Cell-based Toxicity Assay-on-Chip for the Next-Generation CMOS Technology

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SRC Engineering Research Center for Environmentally Benign Semiconductor Manufacturing

Joint School of Nanoscience and Nanoengineering

- Collaboration of North Carolina A&T State University and the University of North Carolina at Greensboro
- Offering graduate degree programs – Professional MS and PhD in Nanoscience (2010-); MS and PhD in Nanoengineering (2011-)
- 105,000 ft^2 facility –
  - Adjacent nanoelectronics and biopharma cleanrooms
  - State-of-art microscopy suite including Helium Ion Microscope
  - Nanobiology, Analytical labs

Research Focus Areas

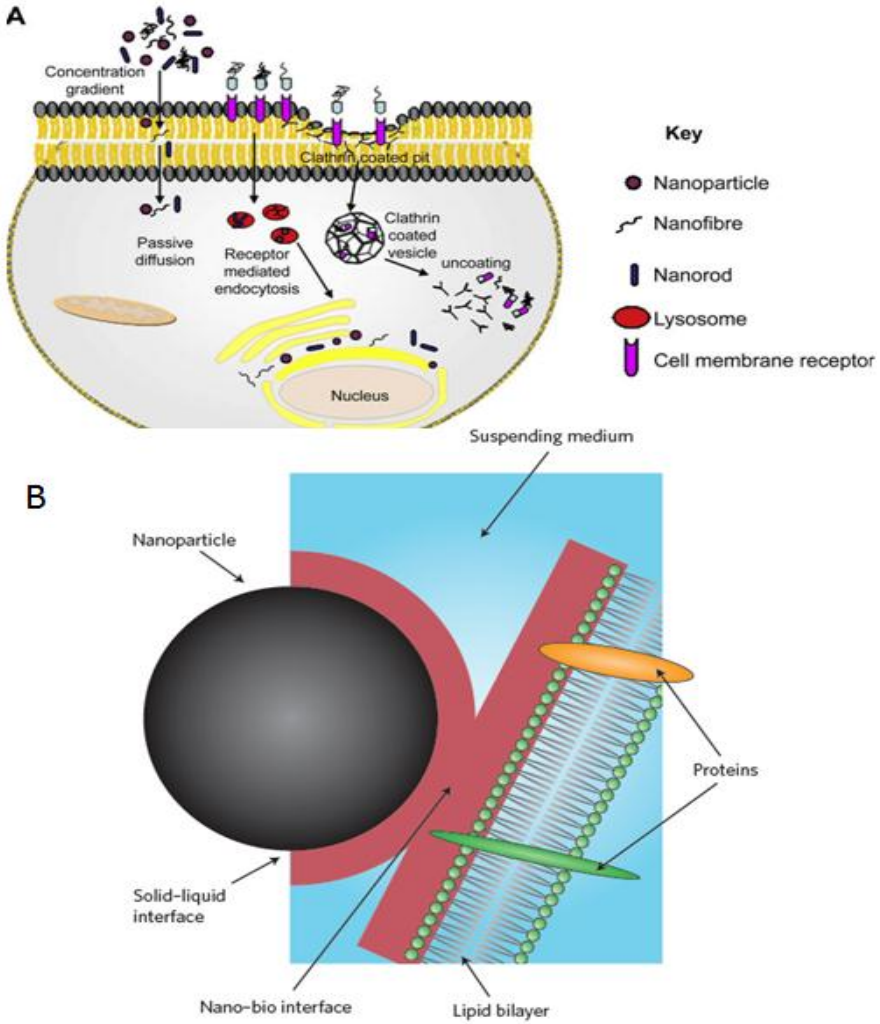
- Nanomaterials
- Nanometrology
- Nanobioelectronics
- Computational Nanotechnology
- Nanobiology & Nanomedicine
- Nanoenergy

Objectives

- Overarching Vision - New approach of scientific integration where nano-ESH is an integral part of Engineered Nanomaterial (EN) design rather than a *post facto* add-on
- Develop a generic, robust, rapid throughout and high-content screening platform where critical biological consequences (CBCs) of Engineered Nanomaterials (ENs) are studied.
  - At acute (< 3 days post-exposure) and chronic time points (up to 2 weeks post-exposure)
  - Combination of conventional assays and novel assessment methods
  - Cyto- and genotoxic assays to identify and quantify associations with specific ENS' physicochemical properties
  - Validate Electrical Impedance Spectroscopy (EIS)
  - Validate Helium Ion Microscopy (HiM)

Motivation and Novelty

- Bio-physico-chemical properties of engineered nanomaterials can alter their biological interface
- Subtle cellular alterations may arise at lower ENs concentrations, which may not result in cell death but could contribute to human health risks
- Orthogonal and longitudinal screening on EN-based CMOS platform for simultaneous study of acute and chronic timepoint device performance in correlation to nano-bio interactions.

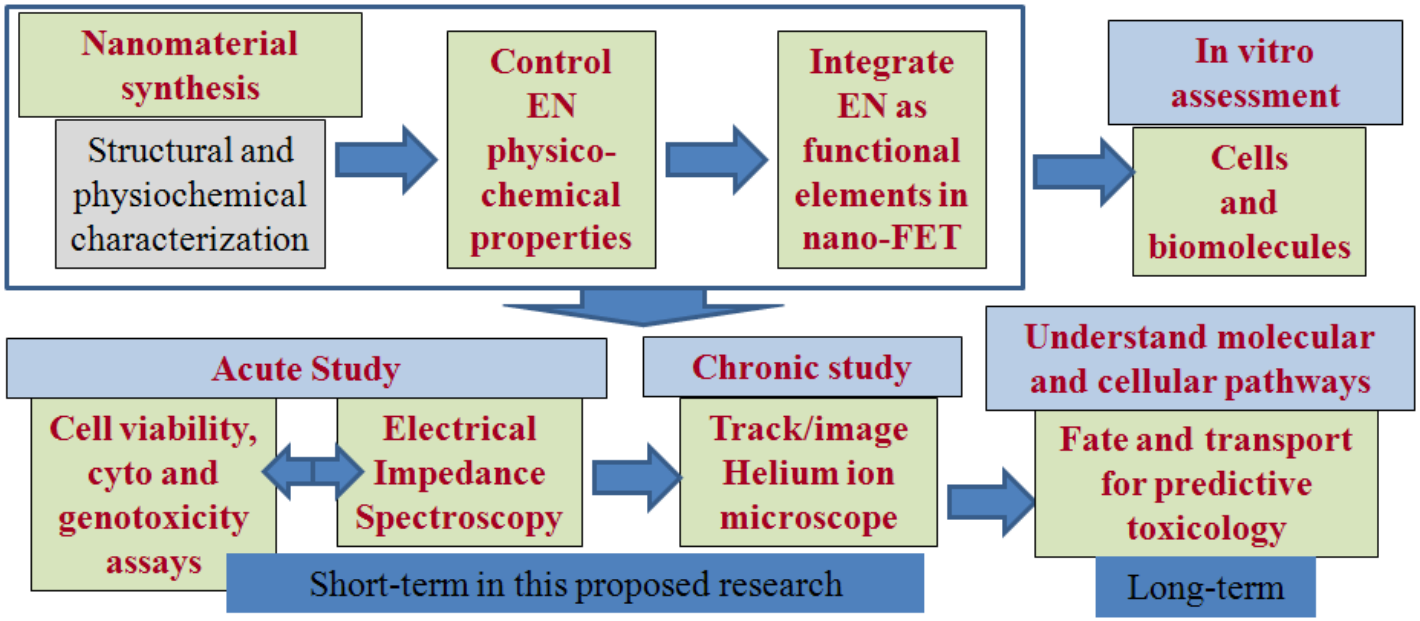


A) Possible mechanisms of cellular uptake of ENs (Singh et al. 2009). B) Nano-bio interface (Nel et al., 2009)

Hypothesis

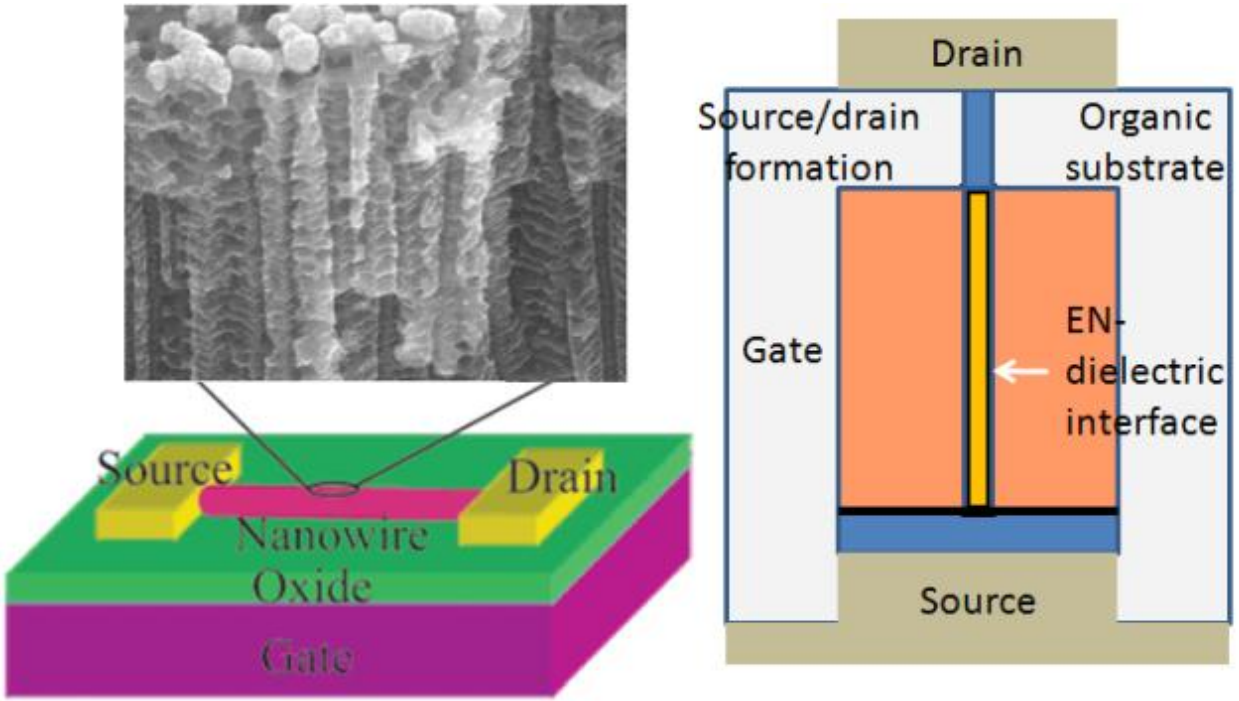
- Non-/semi-invasive methods (such as electrical impedance spectroscopy and helium ion microscopic analysis) will provide quantitative signatures of CBCs, when ENs of varying physicochemical properties are exposed to relevant human cell lines.
- Physico-chemical properties at the nano-scale have very different health implications on a temporal scale (acute to chronic time point)
- Most, if not all potential health risks/hazards will be mediated and/or captured by one or more of these CBCs being adversely affected.
- CBCs are related to cellular uptake, fate, transport, cyto- and genotoxic responses

Approach



- Probe various nano-bio interfaces to allow for the development of predictive relationships between structure and activity that are determined by ENs' bio-physico-chemical properties
- Control over size, shape, phase purity, crystallinity, chemical composition and structure-property correlations
- (a) Cytotoxicity assays – by imaging and monitoring cell function, cell signaling and cell metabolism, which are specific indicators of cell viability and (b) Genotoxicity assays - to understand genotoxic responses, such as DNA strand breakages, oxidative DNA adducts and alterations in gene expression profiles

Approach (contd..)

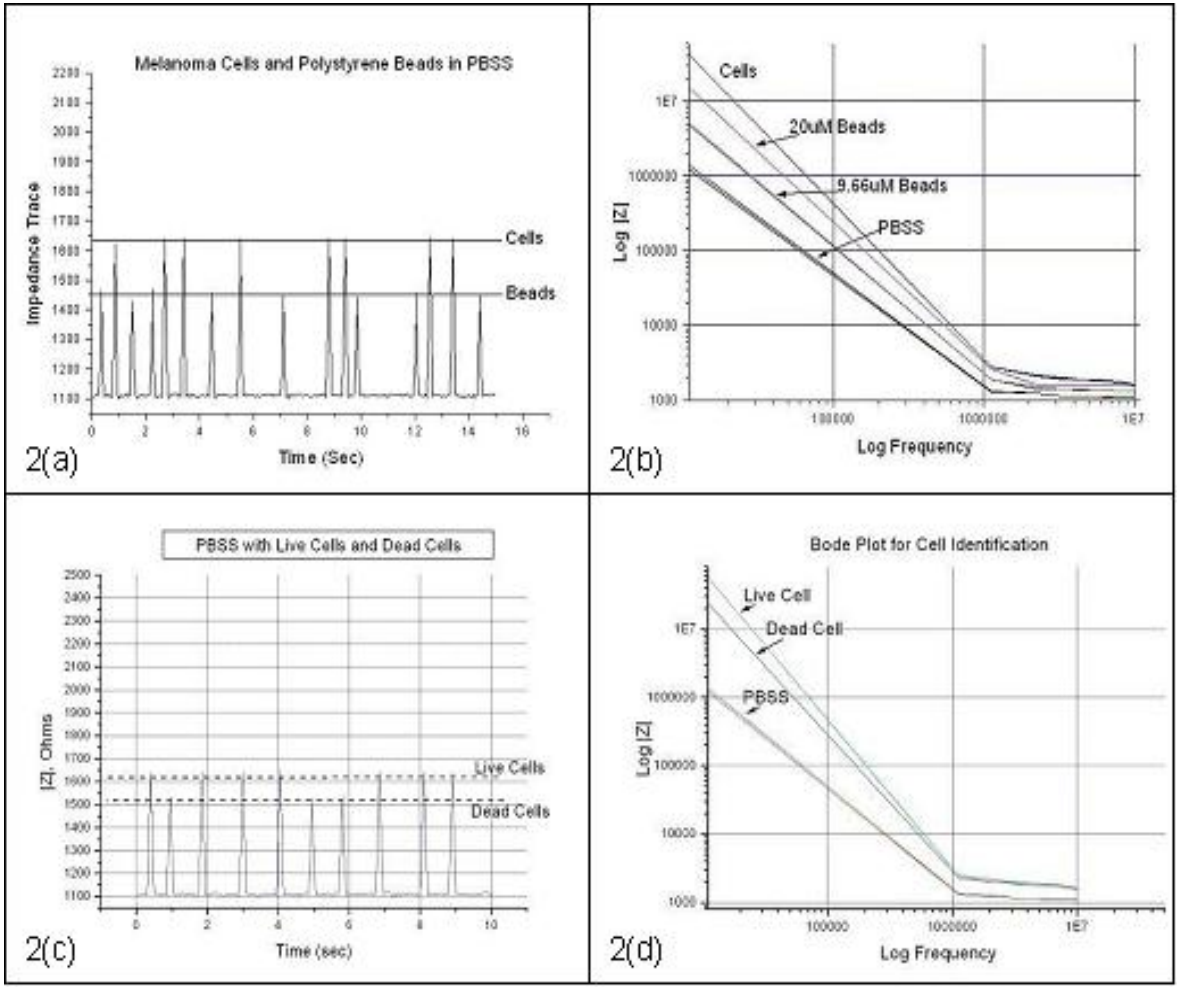


a) Illustration of EN nano-FET architectures – planar and vertical integration (Aravamudhan et al., 2007 and Lu et al., 2006), (b) Electron micrograph of high-density vertical metallic nanowires

- Fabricate Nano-FET architecture in horizontal and vertical configurations
- CNTs, ZnO NWs, Si NWS, Au NWs, GaAsSb NWs - (5-50 nm in diameter and 0.1-1 µm in length)

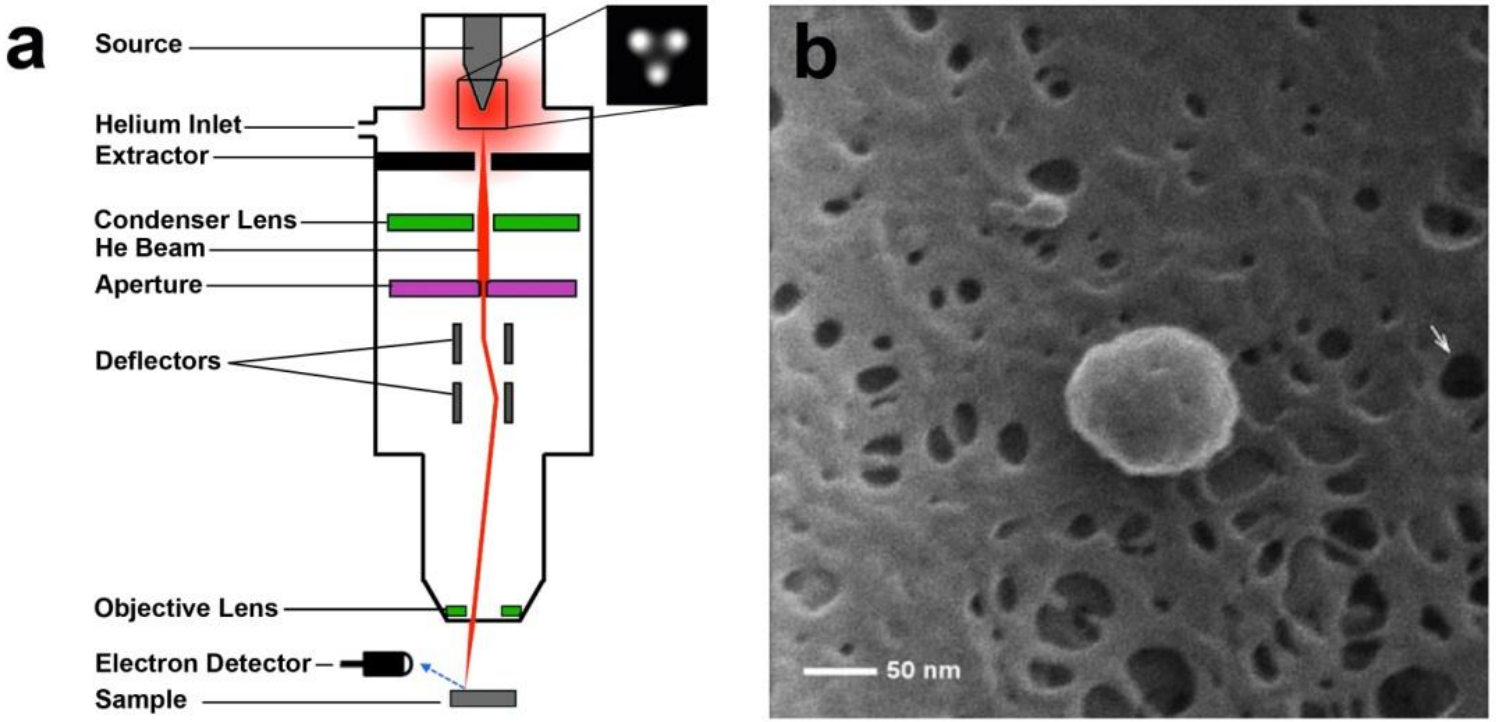
Electrical Impedance Spectroscopy

- To differentially assess cells based on their impedance distribution to facilitate an affordable generic, longitudinal technique to study cellular function, viability and proliferation
- Information about cell structure is obtained at low frequencies since most of the current flows around the dielectric cell membrane. At higher frequencies, the current penetrates the membrane and provides information about the cell interior



(2a) Impedance trace of solution with cells and beads; (2b) Bode Plot of solution, cells, and beads; (2c) Impedance trace of solution with live and dead cells (2d) Bode plot of solution with live and dead cells (Rahman et al. 2008)

Helium Ion Microscopy



(a) Schematic diagram of the Helium Ion Microscope. Top inset: the three metal atoms that form the source tip. (b) Example HIM image of a single nanoparticle on uncoated rodent kidney. Source: Carl Zeiss SMT (Scipioni et al. 2009).

Enabler for cellular and tissue uptake understanding through vastly improved tracking, transportation and aggregation/disaggregation of nanomaterials

Deliverables/Timeline/Students

YEAR 1 - Comprehensive short-term (< 3 weeks) assessment of critical biological consequences (CBCs) using conventional cyto- and genotoxicity assays, where relevant human cells are exposed to varying physico-chemical ENs.

YEAR 2 - Development of novel non-/semi-invasive assessment methods (electrical impedance spectroscopy and Helium Ion Microscopy) to monitor longitudinal CBCs

YEAR 3 - Temporal time course assessment of CBCs (> 3 weeks) with optimized and validated methods

Personnel working on this project

- Multi-disciplinary group of researchers – Nanobioelectronics, nanoelectronics, nanophysics, nanochemistry and toxicology
- 2 Graduate students
- 1 Research Associate