From IBR to GISS - Developing and Commercialising In Vitro Tools for Nanomaterial Safety and Efficacy Testing

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Introduction

- Titanium dioxide and zinc oxide are the major constituents of many sunscreens
- Some years ago, there was controversy about the use of nanomaterials in consumer products
- Our research was directed at some of these nanoparticle issues
- The expertise developed during this project was used quite differently in industry
- This presentation follows the journey from nanosafety and into the commercial world

Titania Photoactivity and Paint

- “BlueScope Steel” study
- Anecdotal evidence of titania damage to paint
- Similar was also seen for ZnO
- Escalation of public concerns

Public Sensitivity to Nanoparticles

- Teachers restrict sunscreens over safety concerns
  - Sunscreen and nanoparticles
  - Escalation of public concerns
The Nanoparticle Research Project

- Project involved 2 industry partners, 2 Universities and government support
- Timeframe was 4 years (2009-2013)
- Budget was approximately $2.4M
- We employed 1 research fellow, 2 post-docs and trained 12 PhD students
- We examined ZnO and TiO$_2$ on both human immune and skin cells

What we Looked at

- In vitro human cell culture systems:
  - Human immune cells (THP-1 cell line and primary cells)
  - Human skin cells (HaCaT cell line and primary cells)
- Major endpoints were:
  - Relative Cytotoxicity, based on particle size and composition
  - Immune modulation by cytokine expression
  - Oxidative stress using fluorometric markers
  - Particle chemistry and dissolution kinetics

Cytotoxicity of ZnO Nanoparticles

ZnO NP cytotoxicities generally increased with smaller NP size

Cytotoxicity of Organic UV Blockers

Organic cytotoxicities were similar to ZnO nanoparticles
**Cytotoxicity of ZnO NPs Relative to Surface Area**

NP cytotoxicity per unit surface area was less than expected.

**ZnCl₂ Nanoparticles in Biological Media**

ZnCl₂ forms 20-60nm particles when exposed to HCO₃⁻ and HPO₄²⁻.

**Cytokine Profile Response of ZnO Nanoparticles**

ZnO NPs and ZnCl₂ show non-specific pro-inflammatory responses.

**Focused Ion Beam Ablation**

Pre-ablated THP-1 Macrophage

900 nm of cellular material removed (3 ablation cycles).
Intracellular Elemental Mapping via Synchrotron XFM

Subtraction of ablative layers allows mapping of the contents of the removed layer.

Human Immune Cells Exposed to UVA and nanoparticles

![Graph showing ROS from UVA and ROS from UVA and treatment for different treatments: No Treatment, Zinc Oxide, Titania (rutile), Titania (anatase), Peroxide solution.]

Some Perspective on Reactive Oxygen

Whitening toothpaste

Hair dyes

Our Key Findings

- ZnO nanoparticles show similar cytotoxicities to standard organic sunscreen active components.
- ZnO inflammatory profiles suggest some enhancement at the nanoscale, though these responses are similar or less than surface area would suggest.
- TiO₂ is an extremely inert material, that is generally unreactive, except for the anatase form, which generates substantial ROS in sunlight.
What does the OECD Think?

- "...that the use of TiO$_2$ nanomaterials with the characteristics as indicated below, at a concentration up to 25% as a UV-filter in sunscreens, can be considered to not pose any risk of adverse effects in humans after application on healthy, intact or sunburnt skin."*
  - Are not contaminated with impurities
  - Are mainly rutile or up to 5% anatase
  - Are 30-100 nm in primary particle size
  - Have an aspect ratio of 1.0 – 4.5
  - Have a coating that does not effect the properties of the material

* SCCS Opinion on Titanium Dioxide (nano form), 22 July 2013

The Potential Perils of Nanomaterials

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- Are mainly rutile or up to 5% anatase
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The Industry Perspective

- Legislation changes altered requirements for broad-spectrum sunscreens
- At the new compliance levels, ZnO is challenging to formulate at SPF50+
- TiO$_2$ is still widely used, though the structural form is not prescribed
- Was it possible to use what we had learned from the research projects to build a testing or ranking system based upon *in vitro* and UV screening?

The Immune Balance Rating

- The IBR™ is a testing system designed to value-add to existing and new products
  - It ranks similar products on a 5-star scale, relative to a reference standard of the same product class
  - It provides a tool for manufactures to compare products after changes or additions
  - It empowers consumers by presenting evidence-based claims about the likelihood of a product to be reactive on the skin
  - It is designed to be rapid and cost effective
The Immune Balance Rating (IBR)

- A testing regime designed to compare fully formulated products
  - Tests for bioactivity
  - Uses skin and immune cells
  - Tests both UVA and UVB
  - Can rank very similar products

How We Test

- The IBR™ is an *in vitro*, biomarker based testing system
  - The product is first emulsified into an aqueous solution
  - This solution is applied in graded concentrations to:
    - Immune cells with/without UVA
    - Skin cells with/without full-spectrum UV
  - Exposures are allowed to run for 24 hours
  - 5 Biomarker endpoints are then measured

What we Measure

- The biomarker endpoints were chosen for applicability and responsiveness
  - Cell viability is used to determine product cytotoxicity
  - Interleukin-8 measures inflammatory response in immune cells
  - Interleukin-6 measures UV stress response in skin cells
  - Reactive oxygen production measures stress in immune cells

The IBR™ Tests

- IBR1: Immunostimulation
  - The capacity of a product to cause cellular inflammation
- IBR2: Immunosuppression
  - The capacity of a product to reduce an inflammatory response
- IBR3: Phototoxicity
  - The capacity of a product to compound or reduce solar damage
- IBR4: Protection from UV
  - The capacity of a product to protect from sunlight-induced inflammation
- IBR5: Protection from UVA
  - The capacity of a product to protect immune cells from UVA induced damage
### Aggregated Scoring of Standard Formulation vs Low Active Sunscreens

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### IBR™ Reports

#### What we’ve done so far…

Visitors Prizes!

Major Customers:

- **ego**
- **Cancer Council Australia**

#### Expanding the IBR into Food

- It is possible to apply the IBR development methodology to the food industry
- Immune and structural cells and micro-organisms are all major contributors to food intolerance
- Elevation in inflammatory signaling or growth-rates (for bacteria) are potential markers of irritancy
- **We can measure potential cellular irritancy this way, but it is not a measure of potentially allergy**
The Gut Inflammatory Scoring System (GISS)

- A 5-7 parameter scoring method for assessing potential food irritancy
- The tests involve *in vitro* cell culture (no animal testing)
  - Human immune cells
  - Human gut epithelial cells
  - Human gut flora
- Test parameters include
  - Cytotoxicity and growth
  - Inflammatory responses
- The tests should be able to discriminate between similar products of the same type
- In the industrial setting the tests should be rapid and cost-effective

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For more information contact Dr Bryce Feltis at Baxter Laboratories (bfeltis@baxterlaboratories.com)

Thank You!