Assessing nanomaterial toxicity with surface-enhanced Raman scattering

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Surface-enhanced Raman scattering can be used as an alternative approach for evaluating the toxicity of nanoparticles in single living cells, bypassing the complications faced by conventional cytotoxicity assays.

Nanomaterials (NMs, i.e., materials that have at least one dimension of less than 100nm) have found application in numerous fields over recent decades. Indeed, ongoing efforts promise the introduction of even more ‘nano’ products onto the market. Concerns about the safety of these materials, however, are growing in parallel. The methods that exist for detecting NM toxicity (i.e., nanotoxicity) are not considered fully compatible with the unique physicochemical properties of NMs (e.g., their altered optical properties and light-absorption capabilities).1 Methods of detection that are not affected by the NM that is to be tested are therefore highly desired.

In terms of current approaches, modifications can be made to conventional toxicity evaluation methods to minimize the false results that are obtained in nanotoxicity studies.1,2 Additionally, data-mining studies are being carried out that attempt to find patterns, related to NM properties, that may help clarify the mechanisms of nanotoxicity.3,4 An additional approach that can quickly and easily collect information from many aspects of the cellular conditions would be very beneficial to the field.

To this end, we have developed a method for evaluating nanotoxicity using surface-enhanced Raman scattering (SERS). SERS is a mode of Raman scattering in which a noble metal surface substrate is used to enhance scattering. The approach is widely used in biomedical research for, e.g., single-molecule detection, tissue classification, cancer staging, and the evaluation of cellular conditions (such as cell cycle and cell death). The technique is label-free and can be carried out with simple sample preparation.5–8

To test the efficacy of SERS for the assessment of NM toxicity,9 we added three types of NMs—zinc oxide NPs (ZnO NPs), titanium dioxide NPs (TiO2 NPs), and single-walled carbon nanotubes (SWCNTs)—to a lung cancer cell line (A549) and a healthy human skin fibroblast cell line (HSF). We introduced these NMs at increasing concentrations: 5, 10, 15, and 20μg/ml for ZnO NPs; 20, 40, 80, and 160μg/ml for TiO2 NPs; and 0.1, 0.25, 0.5, and 1mg/ml for SWCNTs. The cells were seeded on calcium fluoride slides, and after 24 hours, we added gold nanoparticles with diameters of ~50nm, together with the NM to be tested for toxicity, to the incubation medium. The cells were then incubated for a further 24 hours. Prior to SERS measurement, we rinsed the slide in phosphate-buffered saline to remove the cell-culture medium, and added a drop of serum-free culture medium onto the slides to prevent the cells from drying. We then performed measurements on the samples by using a Raman spectrometer equipped with a high-speed encoded stage and an 830nm laser.

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To evaluate the results, we correlated the SERS spectra obtained from these samples with the results from conventional cytotoxicity assays (i.e., WST-1 cell proliferation and apoptosis/necrosis assays). For example, among our results, cell-type-dependent viability fluctuations followed the same trend as the fluctuations of the 1000 cm⁻¹ phenylalanine peak increase. The higher this peak, the less viable the cells. We also visualized the cellular localization of AuNPs and NMs via transmission electron microscopy (TEM) and enhanced dark field microscopy to determine the AuNP distribution, as a good dispersion is crucial for SERS measurements. Figure 1 shows an almost homogeneous distribution of AuNPs in our cell samples.

We preprocessed the spectra (at ~100 spectra per cell) via baseline subtraction, cosmic ray removal, and smoothing. To yield an average spectrum for each test group, we measured and averaged at least 30 single cells to establish statistical reliability. When we compared the average spectra of NM-incubated and non-incubated cells, we observed changes in the peaks related to protein structure and lipid content in both cell lines. These changes can be considered indications of toxic response. An example of this spectral comparison is shown in Figure 2. A549 cells that were incubated with a mixture of AuNPs and 20 µg/ml ZnO NPs show a decrease in the 1130 cm⁻¹ lipid content peak and the 1250-1400 cm⁻¹ protein secondary structure related region compared with the control sample (i.e., A549 cells incubated with only AuNPs).

The A549 and HSF cells that we incubated with increasing concentrations of SWCNTs showed an increase to the 576 cm⁻¹ (i.e., phosphatidylinositol) and the 1061 cm⁻¹ (i.e., ceramide) peaks. It has been shown that endoplasmic reticulum (ER) stress causes ER-mitochondria colocalization, thereby driving the cell into apoptosis by releasing calcium ions. This process is followed by an increase in phosphatidylinositol and ceramide concentrations, which initiates cell-death pathways and certain lipid kinases. We observed this colocalization phenomenon in a TEM image that we obtained of A549 cells that were incubated with AuNPs and 0.25 mg/ml SWCNTs (shown in Figure 3).

In summary, we have proposed SERS as an alternative approach for evaluating NM toxicity in single living cells. Our technique bypasses the complications that are faced when conventional cytotoxicity assays are used for the assessment of NM toxicity. We are currently expanding this work to include various cell types and nanomaterials (e.g., quantum dots and multi-walled carbon nanotubes) to obtain a broader understanding of the applicability of our method.

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References