High throughput genotoxicity assay of nanomaterials with single cell HaloChip assay

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Statement of Purpose: Nanoparticles are used more and more in medical applications as contrast agents and drug delivery vehicles. But, nanoparticles also bring potential toxicity issues to a variety of biological systems such as humans. Nanoparticles enter human body through several ways: they can be taken into body or applied on skin; air-borne nanoparticles can be inhaled involuntarily in lungs; water-borne nanoparticles can enter body through water and food chain; nanoparticles can also be produced during wearing of implanted devices. Once inside body, they will induce immune response, and a range of damage to cells (cytotoxicity) and DNA (genotoxicity). As animal models used to predict the toxicity of nanoparticles (nanotoxicity) are expensive, in vitro toxicity assays are often used for preliminary assessment of nanotoxicity, and data from in vitro studies are extrapolated to predict in vivo responses. However, due to lack of high throughput assay, previous nanotoxicity studies were mostly about cytotoxicity which assess multiple cellular endpoints (membrane integrity, proliferation, metabolic activity and apoptotic cell death), where high doses of nanomaterials are required in many cases to yield observable cytotoxic effects or cell death. It is known that subtle genetic alternations may occur at low nanoparticle dose, which induces reactive oxygen species (ROS) in cells, and cause a variety of DNA damages such as double and single strand breaks, oxidized bases, and DNA-DNA and DNA-protein cross-links. In contrast to small organic molecules whose toxicities are dependent on structures and functional groups, the genotoxicity of nanoparticles depends on their size, shape, composition, surface state, dispersion, and solubility. The large number of parameters increases the number of samples that need to be tested. An ideal assay should have high throughput and be efficient. A variety of in vitro genotoxicity assays can be used to detect chromosomal or clastogenic changes including chromosomal aberrations, micronuclei, sister chromatid exchange and specific base mutation, but the need to accurately predict genotoxicity of nanoparticles at high throughput is largely unmet.

Method: We have developed a high throughput, low-cost single cell array based DNA damage assay (i.e. HaloChip) that can be used to quantify genotoxicity of nanoparticles. This method combines classical halo assay with the state-of-the-art microfabrication technique. A small amount of suspended cells are subjected to nanoparticles for certain time, and patterned onto a solid substrate via electrostatic attraction. After embedding cell array inside an agarose gel, DNA damage will be quantified using fast alkaline halo assay, where DNAs will be stained with fluorescence dye, and damaged DNA fragments will self-diffuse out of cells, forming a halo inside the homogeneous gel matrix. The halo radius is proportional to the amount of damaged DNAs. The symmetrical halo shape will greatly facilitate determination of DNA damage. The technique can also be adapted for the quantification of oxidative base damage, DNA-DNA or DNA-protein cross-linking.

Results: HaloChip assay is used to study genotoxicities of zinc oxide (ZnO) nanoparticles and zinc sulfate-coated cadmium selenide (CdSe/ZnS) nanoparticles with human fibroblast cells. Cells are incubated with nanoparticles (diameter less than 50 nm) for some time and washed to remove excess nanoparticles. Cells are patterned to form single cell array, and embedded in an agarose gel, which provided an inter-connected network for DNA fragments to diffuse. Fast alkaline halo assay is done as follows: the sample is immersed in 0.3 M NaOH for 15 min to remove histone from DNA, and rinsed with water. The substrate is immersed in 5 μg/ml ethidium bromide (EB) solution to stain DNA. After incubation in deionized water for 5 min, a fluorescence microscope is used to image the sample. Fig. 1A showed an image of an array of cells treated with 100 μg/ml ZnO nanoparticles for 2 h and stained with EB, where each nucleus has higher intensity and is surrounded by low intensity diffusive ring. Fig. 1B showed an image of cells that were not treated with ZnO nanoparticles but stained with EB, where DNAs are localized to nucleus as smaller solid circles. DNA damage was quantified using nuclear diffusion factor derived from radii of halo and core. Fig. 1C and 1D show the concentration dependent genotoxicities, where cells are treated with nanoparticles for 2 h at three concentrations (1, 10, and 100 μg/ml). CdSe/ZnS nanoparticles show DNA damage at 10 μg/ml as indicated by a p value significant star sign (*), while ZnO nanoparticles show damage at 100 μg/ml, suggesting CdSe/ZnS nanoparticles is more genotoxic than ZnO nanoparticle. The cytotoxicities of nanoparticles are tested with live/dead assay. In Fig. 1E, ZnO nanoparticles cause little reduction in viability; while CdSe/ZnS nanoparticles show cytotoxicity when concentration is over 100 μg/ml (Fig. 1F), and viability decreases from 100% to 70%. The results show that cytotoxicity assay is not as sensitive as single cell genotoxicity assay: CdSe/ZnS nanoparticles show high genotoxicity at 10 μg/ml; while no cytotoxicity is detected when concentration is lower than 100 μg/ml.

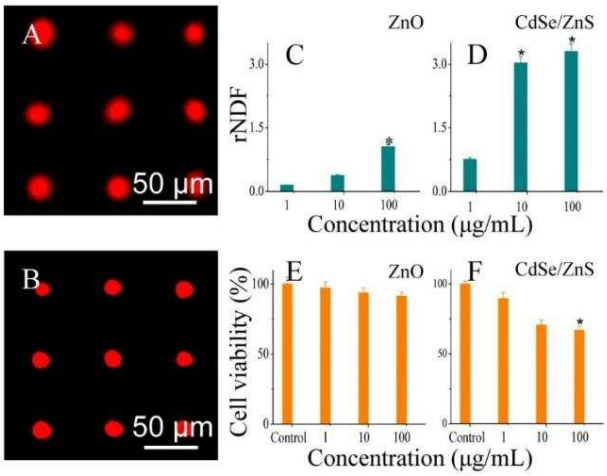

Fig. 1 Genotoxicity and cytotoxicity analysis of metal oxide and quantum dot nanoparticles.