Quantitative assessment of nanoparticle-induced toxicity in embryonic zebrafish

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STRATEGIES FOR NANOTOXICITY ASSESSMENT

**In vitro studies:** cells and proteins

- Isolated systems
- Information not always transferable to in vivo full organisms

**In vivo studies**

- Complex, expensive, time consuming, ethical problems

**Physicochemical Characterization & Surface Analysis**

**Predictive models**

**Physiology of vivo systems exposed to nano**

- Reduced spatial / temporal resolution (10-20 min)
- Real time measurement of physiological changes is difficult
- Off line methods – Microdialysis – Classical measurements
**In vivo Embryonic Studies**

Zebrafish embryos 3 dpf with clearly defined organs

Zebrafish share the same set of genes as humans; signaling and biological processes of vertebrates

Well characterized development, optical transparency, visualization of the organs + accumulation sites of the nanoparticles

Cytotoxicity and screening: organ defects, early drug discovery,

7 dpf in a 96 well-plate

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Ni nanoparticles: Is the toxic effect size/shape dependent?

Nickel has previously shown to be toxic but the exposure studies have mainly been done with aqueous nickel.

Degree of lethality function of Ni NPs concentration and dimensions


N=25 embryos ± standard error
(n= 3-6)
Nanoparticles & soluble Ni generate different intestinal defects

- Skeletal muscle fibers have become separated at concentrations in the 800 mg/L range for embryos exposed to nanoparticles
- No intestinal defect with soluble nickel

*Ispas C. et al., Env. Sci. & Technol., 2009.*
NPs in contact with tissues can induce an inflammatory / oxidative response in situ.

Effect of NPs can vary with the composition, size, shape and exposure time of the material.

Effect of NPs – low concentration exposure:
- Mortality and developmental abnormalities, morphological malformations, behavioral abnormalities.

Mechanism of cytotoxicity: inflammatory response, release of reactive oxygen species:
- Determine markers for inflammation and oxidative stress at the accumulation site – use implantable microelectrodes and perform electrochemical measurements at organ levels.
IN VIVO ELECTROCHEMICAL STUDIES AT ORGAN LEVELS AT THE NANOPARTICLE ACCUMULATION SITE

- Tissue response at the accumulation site
- Dissolution
- Study of inflammation
- Oxidative stress
- Neurological damage

In vivo – implantable
High spatial/temporal resolution at precise locations – particular organs
High sensitivity
Study biochemical events for low concentration exposure
MICROELECTRODES

Boron-doped diamond wire  Carbon Fiber - 5 μm  Pt wire – 50 μm


• Sensor response dependent on sensor and sample size
• Diffusion characteristics, chemical / biochemical reactions vary between macroscopic and ultramicroscopic devices
ELECTROCHEMICAL ASSESSMENT OF NANOPARTICLE INDUCED TOXICITY

1. In vivo assessment of intestinal serotonin neurotransmission in embryonic zebrafish - Electrochemical measurements in live embryos

2. Oxidative stress and inflammation – assessment of NO and superoxide

3. Predictive tools for assessing surface reactivity in vitro

Ni/NiO / CeO2 / CuO NPs 1-100 ppm
VIABILITY OF NPS ON EMBRYOS

A

Percent Surviving Embryos

[CeO₂ NPs]

1 ppm 5 ppm 10 ppm 20 ppm 50 ppm 100 ppm

Day 1 Day 2 Day 3 Day 4 Day 5

B

Percent Surviving Embryos

[CuO NPs]

1 ppm 5 ppm 10 ppm 20 ppm 50 ppm 100 ppm

Day 1 Day 2 Day 3 Day 4 Day 5

1. MEASUREMENT OF INTESTINAL 5-HT IN ZEBRAFISH EMBRYOS

- 5-HT is associated with regulation of mood, some cognitive functions including memory and learning, sleep, appetite and muscle contraction
- Abnormal levels have been implicated in diseases such as Celiac and irritable bowel syndrome

**In vivo** $[5$-HT$] = 30.8 (±3.4)nM.

EFFECT OF NP (50 PPM) EXPOSURE ON 5-HT

FLUORESCENCE VS. ELECTROCHEMISTRY

Effects are concentration dependent

Electrochemical detection of 5-HT in the presence of xeno-material provides quantitative information on their neurotoxic properties.


2. INTESTINAL NO AS A MARKER OF NP-INDUCED OXIDATIVE STRESS AND INFLAMMATION

Cross-validation of NO sensor has been done by fluorescence microscopy utilizing DAF-DA-FM, a specific probe for nitric oxide.

$[\text{NO}]_{\text{WildType}} = 0.79 \pm 0.15 \text{ µM}$

Exposure to NPs induced changes in physiological NO – detected electrochemically within the intestine of live embryonic zebrafish. CuO NPs were found to trigger inflammatory response even at low-level exposure. Ceria NPs affected the levels of NO in a dose-dependent manner:

- Low concentrations: act as inorganic antioxidant
- High concentrations: triggers inflammatory response

Real time monitoring of superoxide and NO radicals in a brain slice model using an electrochemical superoxide microsensor

Effect of ceria on $O_2^{•−}$ in Control Slice

Real time monitoring of superoxide
Effect of ceria on $\text{O}_2^-\text{in ischemic brain}$

3. ELECTROCHEMISTRY AS PREDICTIVE TOOLS OF OXIDATIVE RESPONSE

- Evaluate redox induced changes of ceria nanoparticles associated with oxidative damage by nanoparticle collision at microelectrodes
- Screening approach to assess surface reactivity and ability of NPs to generate or inactivate free radicals (oxidant / prooxidant)

Conclusion

Micro-electrochemical devices - useful tools for studying mechanisms of NPs induced toxicity

✓ neurotoxicity
✓ oxidative stress, ROS, O2-, NO
✓ predictive tool of particle reactivity

Potentially the results could be applied to humans and serve as a general guideline for assessing exposure risks and benefits (nanomedicine) of NPs
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