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Microscopic Analysis of the Interaction of Gold Nanoparticles with Cells of the Innate Immune System

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Gold nanoparticles (AuNPs) can interfere with some of the biochemical processes of macrophage cells but the mechanisms of action of these potentially medically-relevant effects are still unclear. Here we study the fate of AuNPs interacting with cells derived from the innate immune system. To visualize AuNPs with nanometer resolution without losing sight of the whole morphology of cells we developed a convenient approach that uses electron and ion microscopy techniques. The inspection using an ion beam to selectively cut where the AuNPs were found, allows for determining their intracellular localizations. We studied the cellular uptake of AuNPs, with or without exposure of the cells to Latrunculin-A, a phagocytosis inhibitor. Results indicate a size dependence of the internalization mechanisms for THP-1 cells. The internalization of larger AuNPs was blocked in the presence of Latrunculin-A, although they could attach to the membrane. Smaller AuNPs though were not blocked by actin depending processes.

The interaction of gold-based advanced materials with the immune system has been known since the beginning of the last century with the use of organogold compounds to regulate its response in the treatment of rheumatic diseases¹. However there is still much to know about their mechanisms of action² and new methods to study their interactions with cells of the immune system are highly desirable. A novel way to study the role of gold and its interaction with the immune system could be to use AuNPs, since their path can be easily followed and they can be visualized using electron microscopy methods. Citrate-coated AuNPs are more compatible with biological systems compared to other functionalized AuNPs and metallic nanoparticles such as silver³. They are also easy to synthesize in a wide range of monodispersed sizes from 5 to 50 nm, some of which are currently undergoing clinical tests for the treatment of different kinds of tumours⁴. THP-1 cells are a myeloid monocytic immortalized human cell line which can be differentiated to macrophages and display the major functional properties of primary human innate immune cells. They are often used as a model to study human inflammatory responses, which allows for the possibility of elucidating the interactions of NPs with innate immune cells^{5,6}. Localization of metallic NPs within cellular compartments is important in order to understand their role and mechanisms of interaction^{5,7}. However, monitoring NPs in these cells is not an easy task since they may readily change their morphology depending on environmental conditions and are grown as non-adherent cells in suspension, which complicates their fixation process. Moreover, their large size and rounded shape make the detection of NPs with electron transmission microscopy difficult due to the low contrast produced by the mass-thickness (density $\times$ thickness) of the NP in comparison to the surrounding section of the cell.

Methods such as confocal Raman microscopy have allowed the identification and localization of intracellular NPs in adherent cancer cells but with a maximum resolution limited by the wavelength limit of visible light⁸. Recently the internalization pathway and localization of an undetermined number of polystyrene NPs was shown in the case of undifferentiated THP-1 cells and macrophages⁹. Using hyperspectral confocal microscopy it is possible to distinguish AuNPs interacting with living tissues^{10,11}. The first one allows for chemical identification of the NPs using their spectral signature. However, its spatial resolution is limited by the light diffraction limit (in the wavelength of the plasmon resonance $\sim$ 250 nm and $\sim$ 600 nm for lateral and height resolution respectively), and its sensitivity does not allow to detect NPs as small as the electron microscopy; the threshold is typically $\sim$ 60 nm¹⁰. Walkey and coworkers studied the uptake of PEG-grafted and non-coated AuNPs by J77A.1 murine macrophage-like cells prepared in an adherent culture. They

Figure 1 | Panels (A), (B) and (C) show STEM images from the samples of 5, 15 and 35 nm AuNPs, respectively. Magnification and scale bar (50 nm) are common in the three pictures. D shows the particle distribution measured by STEM in a wider population of particles.

showed that the NPs were found in dense aggregated clusters inside endosomal vesicles¹². The conventional detection of NPs in cells using transmission electron microscopy (TEM) allows for superior resolution and contrast between tissues but involves preparing cell slices of around 100 nm thickness and this entails long sample preparation protocols. This method complicates the study of whole cell morphology since exact consequent sequences of microtome slices must be imaged. A novel application of dual beam microscopes performing Scanning Electron Beam – Focused Ion Beam (SEM-FIB) tomography and often called “slice and view” allows the 3D reconstruction of organic tissue with good contrast and depth resolution¹³. However, this method also entails very long processes of cell cutting, the use of sophisticated software and problems associated with locating the correct field of interest in the sample.

Here, we present an alternative method to study the interactions of metal NPs with THP-1 cells. Our procedure takes advantage of an optimized protocol for fixation of the cells and the use of CO₂ critical point drying to remove the solvent from the cell in order to preserve their shape during microscopy without the need to exchange the cell liquid content with microscopy resins. In this way, much of the useless mass of the cell is removed and we can obtain information regarding cell morphology and the quantity of NPs that interact or are internalized by the cell. To achieve this goal we score all the NPs using both traditional SEM, detecting the scattered electrons from the surface and the NPs located on it, and scanning transmission microscopy (STEM), that detects the rest of the NPs. The method is completed by milling into the cell using a FIB on the extra NPs to assure their localization inside the cell. This experimental approach offers speed and lateral resolution versus depth advantages with respect to other tomography techniques that require prior microtome slicing (TEM microtome) or multiple FIB cuts (SEM-FIB tomography). Moreover, we demonstrate that it can be applied to non-adherent cells.

In a recent study, we showed that AuNPs downregulate interleukin-1-Beta-dependent inflammatory responses, which are associated with many autoimmune disorders such as rheumatoid arthritis or psoriasis³. The anti-inflammatory activity of AuNPs was shown to most probably be due to extracellular interactions with IL-1 β . In the present article we now show that AuNPs interact strongly with the cell membrane as well as also being internalized. Using the method described above we studied one of the main possible mechanisms of uptake of AuNPs using Latrunculin-A (LTR), a phagocytosis inhibitor. We compared the results of the uptake of AuNPs of different sizes. The experimental results indicate a size dependence of the internalization mechanisms for THP-1 cells and the accumulation of AuNPs inside specific cellular compartments.

Results

High quality unimodal AuNPs of 5, 15, and 35 nm were successfully synthesized. Representative STEM pictures of AuNPs and statistical analysis from larger populations are shown in Figure 1. An accurate characterization of AuNPs employed in this study was crucial for experiments that determined their cellular distribution and uptake. For this reason, we verified their sizes combining three different methods. Using STEM and image analysis we obtained the sizes close to 35, 15 and 5 nm NPs, in agreement with Dynamic Light Scattering (DLS) and disc centrifuging. All these methods have limitations; SEM because the amount of particles measured is limited; DLS is more sensitive to bigger particles, while the sensitivity of disc centrifuging is very much density dependant. However, the agreement of the three methods allows us to discard aggregation, or the existence of smaller species masked by larger ones, a typical phenomena occurring with DLS measurements¹⁴. Therefore, by using three complementary techniques we carefully characterized the AuNPs according to size, which is critical for the interpretation of results obtained using cellular interactions. Table 1 summarizes the measurements of the three different sizes of AuNPs used in this study. We observed a very good agreement between the electron microscopy and disc centrifuging data, while DLS measured slightly larger diameters, which is consistent with the fact that this technique measures the hydrodynamic diameter rather than the natural one of the AuNP. The three techniques tend to give comparable results in the case of monomodal particle distribution, with DLS giving a slightly larger size compared to the other two techniques^{15,16}.

In order to study cells in vacuum we followed a standard fixation procedure using glutaraldehyde and OsO₄, optimizing their quantities for use with THP-1 cells in suspension followed by critical point drying to remove the water content of the cell to allow their inspection under vacuum conditions.

Although both the *in vivo* and *in vitro* testing of AuNPs of different sizes are known to sometimes produce contradictory results^{17,18}, we first focused on the 35 nm AuNPs as an example for microscopic

Table 1 | AuNP size results of the mean size and standard deviation obtained by STEM, DLS and CPS of the particles reported in panels A, B and C used in our experiments

	STEM		DLS		CPS	
	Mean	σ	Mean	σ	Mean	σ
A	3.3	1.7	7.1	2.1	3.4	1.5
B	12.0	1.0	17	4.7	12.4	1.7
C	31	3	37	10	30	5

detection since this size is easier to examine. Our method was based on correlating the signals of the transmitted high energy electrons and the scattered low energy ones coming mainly from the surface. In our procedure, after detecting a normally-shaped cell (Figure 2 A), we first scanned it using the transmission mode to detect AuNPs. For this we used 30 KeV acceleration voltage (HV) that allowed the highest penetration depth in our system. We also used the ADF detector in dark field configuration that allows for differential detection with increased contrast. AuNPs on thick cells such THP-1 are difficult to detect because the weight of the NPs becomes less relevant compared to the rest of the cellular material that the electrons probe in transmission mode. In order to detect AuNPs, images at various contrast levels were taken. An example of this is illustrated by noticing that the AuNPs visible in figure 2 B disappear when increasing the brightness in figure 2 C, while other NPs were apparent (all the NPs detected in the cell shown in figure 2 using different brightness and contrast configurations are marked with yellow arrows in the respective panels).

After identifying the position of the AuNPs, we explored the surface of the cell using lower HVs that ranged from 5 to 25 KeV to optimize the portion of specific surface signal while avoiding charging effects. If AuNPs detected in transmission mode were not detected on the surface, we proceeded to search inside the cell by performing a cut using FIB milling. Before the cut, and in order to protect the region of the cell containing the AuNPs and avoid the curtain effect (formation of striations across the milling face when the sample has a surface with uneven topography), we deposited a protective metallic layer on top using Pt-GIS activated with the electron gun (the preparation of the cut is shown in Figure 2 F highlighting the deposition with artificial shadowing)¹⁹. The cut was

made using the smallest aperture of our FIB at 1.5 pA current to avoid damage caused by the FIB to the cells. The 52° angle between our SEM and FIB allowed monitoring the section of the cell while milling without repositioning the sample. The final section part of the cell protected by platinum in figure 2 F is shown in Figure 2 G at the observation angle of 52°.

The SEM image taken in surface mode shown in figure 2 A illustrates the shape of the cell, which is similar to the control samples in our experiments and also consistent with previous literature²⁰. None of the cells in this or any other sample of this study showed significant morphological changes with respect to untreated controls. Figures 2 D and E show increased magnifications of the regions marked 1 and 2 in figures 2 A and B. While in figure 2 D AuNPs were not found on the surface, in figure 2 E a single AuNP detected in transmission mode is clearly visible at the surface. The diameters of the detected AuNPs are consistent with the 35 nm size incubated with the cells. AuNPs, attached to the cell membrane, resisted several washing steps as well as centrifugation, indicating a robust interaction with the cell membrane. The absence of AuNPs in figure 2D can be explained after the milling shown in Figure 2 G. The darker area under the Pt-protected region in Figure 2 G corresponds to the lighter material from the cytoplasm. The bright spots marked inside the circled region 1 suggest the presence of AuNPs and this was verified by EDAX analysis.

The Au content of the NPs was controlled by comparing the EDAX signal from the AuNPs with a background from the cell close to the high contrast region that indicates the AuNP position. The signal at 2.12 KeV corresponds to the X-ray emission line from the M- α electron transition of Au atoms, while the peak just below, at 1.9 KeV, corresponds to the signal from M- α -line from the Os used

Figure 2 | Microscopic images of a representative THP-1 cell incubated with nude 35 nm AuNPs. The position of the AuNPs are indicated using yellow arrows: (A) SEM image of the surface; (B) and (C) STEM dark field scans at different brightness contrast indicating different positions where AuNPs were detected. Scale bar is common for the images in the panels (A), (B), and (C). (D) Magnification of the surface of the cell at position 1 indicated in panels (A) and (B). (E) Magnification of the surface of the cell at position 2 indicated in panels (A) and (B). (F) partial blow up of panel A around position 1 after the Pt deposition that has been shadowed in color. (G) Section of the region of the cell under the Pt in panel F, after the milling process, with the AuNPs indicated by the yellow arrow. (H) EDAX signals coming from region 1 (red) after the milling of the cell (panel G)), region 2 (blue) coming from an AuNP at the surface (panel E)) and the background from region 3 (black, panel G)). The EDX intensity marked with an arrow at 2.14 corresponds to the M emission from Au atoms.

Figure 3 | (A) and (B) Surface and transmission images respectively from the same cell treated with LTR and incubated with 35 nm AuNPs. (C) and (D) magnification of the marked regions, AuNPs are indicated with yellow arrows.

during the fixation process. We used this signal to normalize the spectra since Os is approximately equally distributed in regions at a relatively high density. Although the signal from few AuNPs is weak we could detect them in most of our samples. The three spectra shown in figure 2 H correspond to the AuNPs in region 1 in figure 2 G after the cut (red), to the single AuNP detected in region 2 in figure 2 E (blue) and the background chosen from region 3 in figure 2 G, which was close to the cut and the AuNPs (black). The EDAX signal from a single AuNP at the surface of the cell is very difficult to detect and emits a very weak signal slightly above the noise level. Nonetheless, in this case, it was still possible to detect traces of Au content present from a single AuNP of 35 nm. In the case of region 1, it was found that the signal of NPs coming from inside the cell corresponds to a group of four NPs that appeared at the plane of the cut. The measured size of these NPs is also consistent with the

incubated material. In the following experiments the EDAX from smaller NPs were detected only in the case that they form big enough clusters.

We next assessed this experimental approach studying the effects of a known inhibitor of phagocytosis, LTR. LTR is a toxin that causes disruption of actin filaments in the cytoskeleton, thus preventing the internalization of AuNPs. We pre-treated THP-1 cells with 100 nM LTR for 60 min to block phagocytosis before exposure of the cells to AuNPs.

Microscopic observations revealed that the use of LTR did not alter the shape of the THP-1 cells and the plasma membrane appeared to be intact. Figure 3 shows a scan of a cell incubated with LTR and 35 nm AuNPs. Using the same method previously described to detect AuNPs in the cell and on the cell membrane, and comparing Figures 3 A and B, we can confirm that all the AuNPs that we detected were found on the cell surface. In this cell we observed at least 20 AuNPs that were visible both in transmission and surface images, thus in this case AuNPs were clearly not internalized. Figures 3 C and D are magnifications of the marked regions showing representative details of the AuNPs visualized using both methods. We repeated the observation comparing the surface and transmission modes in other cells and performing cuts when particles were not found on the surface, without detecting any particles inside these cells. This experiment indicates that the mechanisms of cellular uptake of 35 nm AuNPs are affected by LTR and involve a strong actin-dependent process, probably phagocytosis.

The behaviour of the 15 nm AuNPs resembled those of 35 nm for all cells that were studied. However, in this range of diameters, individual NPs become more difficult to visualize with SEM because lower voltages are needed to detect smaller particles at the surface and the charging of the non-electronic conductive material of the cell lowers resolution. For this reason the surface analysis is more delicate, although the transmission mode was less challenging. The yellow arrows in Figures 4 A and B indicate 15 nm AuNPs that were located at the surface of the cell. Particles were also found intracellularly as shown using a different cell on Figures 4 C, D, E and F where we illustrate the sequence of detection, cutting and EDAX analysis.

Figure 4 | Images from cells incubated with 15 nm AuNPs. (A) and (B) indicate AuNPs found on the surface of a cell in transmission and surface modes respectively. (C), (D) and (E) show the sequence of detection slicing and analysis of a group of AuNPs found inside a cell. The graph shows the EDAX signal from a group of AuNPs in (E) (red line) compared to the background in an arbitrary point from the cut (black line).

As seen with 35 nm AuNPs, the 15 nm AuNPs particles were not found inside the cells following LTR treatment.

Figure 5 A and B show the surface of a cell incubated with 5 nm AuNPs, and its corresponding magnification of the region where AuNPs were observed. The brighter areas in Figure 5 A were not present in cells not treated with 5 nm AuNPs, and we therefore attributed them to regions containing AuNPs. Figure 5 B shows a magnification of the marked region where a large quantity of these features is present. This accumulation of AuNPs at the surface allowed for EDAX signal detection of Au in cases where we found AuNP clusters, although the EDAX signal of Au was too low when these small AuNPs were in isolation.

The set of smaller size, 5 nm AuNPs exhibited a different behaviour regarding the internalization mechanism than larger AuNPs. Figure 5 C shows a transmission image of a cell incubated with LTR. The horizontal straight stripe highlighted with green is the Pt deposited for the preparation of the cut. Darker dots indicated with the yellow arrow, below the Pt, correspond to the signal from AuNPs in transmission mode. By performing the cross section shown in figure 5 D, we demonstrate that AuNPs were internalized regardless the presence of LTR. Figure 5 E shows the EDAX analysis of the AuNPs found in a cluster inside the cell (shown in figure 5 D). We confirmed this behaviour in our experiments on several THP-1 cells and in different runs.

Discussion

The results of the microscopic analysis of monocytes demonstrate the feasibility of detecting and visualizing how Au organocompounds, and in particular AuNPs, interact with leukocytes. The use of electron microscopy permits superior spatial resolution compared to traditional optical methods^{8–11} which allows for greater

discrimination of NPs that are inside a cell or attached to the plasma membrane. It also allows for chemical identification of nanoparticles by EDS analysis.

The fixation of cells using glutaraldehyde and OsO₄ is a recognized method to analyze cells not only for electron microscopy but also for optical studies. This procedure, which is used for other electron microscopy techniques^{12,13}, exchanges the cellular water content with resins that help to maintain cell morphology. In our case no resin was used. Upon critical point drying the cell loses mass by removing the water, which accentuates the signal contrast from NPs with respect to that of the cell, and thus allows for their detection by comparing the signals of transmitted and scattered electrons. In this way the localization of NPs inside the cells can be performed with a single cut of the ion focused beam.

Literature data shows that LTR has a sequestration effect of monomeric actin and due to this action the dynamic movement of clathrin is inhibited thus preventing endocytosis^{21,22} and recently we reported that AuNPs also modify the acting filament structure of the cell in a different system²³. We used LTR to inhibit or at least slow down the uptake of the cells. Our data, limited to the detailed analysis of a few cells, suggest that this process seems to be essential in the internalization of 35 and 15 nm AuNPs, however LTR was not able to block uptake in the case of the 5 nm AuNPs. Some studies have shown that mammalian cells, contrary to non mammalian cells, can show endocytosis even in the presence of LTR^{24,25}. They are able to perform internalization by clathrin coated pits, albeit at a reduced rate. In our case, whether due to an increase in the hit numbers or due to a size effect on the mechanism of uptake, AuNPs of 5 nm were internalized even in the presence of LTR.

We noticed that in general, AuNPs tended to concentrate together in determined regions of the cell, and this effect tended to be more

Figure 5 | (A) THP-1 cell incubated with 5 nm AuNPs. The brighter dots correspond to regions of AuNPs. (B) magnification of one of these details. (C) Transmission image for a cut of the cell where they were not localized on the surface. The sample was incubated with LTR. Darker dots correspond to the signal from AuNPs in transmission mode. (D) Cross section of the cell demonstrated that AuNPs were internalized regardless the presence of LTR. (E) EDAX analysis, of the selected regions.

noticeable with smaller size. AuNPs on the cell surface and within cross sections of cells were also found as clusters, and for this reason the X-Ray signal could be detected even with small AuNPs. CPS experiments performed with AuNPs in culture medium indicated that there is a slight increase in size of AuNPs but no aggregation. Therefore, the clustering must occur at the cell membrane or inside the cell. We also noted that all the AuNPs, regardless of their size, were found inside these cells grouped in specific areas. This is consistent with experiments by Walkey *et al.* on adherent cells (murine macrophage-like) grown in cell culture dishes¹². Their experiments showed that non functionalized AuNPs were observed as aggregates inside the cells in intracellular vesicles. In contrast to this report, where particles were found predominantly located inside the cells but not adhered to the cell surface, our present study clearly showed particles on the membrane, both in aggregated and dispersed form. The fact that particles resisted several washing and centrifugation steps suggests that there is a significant interaction between them and some - probably specific - membrane sites.

In summary, we have presented a microscopy method to address the problem of studying the uptake and intracellular localization of AuNPs in cells. The method requires observing the surface of the cell using catered electrons and compares the images with a STEM signal to localize the total number of AuNPs and identify internalized AuNP components. The contrast obtained during electron microscopy and thus the detection of the NPs, depends on the mass difference between the NP and the surrounding biological tissue and therefore ultimately on the difference between the atomic number of the atoms constituting the NP and the matrix. The method is completed by milling the cell and observing the position of the AuNPs. Au content was confirmed by EDAX. We used this method to study the uptake of AuNPs, which are increasingly employed in biology and medicine, that were synthesized in three different sizes 5, 15 and 35 nm by THP-1 cells. The interaction of these cells with AuNPs will provide crucial information regarding the effect of Au on the immune response.

We used 35 nm diameter AuNPs as an example to illustrate this novel method and extended the technique to the resolution limits of smaller size AuNPs. We demonstrated the applicability of this method by detecting size-dependent AuNP uptake behaviour of THP-1 using AuNPs with a diameter comparable with the boundaries of the resolution of our microscope. We observed that larger size AuNPs are found dispersed on the cell membrane and aggregated when they are found intracellularly, while smaller size AuNPs are more prone to concentrate in closer aggregates, also on the cell surface. This clustering does not occur in the culture medium. Moreover, AuNPs consistently formed clusters intracellularly, suggesting localization of AuNPs within specialized cell compartments. The microscopy method demonstrated that AuNPs as small as 5 nm could be detected in these cells using a dark-field detector. With the use of ion milling we were able to demonstrate univocally uptake of AuNPs. The method can be applied even to very thick cells, simplifying sample preparation and obtaining clear advantages regarding speed and lateral resolution versus depth.

Methods

Synthesis and characterization of AuNPs. Small AuNPs of 5 nm were produced by NaBH₄ (Sigma) reduction of a starting solution of sodium citrate (Sigma) (2.5 mM) and HAuCl₄ (Sigma) (0.5 mM) in water. 15 nm AuNPs were made by thermal reduction of HAuCl₄ (0.5 mM) in water by rapidly heating the solution to 97°C with a microwave synthesizer (Discover S by CEM). 35 nm Au NPs were made by seed growth increasing the size by addition of NH₂OH·HCl in a solution of HAuCl₄ and NaOH.

Particles were characterized using a disc centrifuge photosedimentometer model DC240000UHR by CPS instruments and with DLS using a Malvern Zetasizer Nano-ZS instrument with temperature control. Each DLS sample was recorded at 25 ± 1°C, in triplicate; each measurement is the average of 20 data sets acquired for 10 s each. Hydrodynamic diameters have been calculated using the internal software analysis from the DLS intensity-weighted particle size distribution. We also analysed the size distribution of the samples using STEM images and statistical analysis performed

with open software Image J. For the size analysis of the bigger samples, we used a circularity filter of 0.8 that brought down the number of particles to ~600 particles for the 15 and 35 nm and to ~250 particles in the case of the smallest 5 nm particles which are very close to the detection limit of our microscope.

Cell preparation. THP-1 human leukaemia monocytic macrophages were purchased from the European collection of Cell Cultures (Salisbury, UK). Cells were grown in RPMI 1640 media supplemented with 10% fetal calf serum, penicillin (50 IU/ml) and streptomycin sulphate (50 µg/ml). Two specimens of 10 ml of sample each with 5 million cells were separated for controls with and without 100 nM LTR. In the case of LTR application, THP-1 cells were pre-treated with 100 nM Latrunculin A (LTR), a phagocytosis inhibitor, for 60 min before 24 h of exposure to AuNPs. Identical samples were incubated with the three different size NPs each one of them using three different treatments i.e. 10 µM nude AuNPs with or without 60 min pre-treatment with 100 nM LTR. Counting with the controls in total we analysed eight different kinds of sample.

The fixation procedure included glutaraldehyde and OsO₄. Glutaraldehyde was used at a concentration of 2.5%. Cells were incubated for 30 minutes, centrifuged at 9000 rpm for 1 minute and then washed two times with water. OsO₄ was used at 1 mg/ml concentration for 20 minutes and then cells were centrifuged at 7,500 g for 1 minute. Cells were then washed again with water.

After cell fixation, we proceeded to solvent exchange using successive solutions of ethanol in water (25, 50, 75, 90 and 100%). After each solvent exchange cells were incubated for 3 minutes and centrifuged at 7,500 g for 2 minutes. The supernatant was discarded and the pellet was used for subsequent exchange. Following liquid exchange, we performed CO₂ critical-point drying of the remaining pellet, which was placed in a vial with a porous lid. Ethanol was exchanged by liquid CO₂ at 5°C and 50 bar using several rinses with an EMITEC critical point dryer. The pressure was increased in the closed volume, raising it to the critical point of CO₂ by increasing the temperature. The pressure was then relieved slowly, reaching ambient conditions in one hour. As result, we obtained powder that contained the cells. Each of the samples was then transferred into individual grids of 300 µm mesh copper coated with graphite for support.

Electron microscopy and EDAX. A double beam scanning electron microscope FEI-Nova nanolab 600I equipped with FIB and Gas injectors (GIS) that includes a platinum precursor (Pt-GIS) was employed. The system is equipped with traditional secondary and in-lens detectors for traditional and immersion imaging of the scattered electrons, as well as an X-ray analyser used for EDAX with element spectral resolution and sensitivity down to the carbon element. We also used a Scanning Transmission Electron Microscope (STEM) detector for transmitted electrons with 8 channels. This detector allows for bright and dark field detection using an annular dark field configuration (ADF).

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Author contributions

C.P.G. performed the electron microscope experiments and wrote the manuscript. V.S. performed the cell experiments and contributed to the writing of the manuscript. D.G. performed the nano-particle synthesis. I.M. Yasinska contributed in the cell preparation. B.F.G. contributed with the cell preparation and supervision of the manuscript. D.M. contributed in the cell fixation protocol and the writing of the manuscript. L.C. contributed in the cell fixation, particle preparation, writing of the article and the coordination of the experiments. F.R. contributed with the supervision of the experiments and the manuscript.

Additional information

Supplementary information accompanies this paper at <http://www.nature.com/scientificreports>

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