New Tools for Real-Time In Vivo Imaging of Effects of Single Nanoparticles on Embryonic Development

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Scientific Problems & Challenges

Nanoparticles (NPs) possess distinctive physicochemical and surface properties, offering the possibility of being used as optical and photonic probes for imaging and sensing, as carriers for smart drug delivery, and as medicines to treat diseases. Their small sizes enable them to penetrate into living cells and organisms, and their unusually high surface-area-to-volume ratios make them highly unstable, subject to aggregation in suspension, and potentially high chemical reactivity, that could lead to cytotoxicity in living organisms and cause adverse environmental impacts.

The primary questions and challenges are: (i) whether the effects of nanomaterials on living organisms are unique or just like other conventional chemicals. (ii) How can one effectively characterize their biological effects and understand their underlying molecular mechanisms, in order to predict and rationally design biocompatible and eco-friendly nanomaterials? Addressing these primary questions are the first and vital step to achieve large-scale manufacture of desired nanomaterials and to ensure the manufacture safety and to eliminate their potential adverse impact on environments.

Innovations & Significance

We have synthesized and characterized a mini library of stable and purified noble metal NPs (e.g., Ag and Au NPs) with controlled sizes, shapes and surface properties. We have developed single nanoparticle optical microscopy and spectroscopy to characterize the sizes, shapes and optical properties of single NPs using their localized surface plasmon resonance (LSPR) spectra, and developed single nanoparticle optical rulers to measure the size and shape of single plasmonic NPs *in situ* in real time. We have shown that single noble metal NPs resist photodecomposition and blinking, and can be directly imaged and characterized in embryos for an extended period of time.

We have demonstrated that the early developing zebrafish embryos can serve as an *in vivo* assay to study the biocompatibility and toxicity of nanomaterials. We have systematically studied the size, dose, chemical, and development stage dependent effects of NPs upon embryonic development.

Photostable Rainbow Colored Single Nanoparticle Probes

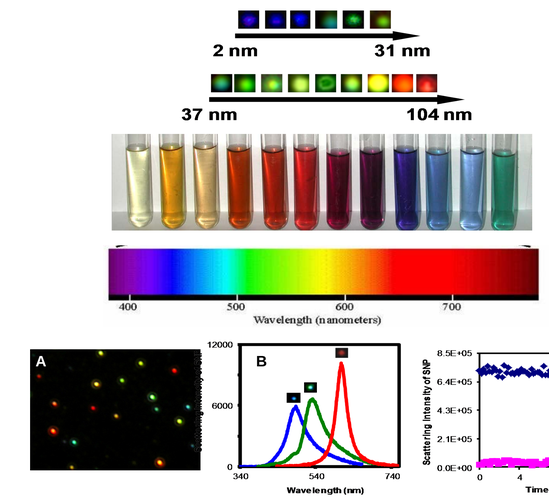

Fig 1: (A) Dark field optical and (B) LSPR spectra of single NPs, and (C) characterization of photostability of single NPs. Scale bar = 2 nm.

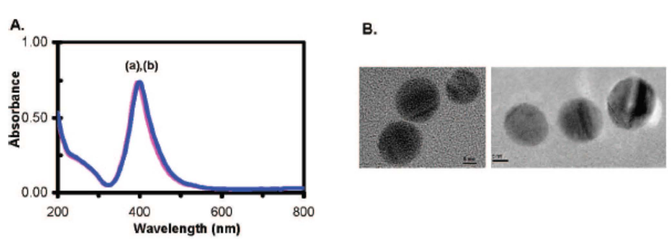

Fig 2: Study of stability of purified single NPs in egg water over 120 h.

Results & Discussion

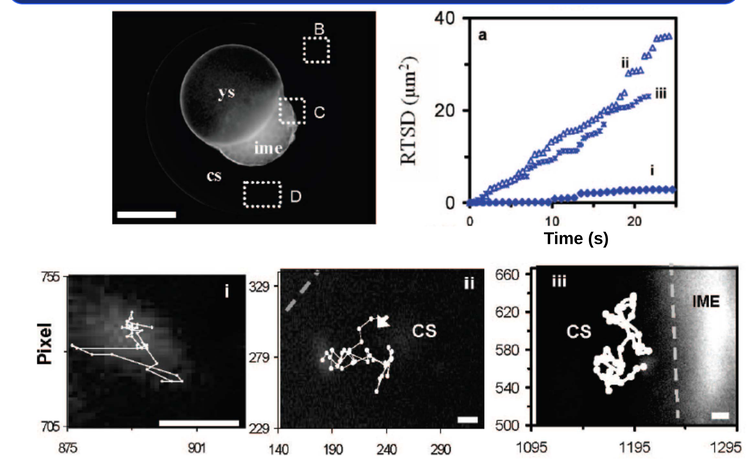

Fig 3: Characterization of transport and diffusion of single Ag NPs in a cleavage-stage (64-cell) living embryo.

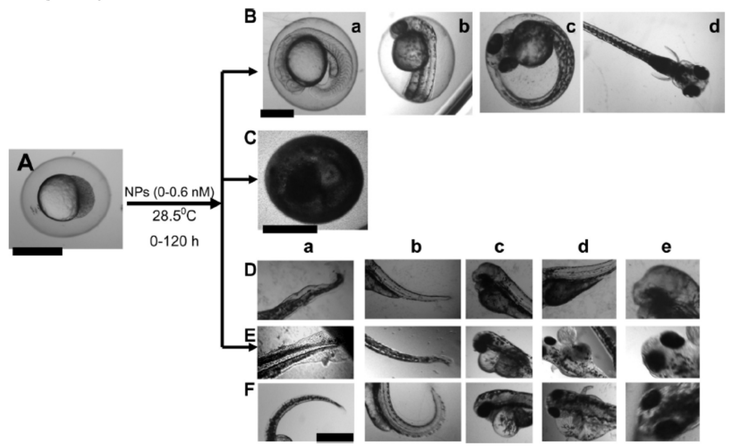

Fig. 4: Study of charge-dependent effects of NPs on embryonic development. Optical images of: (A) Cleavage-stage embryos (0.75−2.25 hpf) incubate with the NPs (0−0.6 nM) over 120 h. (B) Normally developing zebrafish embryos. (C) Dead embryos. (D−F) Deformed zebrafish for (D) Ag-CALNNK NPs+ζ, (E) Ag-CALNNS NPs−2ζ, and (F) Ag-CALNNE NPs−4ζ incubated with the embryos over 120 h show (a) finfold abnormality, (b) tail/spinal cord flexure, (c) cardiac malformation, (d) yolk sac edema, (e) no eye in (D) and small eyes in (E) and (F). Scale bars = 500 μm.

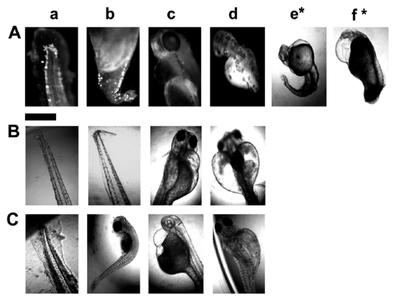

Fig 5: Study of stage-dependent effects of NPs on embryonic development: Optical images of deformed zebrafish observed as (A) stage-I, (B) stage-III, and (C) stage-IV embryos have been incubated with the Ag NPs (13.1 ± 2.5 nm) for 2 h, and developed in egg water over 120 hpf, showing (a) finfold abnormality; (b) tail/spinal cord flexure; (c) cardiac malformation/edema; (d) yolk sac edema, and (e*) and (f*) acephaly (*the severest and rare deformation with no-head, but beating heart). Scale bars are 500 mm.

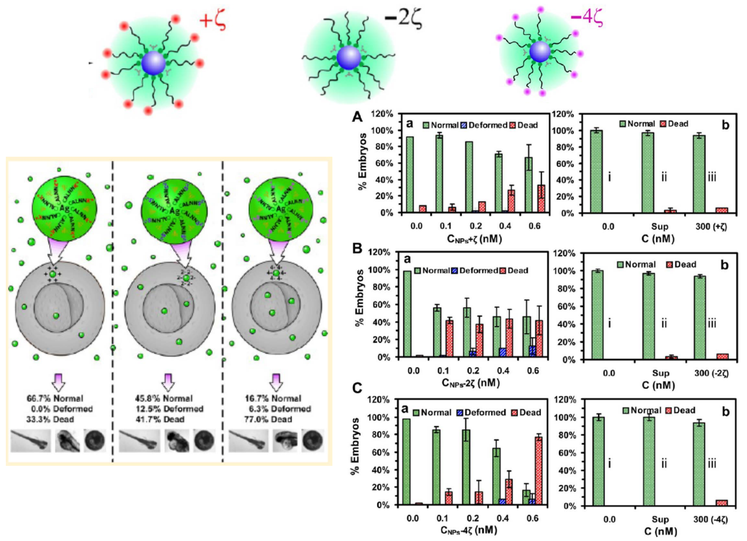

Fig. 6: Study of dose- and charge-dependent toxic effects of Ag-peptide NPs on embryonic developments. Histograms show the percentages of embryos that have been incubated with (a) the NPs: (A) Ag-CALNNK NPs$^{+\zeta}$, (B) Ag-CALNNS NPs$^{-2\zeta}$, and (C) Ag-CALNNE NPs$^{-4\zeta}$; and (b) control experiments (absence of the NPs): (i) egg water, (ii) supernatant (collected from last washing of the NPs), and (iii) 300 nM of the given peptide of (A) CALNNK, (B) CALNNS, or (C) CALNNE, for 120 h, and develop to normal or deformed zebrafish, or become dead.

Summary

In summary, we have designed, synthesized and characterized a mini-library of stable (non-aggregated) and purified NPs, three sized Ag and Au NPs, and functionalized their surfaces with biocompatible peptides to create positively and negatively charged NPs. We have developed single NP dark-field optical microscopy and spectroscopy (DFOMS), single NP optics, and single NP optical rulers for continuous and simultaneous imaging and characterization of multiple single NPs *in vivo* in real time at nm resolution. We also designed early developing zebrafish embryos as *in vivo* assays to study the dependence of transport and effect of NPs on embryonic development. We found single NPs (1-100) with positive and negative charges can passively diffuse into the embryos, and create unique effects on embryonic development that are highly dependent upon size, dose and chemical property of the NPs and embryonic stages. Our studies have offered powerful new tools to study underlying molecular mechanisms of biocompatibility and toxicity of NPs *in vivo* in real time at single NP resolution, and offered new insights into rational design of biocompatible nanomaterials.

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