Evaluation and Comparison of ZnO & TiO2 Nanoparticles Cytotoxicity in Human Cells

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INTRODUCTION

- Nanotechnology involves the science of precise manipulation and control of atomic or molecular structure of materials at nanoscale level to manufacture nanomaterials with unique properties and applications.
Nanoparticles

- Nanoparticles are defined as primary particles with at least one dimension < 100 nm.
Potential Impacts

- It is expected that the rapid expansion of nanotechnology will bring many potential benefits.
- The impacts of nanotechnology will go beyond the industrial revolution estimated to become a one trillion dollar market by 2015.
Nanoparticles Classifications

- Based on their source of production, nanomaterials can be classified in two major categories including:
  - Unintentionally produced ultrafine particles e.g., CDNP or DEP, and
  - Intentionally manufactured nanoparticles e.g., ZnO or TiO$_2$ nanoparticles.
Potential Applications

- Engineered nanomaterials, with their unique physicochemical properties, are rapidly being implemented in the field of medicine, pharmaceutics, biotechnology, energy production, environmental sciences, transportation, housing and electronics.

- Nanoparticles have already been implemented in treatment and drug delivery systems, pharmaceutics, sunscreens, cosmetics, self-cleaning surfaces, disinfectants, and other products.
At the present time the most commercially important class of engineered nanoparticles are the metal oxide nanopowders which include those of zinc (ZnO), titanium (TiO2), silicon (SiO2), aluminium (Al2O3) and iron (Fe3O4 or Fe2O3).

It has been estimated that there are over 400 sunscreen and cosmetic products containing TiO2 or ZnO nanoparticles in the Australian marketplace alone. Despite their conventional powders, ZnO and TiO2 nanoparticles are transparent while retaining the ability to block the UV-A and UV-B radiation.
ZnO & TiO2 Particles & NPs

- ZnO is a mineral, which provides broad-spectrum protection against UVA and UVB rays. ZnO has shown to have anti-inflammatory properties and has been considered one of the safest and most protective sunscreens available (Roselli et al., 2003).

- Similar to ZnO, TiO2 is one of the most effective UV blockers to protect human skin against UV radiation (Popov et al., 2005). TiO2 is commonly used as a whitener in various cosmetics e.g. hand cream, face powders, shampoos, lipsticks and toothpastes (Farrow, 2002).
Although nanomaterials are currently being widely used in modern technology, the possible adverse effects and environmental implications of these nanoscale materials are unknown.

While they may introduce many potential benefits, special emphasis should be placed for safety evaluation of these newly developed nanomaterial products which may differ significantly from their original bulk material.

Therefore, a new field of nanotoxicology has been developed to investigate the possible adverse effects of nanomaterial exposure.
Available toxicity studies have demonstrated that the toxicity of nanoparticles is significantly associated with the unique physicochemical properties of these nanomaterials:

- Very small size distribution
- Large surface area/mass ratio
- Surface chemistry and characteristics
- Insolubility or low water solubility
- Aggregation
# Potential Toxic Effects

Nanoscale properties, that formulate nanomaterials, behave differently and are likely to affect not only its chemistry and physics but also its behavior in biological systems.

<table>
<thead>
<tr>
<th>Nanomaterial Characteristics</th>
<th>Possible Biological Effects</th>
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</table>
| Small size distribution (less than 100 nm) | Crossing tissue and cell membranes  
Cellular injury  
Phagocytosis impairment, breakdown in defence mechanisms  
Migration to other organs  
Transportation of other environmental pollutants |
| Large surface area/mass ratio         | Increased reactivity  
Increased toxicity |
| Surface characteristics               | generation  
Oxidative stress  
Inflammation  
Cytokine production  
Glutathione depletion  
Mitochondrial exhaustion  
Cellular injury  
Protein and damage |
| Insolubility or low water solubility  | Bioaccumulation inside living systems such as human cells, tissues and lungs  
Potential long-term effects |
| Aggregation                           | Interruption of cellular processes  
Cellular injury |

AIM & OBJECTIVES

Comparative Toxicity Assessment of Selected Micro & NPs *In Vitro*

- Characterization of size distribution of selected particles
- Cytotoxicity assessment of ZnO microparticles
- Cytotoxicity assessment of ZnO nanoparticles
- Cytotoxicity assessment of TiO2 microparticles
- Cytotoxicity assessment of TiO2 microparticles
- Comparative toxicity assessment of selected particles and NPs
MATERIALS & METHODS
Test Materials

- TiO2 Particles & NPs; Sigma, Australia
- ZnO Particles & NPs; Sigma, Australia
Analytical Techniques

- **TEM**
  (Transmission Electronic Microscopy)
- **SEM**
  (Scanning Electronic Microscopy)
- **ICP-AES**
  (Inductively Coupled Plasma-Atomic Emission Microscopy)
Application of cell culture techniques in toxicological studies is referred to as *in vitro* toxicology which describes a field of study that applies technology using isolated organs, tissues and cell culture to study the toxic effects of chemicals.

- Basic cell culture techniques are designed to provide a proper support of cells or tissues for their normal survival, growth and function in a vitreous media outside the body.
Human Cell Types

- Skin Fibroblasts
- A549 Lung Cells

Culture Conditions:

All cells were cultured in sterile, vented cell culture flasks with Dulbecco’s modified eagle medium/ Ham’s F12 nutrient mixture (DMEM/F12) supplemented with fetal calf serum (FCS 5%) and L-glutamine (2 mM), penicillin (100 U/ml), streptomycin (0.1 mg/ml) solution. Cultured cells were kept at 37°C in a humidified 5% CO₂ incubator.
The cytotoxic effectes of selected micro & nanoparticles investigated on human target cells using relevant toxicity endpoints:

**MTS (tetrazolium salt, Promega)**
NRU (neutral red uptake, Sigma)
ATP (adenosine triphosphate, Promega)
RESULTS

In Vitro Dose-Response Relationship

\[ y = 100.03e^{-0.0168x} \]

\[ R^2 = 0.9989 \]

Cell Viability %

Concentration (mg/L)

NOAEC

IC50

TLC
Particle Morphology

TEM Images of Selected micro & nanoparticles

ZnO nano-scale
ZnO micro-scale
TiO$_2$ nano-scale
TiO$_2$ micro-scale
Particle Characterisation

Particle size distribution of ZnO and TiO2 particles & NPs determined by TEM (n= 70)

<table>
<thead>
<tr>
<th>Type of particles (nm)</th>
<th>Size and distribution (nm)</th>
</tr>
</thead>
<tbody>
<tr>
<td>ZnO nanoparticles</td>
<td>45 ± 22</td>
</tr>
<tr>
<td>ZnO microparticles</td>
<td>95 ± 35</td>
</tr>
<tr>
<td>TiO2 nanoparticles</td>
<td>63 ± 35</td>
</tr>
<tr>
<td>TiO2 microparticles</td>
<td>132 ± 47</td>
</tr>
</tbody>
</table>

Data presented as mean ± SD
Comparison of the Cytotoxicity of Micro & Nano- Scale Particles on Human A549 Cells

The dose-dependent effects of micro- and nano- zinc oxide and titanium dioxide on human A549 cells using the MTS assay after 24 hours exposure
Comparison of the Cytotoxicity of Micro & Nano- Scale Particles on Human Skin Fibroblasts

The dose-dependent effects of micro- and nano- zinc oxide (a) and titanium dioxide (b) on human skin fibroblasts using the MTS assay after 24 hours exposure
Comparison of the Cytotoxicity of Micro & NPs of ZnO

<table>
<thead>
<tr>
<th>Toxicity Value (ppm; M ± SD)</th>
<th>Zinc Oxide (ZnO)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Nano-sized</td>
</tr>
<tr>
<td>NOAEC</td>
<td>1.12 ± 0.45</td>
</tr>
<tr>
<td>IC&lt;sub&gt;50&lt;/sub&gt;</td>
<td>49.56 ± 12.88</td>
</tr>
<tr>
<td>TLC</td>
<td>298.17 ± 67.35</td>
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Comparison of the Cytotoxicity of Micro & NPs of TiO2

<table>
<thead>
<tr>
<th>Toxicity Value (ppm; M ± SD)</th>
<th>Titanium Dioxide (TiO₂)</th>
<th></th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Nano-sized</td>
<td>Micro-sized</td>
</tr>
<tr>
<td>NOAEC</td>
<td>80.38 ± 13.48</td>
<td>103.52 ± 31.04</td>
</tr>
<tr>
<td>IC₅₀</td>
<td>2,695.57 ± 666.98</td>
<td>N/A</td>
</tr>
<tr>
<td>TLC</td>
<td>N/A</td>
<td>N/A</td>
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## Comparison of the Cytotoxicity of Micro & NPs Of ZnO & TiO2

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<th>Titanium Dioxide (TiO₂)</th>
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<tr>
<td></td>
<td>Nano-sized</td>
<td>Micro-sized</td>
</tr>
<tr>
<td><strong>NOAEC</strong></td>
<td>1.12 ± 0.45</td>
<td>7.25 ± 2.26</td>
</tr>
<tr>
<td><strong>IC₅₀</strong></td>
<td>49.56 ± 12.88</td>
<td>339.94 ± 24.87</td>
</tr>
<tr>
<td><strong>TLC</strong></td>
<td>298.17 ± 67.35</td>
<td>2,085.02 ± 31.65</td>
</tr>
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<td><strong>NOAEC</strong></td>
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</table>
ZnO appeared to be more toxic than TiO₂ NPs on both human skin fibroblasts and A549 lung cells.

Both ZnO and TiO₂ nanoparticles have shown higher toxicities than their normal-sized particles.

The obtained results confirmed that the toxicity of nanoparticles is significantly associated with the unique physicochemical properties of nanomaterials.
Further Directions

- Generate standardized, validated multiple *in vitro* assay systems for toxicity testing of NPs
- Establish *ex vivo* models relevant for specific routes of exposure; dermal,..
- Employ and validate direct exposure techniques at the air-liquid interface relevant for inhalational NPs exposures
- Develop an understanding of ADME knowledge of nanomaterials *in vivo* predicting the interactions of NPs with living systems
CONCLUSION

- Meanwhile, based on the obtained results of this *in vitro* toxicological research and previous knowledge of particle toxicology, tight precautions are required to prevent the possible adverse effects of nanomaterial exposure and provide Safe & Sustainable Nanotechnology.
Publications


Thank you