***Task ID:*** **425.024**

***Project title*:**
Environmental Safety and Health (ESH) Impacts of Emerging Nanoparticles and Byproducts from Semiconductor Manufacturing - Toxicity Assessment and Prediction

***Deliverable:***
Report on toxicity tests for Phase I-nanoparticles using skin organ tests and differentiated lung tissue culture tests

***Project PIs:***

Jim A Field, Farhang Shadman, Reyes Sierra-Alvarez
Dept. Chemical & Environmental Engineering, University of Arizona.

Scott Boitano
Arizona Health Sciences Center, University of Arizona

Buddy Ratner
University of Washington Engineered Biomaterials (UWEB) Center, University of Washington

***Task PIs:***

Scott Boitano
Arizona Health Sciences Center, University of Arizona

Buddy Ratner
University of Washington Engineered Biomaterials (UWEB) Center, Univ. of Washington (UW)

***Students and Postdocs:***

Cara Sherwood, University of Arizona
Mia McCorkel, University of Arizona
Rosa Daneshvar, University of Washington (UW)

***Objective and key findings:***

The goal of this project is to evaluate the potential toxicity of nano-materials from semiconductor manufacturing and it will elucidate the underlying toxicity mechanisms. The impact of surface contaminants and other physicochemical properties on nanoparticle toxicity will be assessed. This research will also develop new methodologies for testing and predicting ESH impacts of nanoparticles.

The objective of this task is to develop advanced organ and tissue cultures for nanotoxicity testing. Validation of toxicity testing, assessment and predictions will be carried out

with real skin using a human foreskin rafted organ culture model developed by UW team. Similar validation will be used on (human) bronchial epithelial cultures that will be developed by Prof. Boitano at the University of Arizona. Both of these cultures allow for examination of NP exposure in multicellular models that better mimic the *in vivo* environment.

*Development of skin organ culture model for testing nanotoxicity:* Newborn foreskin will be used in suspended organ culture to investigate the toxicity of $CeO_2$ nanoparticles (NP) for skin cells. A cut will be made in specimen with scalpel and a known amount of NP will be introduced to the wound. The effect of NP on skin cells will be investigated. Surface properties, size, morphology, charge of different batches of $CeO_2$ NPs will be investigated to find the connection between these properties and toxic effect of NPs.

The challenging part of this project has been to determine the methods to detect the impact of particles on cells. This challenge rises from the fact that, contrary to the versatile and rich cell culture toxicity assays in the literature, organ culture toxicity techniques are not as fully developed. This progress report describes the work completed and work in progress for new batches of 10% $CeO_2$ solution in water (< 25nm) received from the University of Arizona.

*Development of lung cell model for testing nanotoxicity*. Human and mouse primary cultures of lung epithelial cells grown at an air liquid interface that establish a functional airway epithelial barrier in our laboratory have been recently established. These models will be used to test for breakdown of the barrier function (loss of transepithelial resistance) in response to NP exposure. The use of mouse cells provides some significant advantages. Mouse epithelial cells are easier to obtain than human tissue and they grow and differentiate at a faster rate, reducing experimentation time. Similar to the human cells, mouse cells differentiate, express tight junction proteins, and establish epithelial resistance.

***Technical Results and Data:***

**A) Nanoparticle toxicity in a skin organ culture model**

Rosa Daneshvar and Prof. Buddy Ratner, University of Washington

***Physical properties of NPs:*** As the specific properties of NP that may cause the toxicity of these particles are unknown, fully investigating the physical and chemical properties of NP is essential in determining the specific NP properties that might cause toxicity. Size, morphology, surface properties and surface charge of the particles are the desired properties to be investigated.

The particle size and morphology of $CeO_2$ was investigated by scanning electron microscopy (SEM). SEM results showed that particles in solution at the as received concentration exhibited high degrees of agglomeration. The agglomeration pattern in SEM micrographs might be caused by unenclosed ingredient or by a contaminant. Concentration dependency of agglomeration will be further investigated by SEM.

Information about the major chemical elements present in 10nm outermost layer of NP surface was obtained by X-ray photoelectron spectroscopy (XPS). XPS results showed that C, O, Ce were the elements present on the surface of NPs at 24.43%, 58.85% and 19.72% respectively. No other elements were detected. Further surface analysis is required to determine the chemical structures present on the surface of NPs. This information will be obtained for the 2 nm outermost layer of NP surface by time of flight secondary ion mass spectrometry (ToF-SIMS).

To further investigate the physical properties of NP, transmission electron microscopy (TEM) will be conducted to reveal the size and morphology of particles. As TEM and SEM give information about dried state of NPs, information about the size of particles in solution is also required. This information will be acquired by dynamic light scattering (DLS). The effect of surface charge on penetration and localization of particles into cells has been shown in literature.[1-3] Surface charge of NP will be determined with zeta potential measurements.

***Organ culture model:*** Connection has been established to obtain newborn foreskin from Infant and Maternal Care Unit at University of Washington. Culture medium has been selected as used previously by Fukano *et. al.*[4]. Incubation conditions have been selected from work done by Zeltinger et. al. on incubation conditions for suspension organ cultures. [5]

Initial experiments with the organ culture toxicity technique suggested that the most promising tool to examine NP effects is transmission electron microscopy (TEM). The goal of using this technique is to detect the morphological changes in NP-exposed cells as well as detection of NP themselves in the specimen. As TEM is not as commonly used for this purpose as in other applications, the required knowledge and expertise is not as readily available. At this stage of the project, we are gaining the required training and skills to conduct this experiment.

## B) Development of lung cell models for testing nanotoxicity

Prof. Scott Boitano, The University of Arizona

For initial studies we have used lung (16HBE14o-) and skin (HaCat) cell lines to evaluate nanotoxicity. These lines provide a large amount of cells that maintain many characteristic of the native epithelium. In order to better model the native epithelium and we have begun to foster primary cell culture models with native functions for testing toxicity. To this end we have recently established both human and mouse primary cultures of lung epithelial cells grown at an air liquid interface that establish a functional airway epithelial barrier in our laboratory (Figs. 1-3). Human or mouse cells are placed in special culture medium, allowed to come to confluence in submerged medium, then switched to an air/liquid interface after several days in culture (Fig. 1A). Cells will differentiate into the many cell types and take on characteristics similar to that seen in vivo (Fig. 1B). We have tested these tissues for the establishment of an epithelial barrier, a likely first target for nanotoxicity (Figs. 2A, B). The human cells show a transepithelial resistance (TER), a functional indication of a healthy epithelium, and tight junction proteins that line the epithelial cells, a structural component of a healthy epithelium. We will use these models to test for breakdown of the barrier function (loss of TER) in response to NP exposure. Because human tissue is difficult to obtain, and because it is inherently slow in growth and differentiation, we have also developed a mouse model of differentiated primary epithelial cells

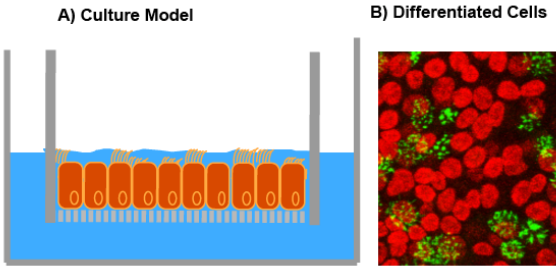


**Fig. 1**. A) Looking sideways at the culture, cells are grown in a chamber that allows for an air/liquid interface. Blue area depicts growth media. B) Cells can fully differentiate: Looking down on the culture, green stain shows ciliated airway epithelial cells and red show nuclei. Note not all cells are ciliated. as observed in vivo.

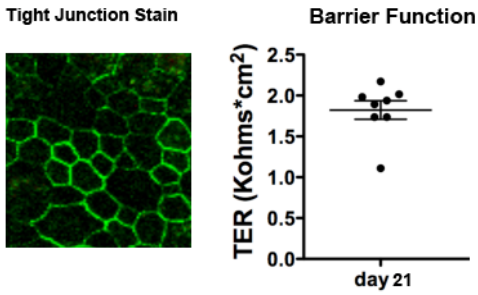


**Fig. 2**. A) Looking down on the culture, cells are stained for occludin, a tight junction protein. Note that the cell borders are well defined. B) Transepithelial resistance of >1000 $\Omega*cm^2$ is indicative of a functional epithelium.

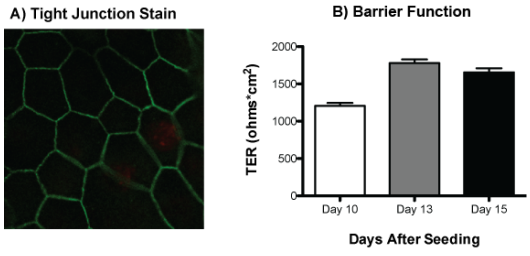


**Fig. 3**. A) Looking down on the culture, mouse cells are stained for occludin, a tight junction protein. Note that the cell borders are well defined. B) Transepithelial resistance of >1500 $\Omega*cm^2$ is indicative of a functional epithelium.

(Figs. 3A, 3B). Similar to the human cells, mouse cells differentiate (not shown), express tight junction proteins (Fig. 3A) and establish epithelial resistance (Fig. 3B). However, they take approximately ½ the time to reach a functional state in culture and are much easier to obtain.