Comparative toxicological analysis of quantum dots and wires on human skin tissue

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Semiconductor quantum dots (QD) and wires are of growing technological relevance with applications in photoelectronics, electronic integrated circuits, radiation tolerant solar cells, and biological labeling. We used an in vitro reconstructed human skin tissue equivalent, typically referred to as 3-D tissue culture or organotypic raft cultures, closely representing the complexity and structural integrity of human skin tissue system. In this study we performed a comparative analysis of different sized QDs and quantum wires, these quantum dots contain a cadmium/selenide core with a cadmium sulfide shell coated with polyethylene glycol (PEG) and are soluble in water. The sizes of these nanoparticles are 3.4 ± 0.2 nm, 5 nm small dots, 16 ± 5 nm large dots and wires that are 10 nm in diameter and > 1 micron in length. In this study, QDs were topically applied to skin tissue at concentrations of 500 nM to 10 µM to assess penetration, cellular viability, cytotoxicity and inflammatory responses. We will also present data showing a possible QD-induced inflammatory response by the exposed tissue. These results implicate that the size and shape properties of QDs impact the ability of these nanomaterials to penetrate skin tissue and influence cytotoxic and inflammatory responses.

***Keywords* — Quantum dots, cell death, toxicology, skin tissue**

I. Overview

Engineered QDs have unique physicochemical properties resulting from a combination of their crystalline metalloid core structure/composition and quantum-size confinement. They are comprised of a metalloid crystalline core (Cd, Zn, In) and a "shell", which can then be further functionalized for a specific application. The unique physical and chemical properties of QDs impact their ability to interact with biological systems. Though several toxicological studies have attributed undesirable toxicity responses to QDs there has been little effort to correlate the physicochemical characteristics of a QD to a biological response. QD-induced toxicity may be potentially due to the metallic core, the shell or the functionalization that in turn influence its physicochemical properties (size, charge, photolysis, oxidation). Ongoing efforts at LANL are focused on delineating and understanding the properties-dependent biological response of engineered nanomaterials. Utilizing an in vitro 3-dimensional organotypic human skin tissue we propose to better understand the bioimpact of QDs on dermal exposure. Reconstructed human tissue equivalents can serve as highly-relevant bio-assessment platforms for the risk assessment of a multitude of new ENMs. Such a platform integrates the reliable, rapid and high-throughput methodology of *in vitro* cell based assays with the accuracy and relevance of *in vivo* platforms making them excellent surrogate models. Preliminary results indicate that the physicochemical properties of semiconductor naomaterials are pivotal in their ability to interact with

II. Methods

Nanocrystal Quantum Dots (NQDs) characterization: Broadband absorption, Narrowband emission (photoluminescence), Efficient absorption , Efficient emission , Tunable optical properties, Photostability, and Chemical reactivity

In vitro 3-D human skin tissue constructs: the immortalized human skin keratinocyte cell line HaCaT and 3T3 fibroblast cells were used to develop the skin tissue construct. Keratinocytes grow at the air-liquid interafce on a fibroblast containing collagen gel. The filter inserts are in contact with the culture medium.

Apoptosis assay: Apoptotic cell death was analyzed by AO/EB cellular staining. The cells were exposed to caged-C_{60} and carboxyfullerenes for 24 hours. Camptothecin (CAM, 1µM) was used as a positive control for apoptosis induction.

Necrotic cell death analysis: The amount of lactate dehydrogenase (LDH) released from damaged cells to medium was used to evaluate the necrotic dell death.

MTT assay: Using MTT assay to evaluate the variability of Organotypic keratinocyte-fibroblast coculture treatd with fullerene derivatives. Skin tissues were treated for 24 hrs with 3nm,16nm and wires, respectively. MTT reagents were loaded on the culture for 3hrs. The cell variability was quantified by colormetric assay.

AFM: Samples were prepared for AFM by aliquoting a small amount of QDs on freshly cleaved mica. Samples were allowed to air dry before imaging. AFM was performed using a PicoPlus II SPM (Molecular Imaging) in MAC mode using type II MAC cantilevers with typical resonance frequencies of 70–80 kHz. Scan rates were 2 Hz. Images were analyzed using Gwyddion software (public domain).

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