307(f)).

__ (53) 52.232-30, Installment Payments for Commercial Items (Oct 1995) (41 U.S.C. 4505, 10 U.S.C. 2307(f)).
_X_ (54) 52.232-33, Payment by Electronic Funds Transfer—System for Award Management (Jul 2013) (31 U.S.C. 3332).
__ (55) 52.232-34, Payment by Electronic Funds Transfer—Other than System for Award Management (Jul 2013) (31 U.S.C. 3332).
__ (56) 52.232-36, Payment by Third Party (May 2014) (31 U.S.C. 3332).
__ (57) 52.239-1, Privacy or Security Safeguards (Aug 1996) (5 U.S.C. 552a).
__ (58)(i) 52.247-64, Preference for Privately Owned U.S.-Flag Commercial Vessels (Feb 2006) (46 U.S.C. Appx. 1241(b) and 10 U.S.C. 2631).
__ (ii) Alternate I (Apr 2003) of 52.247-64.
(c) The Contractor shall comply with the FAR clauses in this paragraph (c), applicable to commercial services, that the Contracting Officer has indicated as being incorporated in this contract by reference to implement provisions of law or Executive orders applicable to acquisitions of commercial items:
__ (1) 52.222-17, Nondisplacement of Qualified Workers (May 2014)(E.O. 13495).
__ (2) 52.222-41, Service Contract Labor Standards (May 2014) (41 U.S.C. chapter 67).
__ (3) 52.222-42, Statement of Equivalent Rates for Federal Hires (May 2014) (29 U.S.C. 206 and 41 U.S.C. chapter 67).
__ (4) 52.222-43, Fair Labor Standards Act and Service Contract Labor Standards-Price Adjustment (Multiple Year and Option Contracts) (May 2014) (29 U.S.C. 206 and 41 U.S.C. chapter 67).
__ (5) 52.222-44, Fair Labor Standards Act and Service Contract Labor Standards—Price Adjustment (May 2014) (29 U.S.C. 206 and 41 U.S.C. chapter 67).
__ (6) 52.222-51, Exemption from Application of the Service Contract Labor Standards to Contracts for Maintenance, Calibration, or Repair of Certain Equipment—Requirements (May 2014) (41 U.S.C. chapter 67).
__ (7) 52.222-53, Exemption from Application of the Service Contract Labor Standards to Contracts for Certain Services—Requirements (May 2014) (41 U.S.C. chapter 67).
__ (8) 52.222-55, Minimum Wages Under Executive Order 13658 (Dec 2015).
__ (9) 52.226-6, Promoting Excess Food Donation to Nonprofit Organizations (May 2014) (42 U.S.C. 1792).
__ (10) 52.237-11, Accepting and Dispensing of $1 Coin (Sept 2008) (31 U.S.C. 5112(p)(1)).
(d) Comptroller General Examination of Record. The Contractor shall comply with the provisions of this paragraph (d) if this contract was awarded using other than sealed bid, is in excess of the simplified acquisition threshold, and does not contain the clause at 52.215-2, Audit and Records—Negotiation.

(1) The Comptroller General of the United States, or an authorized representative of the Comptroller General, shall have access to and right to examine any of the Contractor's directly pertinent records involving transactions related to this contract.
(2) The Contractor shall make available at its offices at all reasonable times the records, materials, and other evidence for examination, audit, or reproduction, until 3 years after final payment under this contract or for any shorter period specified in FAR Subpart 4.7, Contractor Records Retention, of the other clauses of this contract. If this contract is completely or partially terminated, the records relating to the work terminated shall be made available for 3 years after any resulting final termination settlement. Records relating to appeals under the disputes clause or to litigation or the settlement of claims arising under or relating to this contract shall be made available until such appeals, litigation, or claims are finally resolved.
(3) As used in this clause, records include books, documents, accounting procedures and practices, and other data, regardless of type and regardless of form. This does not require the Contractor to create or maintain any record that the Contractor does not maintain in the ordinary course of business or pursuant to a provision of law.
(e)(1) Notwithstanding the requirements of the clauses in paragraphs (a), (b), (c), and (d) of this clause, the Contractor is not required to flow down any FAR clause, other than those in this paragraph (e)(1) in a subcontract for commercial items. Unless otherwise indicated below, the extent of the flow down shall be as required by the clause—
(i) 52.203-13, Contractor Code of Business Ethics and Conduct (Oct 2015) (41 U.S.C. 3509).
(ii) 52.219-8, Utilization of Small Business Concerns (Oct 2014) (15 U.S.C. 637(d)(2) and (3)), in all subcontracts that offer further subcontracting opportunities. If the subcontract (except subcontracts to small business concerns) exceeds $700,000 ($1.5 million for construction of any public facility), the subcontractor must include 52.219-8 in lower tier subcontracts that offer subcontracting opportunities.
(iii) 52.222-17, Nondisplacement of Qualified Workers (May 2014) (E.O. 13495). Flow down required in accordance with paragraph (l) of FAR clause 52.222-17.
(iv) 52.222-21, Prohibition of Segregated Facilities (Apr 2015)
(v) 52.222-26, Equal Opportunity (Apr 2015) (E.O. 11246).
(vi) 52.222-35, Equal Opportunity for Veterans (Oct 2015) (38 U.S.C. 4212).
(vii) 52.222-36, Equal Opportunity for Workers with Disabilities (Jul 2014) (29 U.S.C. 793).
(viii) 52.222-37, Employment Reports on Veterans (Feb 2016) (38 U.S.C. 4212)
(ix) 52.222-40, Notification of Employee Rights Under the National Labor Relations Act (Dec 2010) (E.O. 13496). Flow down required in accordance with paragraph (f) of FAR clause 52.222-40.

(x) 52.222-41, Service Contract Labor Standards (May 2014) (41 U.S.C. chapter 67).
(xi) 52.222-50, Combating Trafficking in Persons (Mar 2015) (22 U.S.C. chapter 78 and E.O 13627). Alternate I (Mar 2015) of 52.222-50 (22 U.S.C. chapter 78 and E.O 13627).
(xii) 52.222-51, Exemption from Application of the Service Contract Labor Standards to Contracts for Maintenance, Calibration, or Repair of Certain Equipment-Requirements (May 2014) (41 U.S.C. chapter 67).
(xiii) 52.222-53, Exemption from Application of the Service Contract Labor Standards to Contracts for Certain Services-Requirements (May 2014) (41 U.S.C. chapter 67).
(xiv) 52.222-54, Employment Eligibility Verification (OCT 2015) (E.O. 12989).
(xv) 52.222-55, Minimum Wages Under Executive Order 13658 (Dec 2015).
(xvi) 52.225-26, Contractors Performing Private Security Functions Outside the United States (Jul 2013) (Section 862, as amended, of the National Defense Authorization Act for Fiscal Year 2008; 10 U.S.C. 2302 Note).
(xvii) 52.226-6, Promoting Excess Food Donation to Nonprofit Organizations (May 2014) (42 U.S.C. 1792). Flow down required in accordance with paragraph (e) of FAR clause 52.226-6.
(xviii) 52.247-64, Preference for Privately Owned U.S.-Flag Commercial Vessels (Feb 2006) (46 U.S.C. Appx. 1241(b) and 10 U.S.C. 2631). Flow down required in accordance with paragraph (d) of FAR clause 52.247-64.
(2) While not required, the Contractor may include in its subcontracts for commercial items a minimal number of additional clauses necessary to satisfy its contractual obligations.

(End of clause)

52.227-14 Rights in Data—General (MAY 2014)

(a) Definitions. As used in this clause—
"Computer database" or "database means" a collection of recorded information in a form capable of, and for the purpose of, being stored in, processed, and operated on by a computer. The term does not include computer software.
"Computer software"—
(1) Means
(i) Computer programs that comprise a series of instructions, rules, routines, or statements, regardless of the media in which recorded, that allow or cause a computer to perform a specific operation or series of operations; and
(ii) Recorded information comprising source code listings, design details, algorithms, processes, flow charts, formulas, and related material that would enable the computer program to be produced, created, or compiled.
(2) Does not include computer databases or computer software documentation.

"Computer software documentation" means owner's manuals, user's manuals, installation instructions, operating instructions, and other similar items, regardless of storage medium, that explain the capabilities of the computer software or provide instructions for using the software.

"Data" means recorded information, regardless of form or the media on which it may be recorded. The term includes technical data and computer software. The term does not include information incidental to contract administration, such as financial, administrative, cost or pricing, or management information.

"Form, fit, and function data" means data relating to items, components, or processes that are sufficient to enable physical and functional interchangeability, and data identifying source, size, configuration, mating and attachment characteristics, functional characteristics, and performance requirements. For computer software it means data identifying source, functional characteristics, and performance requirements but specifically excludes the source code, algorithms, processes, formulas, and flow charts of the software.

"Limited rights" means the rights of the Government in limited rights data as set forth in the Limited Rights Notice of paragraph (g)(3) if included in this clause.

"Limited rights data" means data, other than computer software, that embody trade secrets or are commercial or financial and confidential or privileged, to the extent that such data pertain to items, components, or processes developed at private expense, including minor modifications.

"Restricted computer software" means computer software developed at private expense and that is a trade secret, is commercial or financial and confidential or privileged, or is copyrighted computer software, including minor modifications of the computer software.

"Restricted rights," as used in this clause, means the rights of the Government in restricted computer software, as set forth in a Restricted Rights Notice of paragraph (g) if included in this clause, or as otherwise may be provided in a collateral agreement incorporated in and made part of this contract, including minor modifications of such computer software.

"Technical data" means recorded information (regardless of the form or method of the recording) of a scientific or technical nature (including computer databases and computer software documentation). This term does not include computer software or financial, administrative, cost or pricing, or management data or other information incidental to contract administration. The term includes recorded information of a scientific or technical nature that is included in computer databases (See 41 U.S.C. 116).

"Unlimited rights" means the rights of the Government to use, disclose, reproduce, prepare derivative works, distribute copies to the public, and perform publicly and

display publicly, in any manner and for any purpose, and to have or permit others to do so.

(b) Allocation of rights.

(1) Except as provided in paragraph (c) of this clause, the Government shall have unlimited rights in—

(i) Data first produced in the performance of this contract;

(ii) Form, fit, and function data delivered under this contract;

(iii) Data delivered under this contract (except for restricted computer software) that constitute manuals or instructional and training material for installation, operation, or routine maintenance and repair of items, components, or processes delivered or furnished for use under this contract; and

(iv) All other data delivered under this contract unless provided otherwise for limited rights data or restricted computer software in accordance with paragraph (g) of this clause.

(2) The Contractor shall have the right to—

(i) Assert copyright in data first produced in the performance of this contract to the extent provided in paragraph (c)(1) of this clause;

(ii) Use, release to others, reproduce, distribute, or publish any data first produced or specifically used by the Contractor in the performance of this contract, unless provided otherwise in paragraph (d) of this clause;

(iii) Substantiate the use of, add, or correct limited rights, restricted rights, or copyright notices and to take other appropriate action, in accordance with paragraphs (e) and (f) of this clause; and

(iv) Protect from unauthorized disclosure and use those data that are limited rights data or restricted computer software to the extent provided in paragraph (g) of this clause.

(c) Copyright—

(1) Data first produced in the performance of this contract.

(i) Unless provided otherwise in paragraph (d) of this clause, the Contractor may, without prior approval of the Contracting Officer, assert copyright in scientific and technical articles based on or containing data first produced in the performance of this contract and published in academic, technical or professional journals, symposia proceedings, or similar works. The prior, express written permission of the Contracting Officer is required to assert copyright in all other data first produced in the performance of this contract.

(ii) When authorized to assert copyright to the data, the Contractor shall affix the applicable copyright notices of 17 U.S.C. 401 or 402, and an acknowledgment of Government sponsorship (including contract number).

(iii) For data other than computer software, the Contractor grants to the Government, and others acting on its behalf, a paid-up, nonexclusive, irrevocable, worldwide license in such copyrighted data to reproduce, prepare derivative works, distribute copies to the public, and perform publicly and display publicly by or on behalf of the Government. For computer software, the Contractor grants to the Government, and others acting on its behalf, a paid-up, nonexclusive, irrevocable, worldwide license in such copyrighted computer software to reproduce, prepare derivative works, and perform publicly and display publicly (but not to distribute copies to the public) by or on behalf of the Government.

(2) Data not first produced in the performance of this contract. The Contractor shall not, without the prior written permission of the Contracting Officer, incorporate in data delivered under this contract any data not first produced in the performance of this contract unless the Contractor—

(i) Identifies the data; and

(ii) Grants to the Government, or acquires on its behalf, a license of the same scope as set forth in paragraph (c)(1) of this clause or, if such data are restricted computer software, the Government shall acquire a copyright license as set forth in paragraph (g)(4) of this clause (if included in this contract) or as otherwise provided in a collateral agreement incorporated in or made part of this contract.

(3) Removal of copyright notices. The Government will not remove any authorized copyright notices placed on data pursuant to this paragraph (c), and will include such notices on all reproductions of the data.

(d) Release, publication, and use of data. The Contractor shall have the right to use, release to others, reproduce, distribute, or publish any data first produced or specifically used by the Contractor in the performance of this contract, except—

(1) As prohibited by Federal law or regulation (e.g., export control or national security laws or regulations);

(2) As expressly set forth in this contract; or

(3) If the Contractor receives or is given access to data necessary for the performance of this contract that contain restrictive markings, the Contractor shall treat the data in accordance with such markings unless specifically authorized otherwise in writing by the Contracting Officer.

(e) Unauthorized marking of data.

(1) Notwithstanding any other provisions of this contract concerning inspection or acceptance, if any data delivered under this contract are marked with the notices specified in paragraph (g)(3) or (g) (4) if included in this clause, and use of the notices is not authorized by this clause, or if the data bears any other restrictive or limiting markings not authorized by this contract, the Contracting Officer may at any time either return the

data to the Contractor, or cancel or ignore the markings. However, pursuant to 41 U.S.C. 4703, the following procedures shall apply prior to canceling or ignoring the markings.
(i) The Contracting Officer will make written inquiry to the Contractor affording the Contractor 60 days from receipt of the inquiry to provide written justification to substantiate the propriety of the markings;
(ii) If the Contractor fails to respond or fails to provide written justification to substantiate the propriety of the markings within the 60-day period (or a longer time approved in writing by the Contracting Officer for good cause shown), the Government shall have the right to cancel or ignore the markings at any time after said period and the data will no longer be made subject to any disclosure prohibitions.
(iii) If the Contractor provides written justification to substantiate the propriety of the markings within the period set in paragraph (e)(l)(i) of this clause, the Contracting Officer will consider such written justification and determine whether or not the markings are to be cancelled or ignored. If the Contracting Officer determines that the markings are authorized, the Contractor will be so notified in writing. If the Contracting Officer determines, with concurrence of the head of the contracting activity, that the markings are not authorized, the Contracting Officer will furnish the Contractor a written determination, which determination will become the final agency decision regarding the appropriateness of the markings unless the Contractor files suit in a court of competent jurisdiction within 90 days of receipt of the Contracting Officer's decision. The Government will continue to abide by the markings under this paragraph (e)(l)(iii) until final resolution of the matter either by the Contracting Officer's determination becoming final (in which instance the Government will thereafter have the right to cancel or ignore the markings at any time and the data will no longer be made subject to any disclosure prohibitions), or by final disposition of the matter by court decision if suit is filed.
(2) The time limits in the procedures set forth in paragraph (e)(1) of this clause may be modified in accordance with agency regulations implementing the Freedom of Information Act (5 U.S.C. 552) if necessary to respond to a request thereunder.
(3) Except to the extent the Government's action occurs as the result of final disposition of the matter by a court of competent jurisdiction, the Contractor is not precluded by paragraph (e) of the clause from bringing a claim, in accordance with the Disputes clause of this contract, that may arise as the result of the Government removing or ignoring authorized markings on data delivered under this contract.
(f) Omitted or incorrect markings.
(1) Data delivered to the Government without any restrictive markings shall be deemed to have been furnished with unlimited rights. The Government is not liable for the disclosure, use, or reproduction of such data.

(2) If the unmarked data has not been disclosed without restriction outside the Government, the Contractor may request, within 6 months (or a longer time approved by the Contracting Officer in writing for good cause shown) after delivery of the data, permission to have authorized notices placed on the data at the Contractor's expense. The Contracting Officer may agree to do so if the Contractor—

(i) Identifies the data to which the omitted notice is to be applied;

(ii) Demonstrates that the omission of the notice was inadvertent;

(iii) Establishes that the proposed notice is authorized; and

(iv) Acknowledges that the Government has no liability for the disclosure, use, or reproduction of any data made prior to the addition of the notice or resulting from the omission of the notice.

(3) If data has been marked with an incorrect notice, the Contracting Officer may—

(i) Permit correction of the notice at the Contractor's expense if the Contractor identifies the data and demonstrates that the correct notice is authorized; or

(ii) Correct any incorrect notices.

(g) Protection of limited rights data and restricted computer software.

(1) The Contractor may withhold from delivery qualifying limited rights data or restricted computer software that are not data identified in paragraphs (b)(1)(i), (ii), and (iii) of this clause. As a condition to this withholding, the Contractor shall—

(i) Identify the data being withheld; and

(ii) Furnish form, fit, and function data instead.

(2) Limited rights data that are formatted as a computer database for delivery to the Government shall be treated as limited rights data and not restricted computer software.

(3) [Reserved]

(h) Subcontracting. The Contractor shall obtain from its subcontractors all data and rights therein necessary to fulfill the Contractor's obligations to the Government under this contract. If a subcontractor refuses to accept terms affording the Government those rights, the Contractor shall promptly notify the Contracting Officer of the refusal and shall not proceed with the subcontract award without authorization in writing from the Contracting Officer.

(i) Relationship to patents or other rights. Nothing contained in this clause shall imply a license to the Government under any patent or be construed as affecting the scope of any license or other right otherwise granted to the Government.

(End of clause)

LC1A CONTRACTOR'S NOTE

Deliveries and/or shipments shall not be left at the Loading Dock. **All deliveries shall be considered "inside deliveries"** to the appropriate room at the Consumer Product Safety Commission (CPSC) and in accordance with the instructions below. When scheduling deliveries the purchase order number shall always be referenced and all packages shall clearly display the Purchase Order Number on the outside of the cartons and/or packages, to include the packing slip.

ATTENTION GOVERNMENT VENDOR

A. DELIVERY INSTRUCTIONS:

1. DELIVERY INSTRUCTIONS FOR LARGE OR HEAVY ITEMS:

If the shipment or item being delivered requires use of a loading dock, advance notification is required. The contractor shall contact the Shipping and Receiving Coordinator at 301-892-0586 or Constantia Demas (301) 504-7544 forty-eight (48) hours in advance of the date the items are to arrive to schedule use of the loading dock.

LOADING DOCK HOURS OF OPERATION:

9:00 am to 11:00 am or 1:30 pm to 4:00 pm
Monday through Friday (except holidays)

Please notify contact person if there is a change in the delivery date. For changes, delays, or assistance please contact CPSC as follows:

Facilities Management Support Services (301) 504-7091 and

The COR – See page 1 of award.

Upon arrival, the driver should contact the CPSC Guard, 301-504-7721, at the loading dock to obtain assistance in using freight elevators and to gain access to CPSC security areas.

2. DELIVERY INSTRUCTION FOR SMALL ITEMS

When delivering or shipping small items, the contractor and/or carrier service shall report to the 4th floor lobby, North Tower, 4330 East West Highway, to sign in with the CPSC guard. Upon completion of signing in, the contractor shall deliver all shipments to the Mail Room, Room 410. After delivery, delivery personnel shall promptly depart the building.

MAIL ROOM HOURS OF OPERATION:

Monday through Friday (except holidays) – 7:30 am to 5:00 pm

B. BILLING INSTRUCTIONS

Pursuant to the Prompt Payment Act (P.L. 97-177) and the Prompt Payment Act Amendments of 1988 (P.L. 100-496) all Federal agencies are required to pay their bills on time, pay interest penalties when payments are made late, and to take discounts only when payments are made within the discount period. To assure compliance with the Act, vouchers and/or invoices shall be submitted on any acceptable invoice form which meets the criteria listed below. Examples of government vouchers that may be used are the Public Vouchers for Purchase and Services Other Than Personal, SF 1034, and Continuation Sheet, SF 1035. At a minimum, each invoice shall include:

1. The name and address of the business concern (and separate remittance address, if applicable).

2. **Do NOT** include Taxpayer Identification Number (TIN) on invoices sent via e-mail.

3. Invoice date.

4. Invoice number.

5. The contract or purchase order number (see block 2 of OF347 and block 4 of SF1449 on page 1 of this order), or other authorization for delivery of goods of services.

6. Description, price and quantity of goods or services actually delivered or rendered.

7. Shipping cost terms (if applicable).

8. Payment terms.

9. Other substantiating documentation or information as specified in the contract or purchase order.

10. Name, title, phone number and mailing address of responsible official to be notified in the event of a deficient invoice.

ORIGINAL VOUCHERS/INVOICES SHALL BE SENT TO:

PREFERED: Via email to:

9-AMC-AMZ-CPSC-Accounts-Payable@faa.gov

OR

U.S. Mail
Enterprise Service Center, c/o CPSC, Accounts Payable Branch, AMZ-160
PO Box 25710
Oklahoma City, Ok. 73125

FEDEX
Enterprise Service Center, c/o CPSC, Accounts Payable Branch, AMZ-160
6500 S. MacArthur Blvd.
Oklahoma City, Ok. 73169

Invoices not submitted in accordance with the above stated minimum requirements will not be processed for payment. Deficient invoices will be returned to the vendor within seven days or sooner. Standard forms 1034 and 1035 will be furnished by CPSC upon request of the contractor.

Inquiries regarding payment should be directed to the Enterprise Service Center (ESC), Office of Financial Operations, Federal Aviation Administration (FAA) in Oklahoma City,9-AMC-AMZ-CPSC-Accounts-Payable@faa.gov.

C. PAYMENT

Payment will be made as close as possible to, but not later than, the 30th day after receipt of a proper invoice as defined in "Billing Instructions," except as follows:

When a time discount is taken, payment will be made as close as possible to, but not later than, the discount date. Discounts will be taken whenever economically justified. Otherwise, late payments will include interest penalty payments. Inquiries regarding payment should be directed to 9-AMC-AMZ-CPSC-Accounts-Payable@faa.gov or at the U.S. Mail and Fedex addresses listed above:

Complaints related to the late payment of an invoice should be directed to Ricky Woods at the same the same address (above) or 405-954-5351.

Customer Service inquiries may be directed to Adriane Clark at AClark@cpsc.gov.

D. INSPECTION & ACCEPTANCE PERIOD

Unless otherwise stated in the Statement of Work or Description, the Commission will ordinarily inspect all materials/services within seven (7) working days after the date of receipt. The CPSC representative responsible for inspecting the materials/services will transmit disapproval, if appropriate, to the contractor and the contract specialist listed below. If other inspection information is provided in the Statement of Work or Description, it is controlling.

E. ALL OTHER INFORMATION RELATING TO THE PURCHASE ORDER

Contact: Contract specialist Cassandra Sterba at 301-504-7837.

F. PROCESSING INSTRUCTIONS FOR REQUESTING OFFICES

The Purchase Order/Receiving Report (Optional Form 347 or Standard Form 1449) must be completed at the time the ordered goods or services are received. Upon receipt of the goods or services ordered, each item should be inspected, accepted (partial or final) or rejected. The Purchase Order/Receiving Report must be appropriately completed, signed and dated by the authorized receiving official. In addition, the acceptance block shall be completed (Blocks 32 a, b & c on the SF 1449 and column G and page 2 of the OF 347). The receiving report shall be retained by the requesting office for confirmation when certifying invoices.

G. PROPERTY/EQUIPMENT PURCHASES

In the case of Purchase Orders/Receiving Reports involving the purchase and receipt of property/equipment, a copy of the Purchase Order/Receiving Report must also be immediately forwarded directly to the Property Management Officer (Constantia Demas) in the Facilities Management Support Services Branch (Room 425). The transmittal of Purchase Orders/Receiving Reports to the property management officer is critical to the integrity and operation of CPSC's Property Management System. Receiving officials should also forward copies to their local property officer/property custodian consistent with local office procedures.

LC 5 Contracting Officer's Representative (COR) Designation

a. The following individual has been designated at the Government's COR for this contract:
Name: Joanna Matheson
Division: Office of Hazard Identification and Reduction
Telephone: 301-987-2564
Email: jmatheson@cpsc.gov

b. The CPSC COR is responsible for performing specific technical and administrative functions, including:

(1) performing technical evaluation as required;

(2) assisting the Contractor in the resolution of technical problems encountered during performance; monitoring the Contractor's technical progress, including surveillance and assessment of performance, and notifying the Contracting Officer within one week when deliverables (including reports) are not received on schedule in accordance with the prescribed delivery schedule; and

(3) inspection and acceptance of all items required by the contract.

c. The COR, who may be personally liable for unauthorized acts, is not authorized to and shall not:

(1) make changes in scope of work, contract schedules, and/or specifications, or to make changes that affect price, quality, quantity or delivery,

(2) direct or negotiate any change in the terms, conditions, or amounts cited in the contract; and

(3) make commitments or changes that affect price, or take any action that commits the Government or could lead to a claim against the Government.

d. This delegation is not redelegable and remains in effect during the period of performance of the contract.

e. A clear distinction is made between Government and Contractor personnel. No employer-employee relationship will occur between government employees and contractor employees. Contractor employees must report directly to their company (employer) and shall not report to Government personnel.

LC 9 Key Personnel

a. The following individuals, listed by name and title, have been identified as key personnel for performance under this contract:

Christopher Volpe, Professor of Physiological Sciences

b. The personnel specified above and/or in the schedule of the contract are considered to be essential to the work being performed hereunder. If these individuals are unavailable for assignment for work under the contract, or it is anticipated that their level of involvement will be significantly different from the negotiated level, the Contractor shall immediately notify the Contracting Officer and shall submit justifications (including proposed substitutions) in sufficient detail to permit evaluation of the impact on the program. The Contracting Officer shall review, and may ratify in writing, such substitutions. Prior to substitution of key personnel, the Contractor shall obtain the written consent of the Contracting Officer.

LC 21B Disclosure of Information - Restricted Publication

a. The Contractor shall submit to the Commission any report, manuscript or other document containing the results of work performed under this contract. This document shall not be published or otherwise disclosed by the contractor.

b. Should the contractor subsequently apply to the Consumer Product Safety Commission for permission to publish documents containing the results of this work and the release is approved in writing, any publication of, or publicity pertaining to, the Contractor's document shall include the following statement: "This project has been funded with federal funds from the United States Consumer Product Safety Commission under contract number CPSC-S-16-0060. The content of this publication does not necessarily reflect the views of the Commission, nor does mention of trade names, commercial products, or organizations imply endorsement by the Commission.

LC 22 Handling of Confidential Information

a. If the Contractor obtains confidential business information about any company in connection with performance of this contract, either from the CPSC, the other company itself, or any other source, the Contractor agrees that it will hold the information in confidence and not disclose it either to anyone outside the CPSC or to any Contractor employee not involved in performance of this contract.

b. At the completion of performance of this contract, the Contractor shall return any confidential information, obtained as described above, either to its owner or to the CPSC. No such information shall be retained by the Contractor. Furthermore, the Contractor agrees not to use any such confidential business information for any purpose other than performance of this contract. During contract performance, the Contractor shall maintain confidential business information obtained as described in this article in a safe or locked file cabinet to which only employees performing work under this contract shall have access. A log shall be maintained to reflect each entry to the safe or cabinet. The Contractor shall provide to the COR, and keep current, a list of all employees with such access. The Contractor shall require each such employee to execute an affidavit as set forth in the attached "Affidavit of Disclosure" and the original and one copy of each affidavit shall be sent to the COR.

c. A site inspection of the Contractor's security measures for confidential information may be performed by the CPSC COR prior to contract award and at any time during contract performance as deemed necessary by the COR. Approval of the security measures may be a prerequisite to contract award and continued performance.

d. Failure by the contractor to comply with the terms of this clause may be treated as a default pursuant to the terms of this contract.

LC 24 Nondisclosure of any Data Developed Under this Contract

a. The Contractor agrees that it and its employees will not disclose any data obtained or developed under this contract to third parties without the consent of the U. S. Consumer Product Safety Commission Contracting Officer.

b. The Contractor shall obtain an agreement of non-disclosure from each employee who will work on this contract or have access to data obtained or developed under this contract.

LC 31 Restrictions on Use of Information

a. If the Contractor, in the performance of this contract, obtains access to information such as CPSC plans, reports, studies, data protected by the Privacy Act of 1974 (5 U.S.C. 552a), or personal identifying information which has not been released or otherwise made public, the Contractor agrees that without prior written approval of the Contracting Officer it shall not: (a) release or disclose such information, (b) discuss or use such information for any private purpose, (c) share this information with any other party, or (d) submit an unsolicited proposal based on such information. These restrictions will remain in place unless such information is made available to the public by the Government.

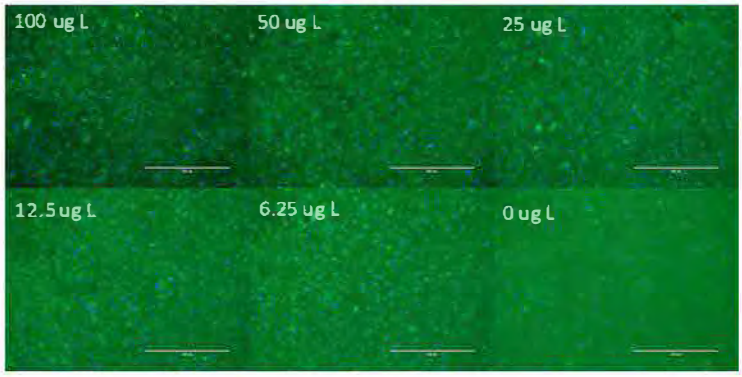
b. In addition, the Contractor agrees that to the extent it collects data on behalf of CPSC, or is given access to, proprietary data, data protected by the Privacy Act of 1974, or other confidential or privileged technical, business, financial, or personal identifying information during performance of this contract, that it shall not disclose such data. The Contractor shall keep the information secure, protect such data to prevent loss or dissemination, and treat such information in accordance with any restrictions imposed on such information.

1. Work progress update

Progress has been made on the objectives that are outlined in the SIINN Nanowires proposal. To investigate the impact of length on silver nanowire environmental toxicity, nanowires that have the same diameter (90 nm) but are short (9 microns) or long (25 microns) were synthesized and provided by the collaboration at the University of Grenoble Alps. RTgillW1 cells have been successfully cultured in the laboratory at the University of Florida, and cytotoxicity assays show evidence for the cellular toxicity of silver nanowires. The CellTiterGlo assay measures ATP production of live cells by fluorescence, and this assay indicated decreased ATP production by RTgillW1 cells after 48-hour exposure to short and long silver nanowires. This decrease was significant for both short and long nanowires, but was more pronounced for short nanowires. Lysosomal activity was evaluated using the lysosensor green dye and GFP fluorescent filter (Figure 1). Results indicated increased in lysosomal activity in RTgillW1 cells after exposure to both short and long nanowires, and this observation was noted in concentrations as low as 6.25 ug/L. This assay will be repeated using lower concentrations. Mitochondrial membrane potential was evaluated with the Mitotracker dye and Cy5 fluorescent filter, and this endpoint was also increased in short and long nanowire exposures (Figure 2).

Figure 1 Increased lysosomal activity in RTgillW1 cells exposed to A. long-L and B. short-S silver nanowires for 48 hours.

A



B

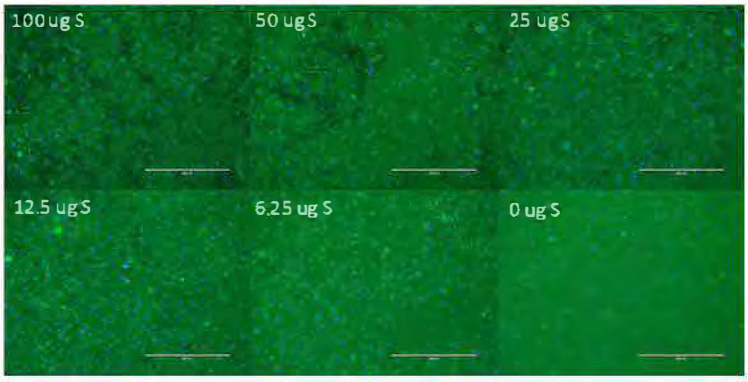
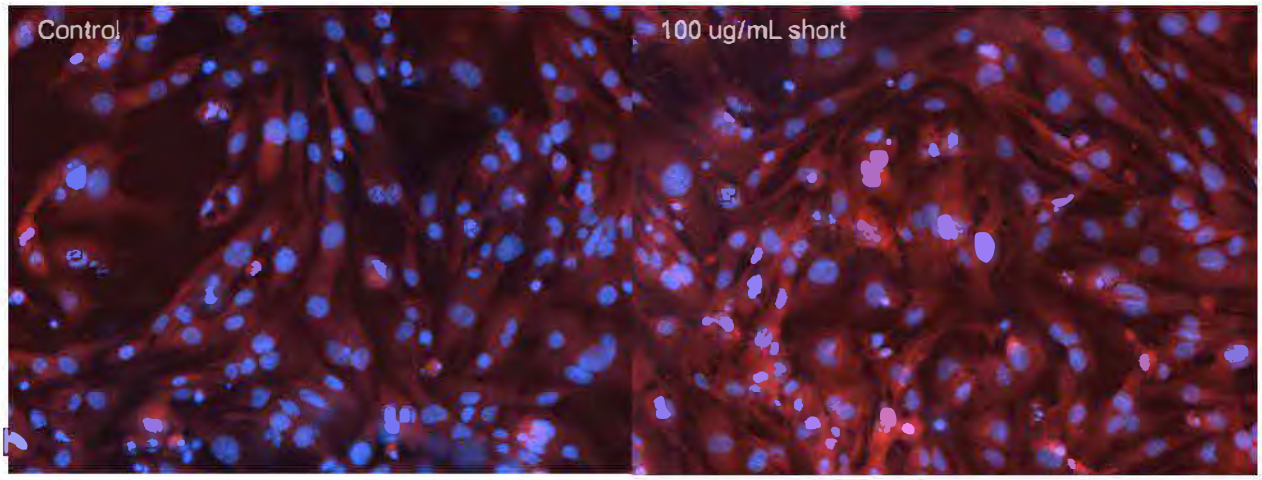


Figure 2 Increased mitochondrial membrane potential in RTgillW1 cells after exposure to short silver nanowires. Images were acquired at 20X magnification.



2. List of presentations and publications related to the work
    a. Publications: None
    b. Presentations
        i. Arndt, D. and Vulpe, C. Silver nanowire toxicity to cells and organisms: A silver bullet to ecosystem health? University of Lille, France. May 23, 2016.
        ii. Arndt, D., Tagmount, A., Vulpe, C. Impact of silver nanowire length and diameter on rainbow trout RTgillW1 cells. Southeast Society of Environmental Toxicology and Chemistry Meeting. Gainesville, FL. September 22, 2016.
        iii. Arndt, D., Tagmount, A., Vulpe, C. Impact of silver nanowire length and diameter on rainbow trout RTgillW1 cells. Nanosafe meeting. University of Grenoble Alps, Grenoble, France. November 11, 2016.

3. Issues that could delay the work

There are three issues that require some troubleshooting to generate high quality data for the project. First, the RTgillW1 cell lines are grown in L15 growth medium, and silver ions have been observed to precipitate out of solution in the L15 medium. Additional characterization of the medium and the nanowires will be required to understand what the concentration of dissolved silver in the medium. Second, the rainbow trout was chosen as a model organism because of its environmental sensitivity and well-known role as an indicator species, and because cell lines for the gill and gut are available (allowing for investigation of specific exposure pathways of silver nanowires into the organism). However, the rainbow trout is also a less traditional model organism, and acquiring antibodies for some of the cytotoxicity assays (H2AX DNA damage and cytochrome C assay) will be difficult and/or more expensive. Alternative assays for examining these endpoints are being evaluated. Third, it was proposed that the bioenergetics of the cells be examined using the Seahorse XF96 instrument. This instrument allows for high throughput

investigation of changes in bioenergetics of cells, and although it is advertised to have temperature control, it does not have a cooling system to decrease temperature to that where rainbow trout cells grow at (19 °C). Preliminary runs of RTgillW1 cells in this instrument are promising, and indicate that temperatures can be decreased to 22-25 °C, and that the cells survive the run and accurate bioenergetics measurements can be made. Further optimization of this assay for RTgillW1 cells is needed, and it is expected that this instrument can be successfully used to measure the bioenergetics of rainbow trout cells after exposure to silver nanowires.

SIINN Nanowires Project Quarterly Update

09.26.17

1. Work Progress Update

Experiments that were conducted with RTgutGC cells were repeated with RTgillW1 cells. Toxicity trends were similar in gill cells compared to gut cells (thin and short AgNWs were less toxic than longer or thicker AgNWs). In addition, short AgNWs (10 um length) induced slightly more toxicity in RTgillW1 cells compared to RTgutGC cells (Figure 1 left), but long AgNWs induced similar levels of toxicity in both RtgillW1 and RTgutGC cells (Figure 1 right). Further progress has been made on mechanistic toxicity experiments. RTgillW1 and RTgutGC cells were exposed to AgNWs and the cells have been frozen for RNA extraction and RNA seq analysis. To move forward on ICPMS studies with AgNWs, a standardized AgNP has been ordered from NIST to optimize concentrations and methods.

Our collaborators used the European synchrotron facility to image the AgNW exposures with cells. The images indicate differential bending behaviors of thin nanowires compared to thick nanowires, where thin AgNWs appear to fold up and thick AgNWs maintain their high aspect ratio and wire-like structure. This could have significant implications for differential toxicity regarding uptake and elimination mechanisms. We plan to take images of the cells using confocal microscopy, which will allow better visualization of nanowire behavior at the cell surface.

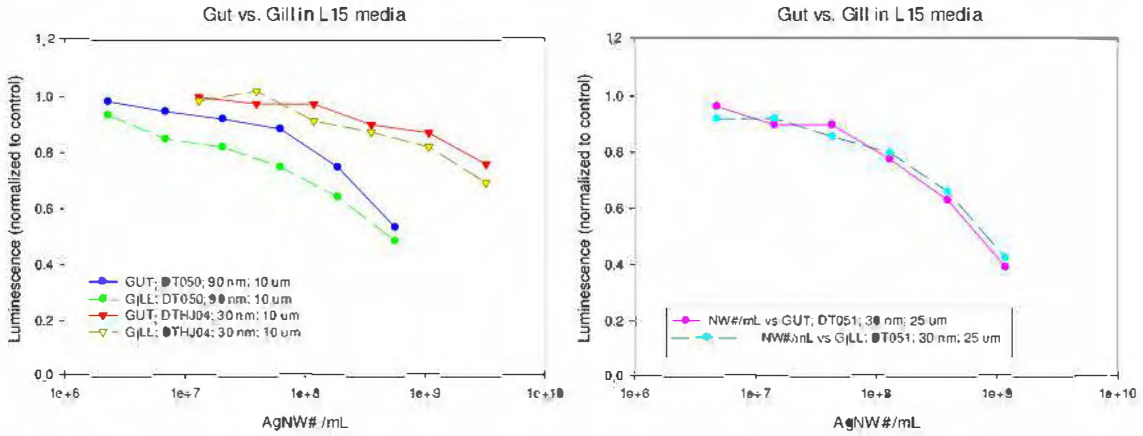


Figure 1 AgNW toxicity in gill and gut cells after 24 hour exposure. Gill cells are more susceptible to toxicity by short AgNWs (left) compared to long AgNWs (right).

2. List of presentations and publications related to the work

Publications: No publications this quarter

Presentations: No presentations this quarter. A presentation is prepared for the Southeast SETAC regional conference in Georgia (Sept. 28-30th) and a poster is being prepared for the national SETAC conference in Minnepolis, MN (November 13-17th).

3. Issues that could delay the work.

Hurricane Irma set the laboratory back about two weeks. It is expected that metabolic assays with the Seahorse instrument will be caught up during the next quarter. The aquarium for rainbow trout larva

is set up, but experimental methods need to be modified to accommodate limited quantities of nanowires, especially DT051 (long and thin AgNW; 25 um length and 30 nm diameter).

SIINN Nanowires Project Quarterly Update

06.26.17

1. Work Progress Update

Progress has been made on the objectives that are outlined in the SIINN nanowires proposal. RTgutGC cells arrived from EAWAG in Switzerland in good condition and were successfully transitioned to cultures at our laboratory. As will RTgillW1 cells, RTgutGC cell viability was evaluated by the Cell Titer Glo luminescent assay for detecting ATP production. For these experiments, a protocol was developed and distributed to the SIINN Nanowires collaborators for concentrating the stock nanowires suspensions. As a consequence, ATP production was evaluated in RTgutGC after exposure to all types of nanowires (previous assays with RTgillW1 were only conducted with the most concentrated nanowire samples DT050, DT067, and DTHJ04) at higher concentrations (up to 360 ug/mL, compared to 80 ug/mL in previous experiments). Results indicate less toxicity associated with thin nanowires compared to thick nanowires, and this result was consistent regardless of whether the exposure was calculated by mass or by AgNW number (Figure 1). These results somewhat contrast our previous experiments, which didn't show much differential toxicity regarding AgNW diameter. However, exposure of cells at the higher concentrations elucidated the differential toxicity of AgNWs regarding diameter.

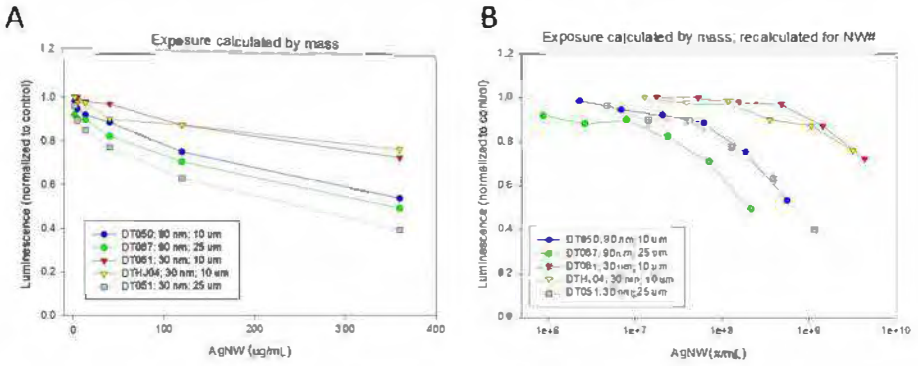


Figure 1: ATP production in RTgutGC cells after exposure to AgNWs that differ in length and diameter, calculated by A) mass and B) NW# (NW dimensions represented as diameter, length).

An issue stated in the 033017 quarterly report has been resolved. Amino acids and chloride components in the L15 media (required for culturing rainbow trout cells) form complexes with silver ions and precipitate out of the L15 media – resulting in very little ionic toxicity in $AgNO_3$ ionic controls. It was found that L15 media can be modified to remove many/most of these interfering components with little to no effect on cell viability over a 72 hour period. The modified L15 media (L15/ex) was prepared and another set of assays with Cell Titer Glo were conducted on RTgutGC cells using L15/ex. Results indicated significantly higher toxicity of the ionic silver control, $AgNO_3$ (significant toxicity observed at 5 ug/mL in L15/ex compared to 175 ug/mL in L15). Toxicity of long AgNWs (DT067 and DT051) was significantly increased in L15/ex compared to L15 media, indicating an ionic component of toxicity associated with long AgNWs but not short AgNWs (Figure 2). L15 and L15/ex media create an optimal model system for differentiating AgNW toxicity from ionic toxicity, and they show that media components do not have any protective effects on NW-only toxicity. These experiments also highlight the potential for differential toxicity of some AgNWs depending on the type of exposure (ie. freshwater versus saltwater environment).

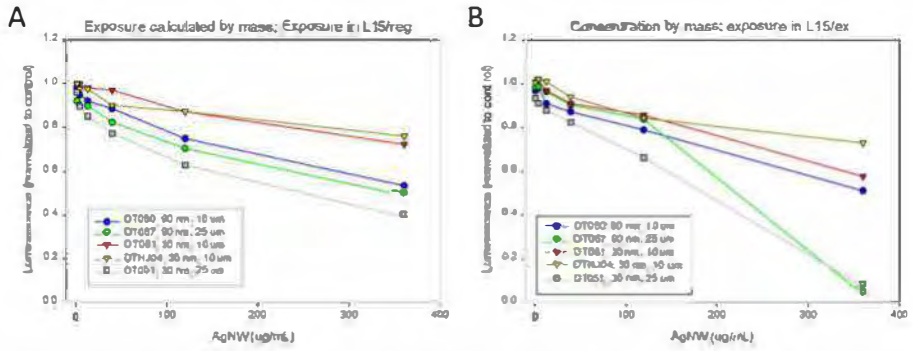


Figure 2: Toxicity of AgNWs to RTgutGC cells in A) L15 media and B) L15/ex media (NW dimensions represented as diameter; length).

The facility where whole organism exposures will take place has been IACUC approved. The space for the aquariums did not have any available outlets for the aquariums, but electrical outlets were recently installed on 6/16/2017. Aquariums can now be set up for whole organism exposures in the new aquatic facility.

2. List of presentations and publications related to the work

Publications: No publications this quarter

Presentations:

Devrah Arndt, Abderrahmane Tagmount, Djadidi Toybou, Laurent Charlet, Christopher Vulpe. Impact of silver nanowire length and diameter on the rainbow trout RTgillW1 cell line. North American ACS Meeting. San Francisco, CA. April 2-6th, 2017.

3. Issues that could delay the work.

There has been some resolution to the issue of finding antibodies that are compatible with rainbow trout for some of the cytotoxicity assays. A recent discussion with a colleague indicated that a specific human antibody for the Annexin V assay produces good fluorescence in rainbow trout. In addition, the comet assay doesn't require the use of an antibody, and might be a good replacement for the H2AX DNA damage assay for testing genotoxicity. We are still looking for options for cytochrome C assays.

An issue with ICPMS could delay some ICPMS-related work. In the ICPMS instrument, particles that are present in aqueous solution are ionized to the plasma in droplets that have maximum diameters of 4 microns. Our nanowires are much larger than this at 10 and 25 microns. It is likely that some significant troubleshooting will be required to optimize experiments using AgNWs and ICPMS.

SIINN Quarterly Report

03.31.18

1. Work Progress Update

Progress has been made on the metabolic assays with the Seahorse XF instrument. Results indicate that thick silver nanowires significantly reduce basal metabolism and ATP production in rainbow trout gill cells at higher concentrations (above 120 ug/mL). Thin silver nanowires have no effect on basal metabolism or ATP production in rainbow trout gill cells (Figure 1a and 1b). This result is consistent with the CellTiter-GLO assay, which previously indicated decreased ATP production (by measured luminescence at 450 nm) by rainbow trout gill cells exposed to higher concentrations of thick silver nanowires.

Proton leakage was increased in thick silver nanowire-exposed cells compared to control cells and was decreased in thin silver nanowire-exposed cells compared to control cells. While these observations weren't statistically significant compared to the control, the cells from thin and thick silver nanowire treatments were statistically different from each other (Figure 1c).

All treatments of silver nanowires significantly reduced the reserve capacity of rainbow trout gill cells (Figure 1d). The reserve capacity makes up the energetic reserve that can be used by the cell in times of stress. Since the reserve capacity is reduced in all treatments despite the fact that we have not observed reduced toxicity with thin silver nanowires at these concentrations, it is possible that the reserve capacity is not a mechanism of silver nanowire toxicity. It is also possible that silver nanowire residue is interfering with this measurement. Control assays with 1) only silver nanowires and no cells and 2) silver nanowires added to the cells directly before starting the assay will clarify any possible interference of the silver nanowires with the readings.

Our aquarium system had a leak, but the leak has been repaired and whole organism experiments with rainbow trout embryos and fry are expected to be started in late April or early May.

2. List of presentations and publications related to the work

Publications: The following manuscript has been submitted to Science for publication and is currently in the review process.

Crumpling versus Puncturing: A Biomechanical Threshold for the Acute Cellular Toxicity of Silver Nanowires by Sylvia G Lehmann[1], Djadidi Toybou, Ana Pradas Del Real, Muriel Viau[1]; Devrah Arndt, Christopher Vulpe, Alexandra Pacureanu, Peter Cloetens, M. Salome, H. Castillo, J.P. Simonato, Caroline Celle, Laurent Charlet[1] and Benjamin Gilbert[1]

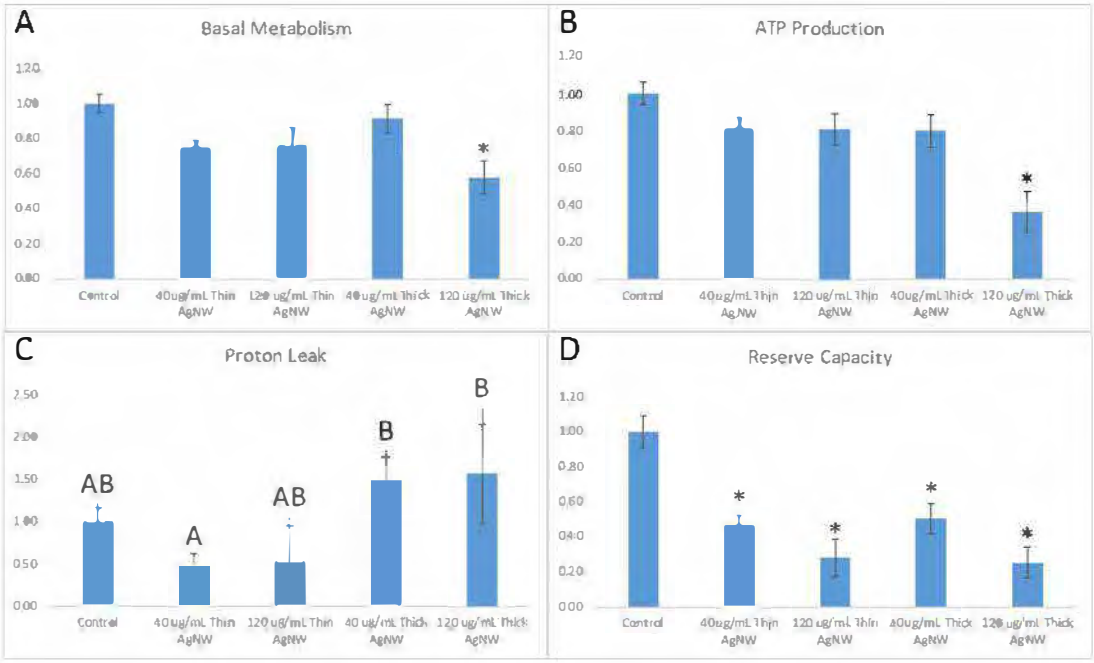
Presentations: No presentations this quarter

3. Issues that could delay the work

The seahorse XF instrument had a major mechanical failure and the machine needs to be sent to Agilent for repair. The Seahorse XF will likely be out of commission for several months, so the control assays described in the first section will be delayed until the machine is repaired.

We are planning to do confocal microscopy to explore silver nanowire uptake, transport, and bioaccumulation in rainbow trout cells, but our silver nanowires are not functionalized and therefore cannot be labeled with fluorescent probes. The confocal microscope in our department is not equipped with darkfield capabilities (which is necessary to view non-labeled silver nanowires), but a microscope with darkfield capabilities was purchased and is being set up at the VA. When this microscope is available we plan to image the silver nanowire exposure with the rainbow trout cells.

Figure 1 Impact of silver nanowire exposure on A) basal metabolism, B) ATP production, C) proton leakage, and D) reserve capacity in rainbow trout cells. Data are normalized to the control and the y-axis represents the oxygen consumption rate (OCR) (pmol/min) relative to the control oxygen consumption rate.



SIINN Nanowires Project Quarterly Update

03.26.17

1. Work Progress Update

Further progress has been made on the objectives that are outlined in the SIINN Nanowires proposal. Collaborators at the University of Grenoble Alps synthesized another nanowire for our studies that has a smaller diameter compared to the previous nanowires that were provided (30 nm diameter compared to 90 nm diameter), making it possible for us to investigate the impact of diameter on silver nanowire toxicity. The cell titer assay measures ATP production by live cell luminescence. This assay previously indicated decreased ATP production for short and long AgNWs with a diameter of 90 nm, with a slightly more pronounced effect on short AgNWs. Recent data indicates that nanowires with a smaller diameter (30 nm) induce similar changes to ATP production as the wide diameter nanowires (Figure 1). Although not significant, data trends show that small diameter AgNWs are slightly more toxic to gill cells that wide diameter AgNWs. In contrast, lysosome and mitochondrial activity were found to be significantly higher in RTgillW1 cells exposed to AgNWs with wide diameters compared to AgNWs with thin diameters (Figure 2 and Figure 3), indicating less toxicity to these organelles associated with thin-diameter AgNWs. A LDH cytotoxicity assays showed little effect of all tested AgNW treatments to RTgillW1 cells, indicating that AgNWs do not significantly impact plasma membrane permeability.

Work has been done to troubleshoot the previously mentioned problem with the rainbow trout gill and gut cell line in the Seahorse Instrument. The Seahorse instrument is designed to test mammalian cell lines that are incubated at 37 C, but our cells require incubation at 19 C. By shutting off the heating equipment in the Seahorse Instrument and shortening the run, we were able to successfully run the gill cells in the Seahorse instrument. Although temperatures within the instrument reached 24 C during the run, the cells survived the run and data output looks good. We are also working with an Agilent technician to optimize experiments in this instrument.

Rainbow trout gut cells were shipped from EAWAG in Switzerland and have arrived in good condition at our facility. These cells are being cultured and grown in our incubation system and future experiments will include this cell line in analysis.

Figure 1 AgNW diameter does not have a strong effect on cell viability. It appears that wide and thin diameter AgNWs can induce significant toxicity to cells at concentrations as low as 1.25 ug/mL.

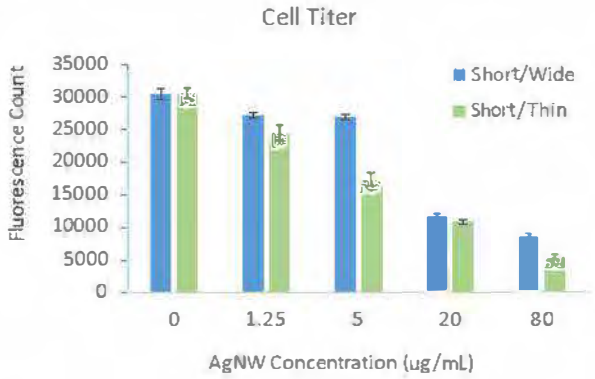
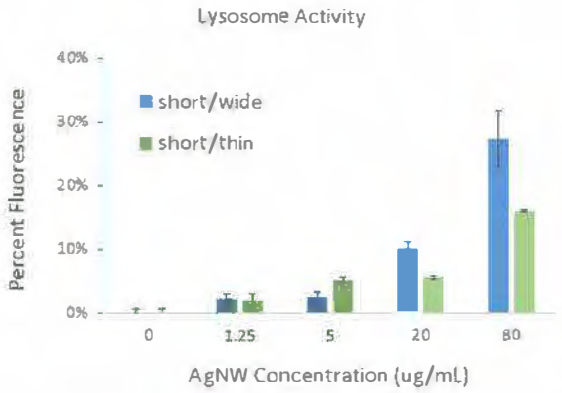


Figure 2 Lysosomal activity is higher in wide-diameter AgNWs compared to thin-diameter AgNWs, indicating elevated degradation of enzymes, proteins, or foreign material in the cell after exposure to wide diameter nanomaterials.

A



B

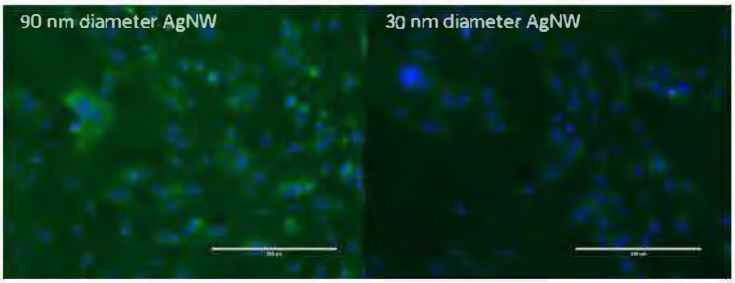
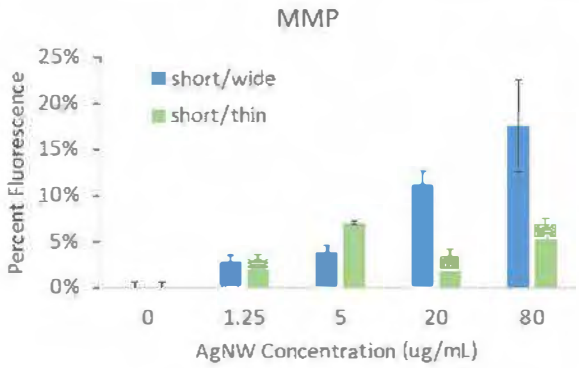


Figure 3 Mitochondrial membrane potential is elevated in wide-diameter AgNWs compared to thin-diameter AgNWs.



2. List of presentations and publications related to the work

Publications: No publications this quarter

Presentations: No presentations this quarter. A presentation will be given at the American Chemical Society meeting April 5th, 2017 in San Francisco. This presentation will be included in the next quarterly report.

3. Issues that could delay the work

A literature search on the topic of L15 media (optimal media for rainbow trout cells) and silver ion toxicity has revealed that the high amino acid content of L15 media results in complexation of silver ions with thiols and other media components. A plan to make L15 media with a lower amino acid content and perform additional cytotoxicity experiments in this new media is underway. These experiments will be very informative about silver nanowire toxicity relative to environmental conditions of the exposure.

Aquariums are being set up for whole organism exposures. The facility where the experiments will take place is new and currently scheduled for IACUC approval by the end of April. Other investigators will have fish cultures in this room, and IACUC approval will depend on the shared preparations of these researchers who will culture fish in the same facility.

Finally, the rainbow trout model was chosen because individual cell lines are available that allow for investigation of two primary routes of exposure of AgNWs (the gill and the gut), but antibodies for some of the cytotoxicity assays (H2AX DNA damage, cytochrome C) are not readily available or are expensive. We are still investigating the best way to complete the rest of the experiments using the non-traditional rainbow trout model.