7. Highlights

University Name: The Ohio State University (OSU)
Cooperative Agreement Number: NSEC-CANPBD Grant #0914790

Science Highlights:

- Highlight Title: Multifunctional Nanoparticles: Nanomedicines for Cancer
  Author Names: Robert Lee and L. James Lee

Education Highlight

- Highlight Title: Outreach on a Massive Scale: Making Nanotechnology and Biotechnology Understandable to Young Scientists
  Author Name: Sherwin Singer

- Highlight Title: Nano/Biotechnology Comes Alive for High School Students
  Author Name: Sherwin Singer

Shared Facilities Highlight

- Highlight Title: On-chip Capture/Sorting and Characterization of Cancerous Cells
  Author Names: L. James Lee, Greg Lafyatis, John Lannutti

Multifunctional Nanoparticles: Nanomedicines for Cancer

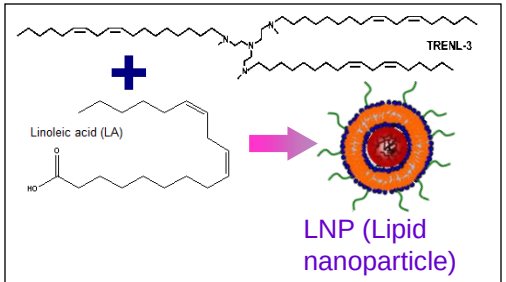


Fig 1. Novel lipid TRENL-3 and LNPs for siRNA delivery

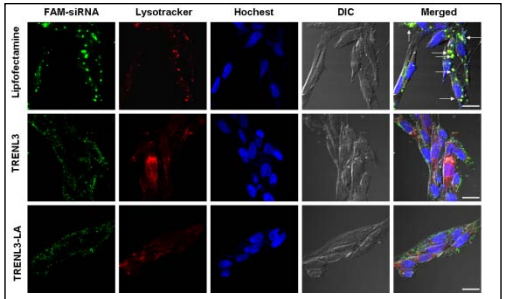


Fig 2. Intracellular trafficking of LNPs

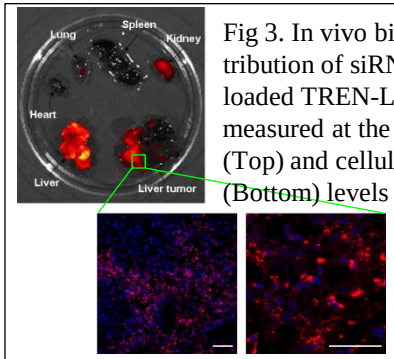


Fig 3. In vivo biodistribution of siRNA loaded TREN-LNPs, measured at the organ (Top) and cellular (Bottom) levels

Outcome: RNA interference by siRNA or miRNA has great therapeutic potential against serious human diseases such as cancer and leukemia. Their clinical implementation, however, has been hampered by the lack of effective delivery systems. Targeted multifunctional nanoparticles present a promising solution to this challenging problem. Much progress has been made through the Multifunctional Nanoparticle Design and Synthesis (MNDS) platform, through rational design and selection of novel lipids and helper lipids in lipid nanoparticles (LNPs), in depth analysis of their intracellular pathways and structure-function relationships, and interdisciplinary collaboration with disease specialists at the OSU Comprehensive Cancer Center. This effort has led to a number of breakthroughs. For example, a TREN-based series of novel lipids have been synthesized (Fig 1), along with LNPs composed of novel co-lipids (e.g., in SPANosomes), leading to outstanding siRNA and miRNA delivery efficiencies both in vitro and in vivo. Fig 2 shows intracellular trafficking of the novel LNPs studied by confocal microscopy. Fig 3 shows the in vivo delivery of siRNA to the liver tumor visualized by IVIS imaging and by confocal microscopy of tissue sections, which demonstrates excellent delivery efficiency.

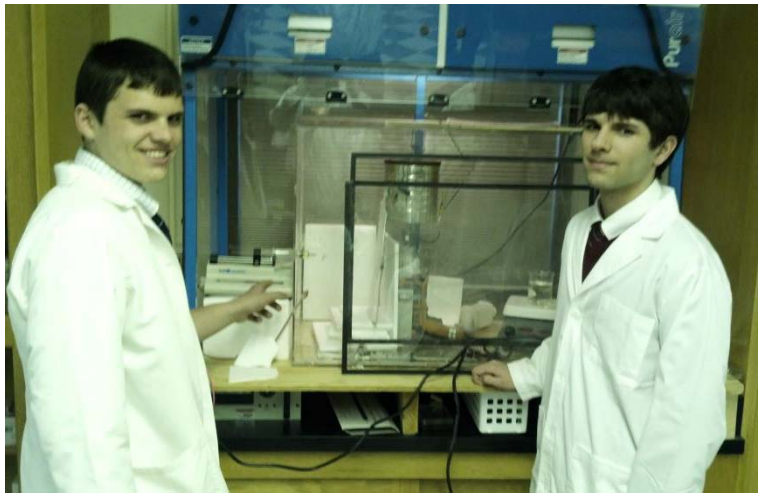
The results of this research have been presented at national conferences and have led to a number of publications in high quality journals. We have established strategic collaborations with physician scientists working on leukemias, lung cancer, and liver cancer, which has led to several NIH R01 and R21 grants.

Impact/Benefits: This work could improve the efficiency of delivery for RNAi agents in the clinical setting with strong potential for future clinical translation and commercialization. The multifunctional LNPs are being used for the delivery of multiple classes of agents, including siRNA, miRNA, and anti-miR, against several diseases. The studies on intracellular trafficking are likely to improve understanding of mechanisms of nanoparticle-cell interaction and promote further innovations through rational design of the nanoparticles, thus advancing the development of this class of nanomedicines.

Explanation: The LNPs are capable of delivering siRNA or other nucleic acid drugs that prevent cancer cells from producing certain proteins, thus killing them. However, effective and non-toxic delivery of nucleic acid drugs remains a great challenge for their therapeutic application. Our novel LNP formulation is a platform technology that can deliver small/large nucleic acids into cancer tissues. This is a powerful innovation in the development of nanomedicines to combat cancer.

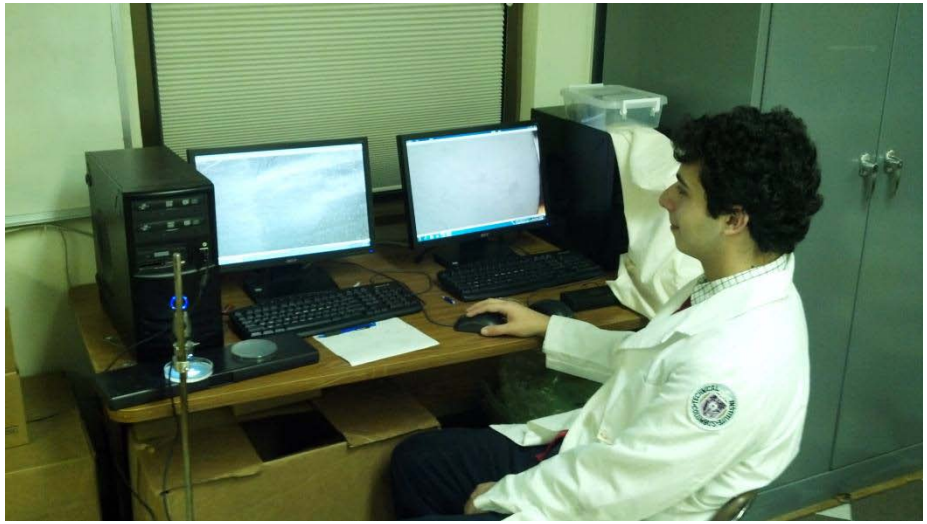
NSF Award Number: 0914790
Institution Name: The Ohio State University
Center for Affordable Nanoengineering of Polymeric Biomedical Devices (CANPBD)

Nano/Biotechnology Comes Alive for High Schools Students



Outcome: Through a summer internship at Ohio State sponsored by CANPBD, high school physics teacher, Dr. Sarah Vandermeer, was introduced to techniques of nanofiber synthesis, and their applications in biotechnology. Dr. Vandermeer assembled her own electrospinning apparatus at St. Charles High School, where her students have performed original research on the physical properties of nanospun fibers, and observed cell mobility on nanofiber scaffolds. Most significantly, confidence and enthusiasm for scientific investigation is running high at St. Charles.

Impact/Benefits: Dr. Vandermeer's crew of budding scientists have learned many fundamental concepts spanning several fields of science in the course of their investigations – principles of electrostatics, mechanical properties of materials, chemical modification of materials, and cell culture techniques. She reports that the most important impact on her students is to instill enthusiasm for scientific investigation, and the development of problem-solving abilities. The students encountered obstacles at many points during this long investigation. Now they have the confidence in their abilities to reason their way to solutions.



Explanation: The investigation is has proceeded through several stages over two years, during which students have developed skills ranging from problem-solving and data analysis, to literature searching. The group first investigated the tensile properties of their nanofiber sheets. The final stage of the investigation is observing the effects of aligned nano-fibers on cell mobility. The St. Charles students grew amoebazoan slime molds from spores and set up a PC microscope to record time lapse images of cell motion. The students have observed different cell motion on aligned and randomly oriented nano-fiber substrates.

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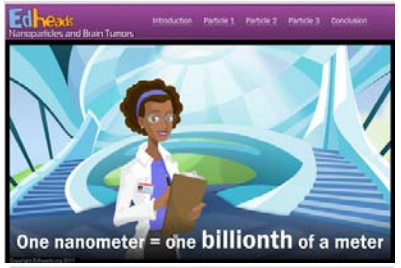
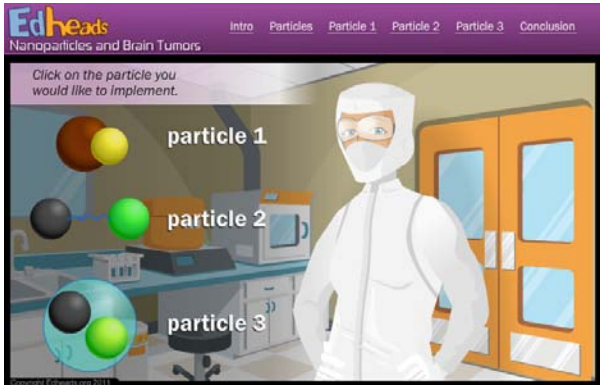
Outreach on a massive scale: Making nanotechnology and biotechnology understandable to young scientists



Outcome: Through a collaboration with Edheads (www.edheads.org), an award-winning, fun, distinctive and highly successful 501(3)(c) educational web development company, the NSF-supported CANPBD Center at Ohio State has been able to explain the benefits and excitement of nanotechnology to many thousands of potential future scientists. The response to the web-based activity has been very enthusiastic.

Impact/Benefits: The Nanoparticles and Brain Tumors activity was launched on December 16, 2012. In four weeks since the launch, 79,325 different viewers participated in the activity. A broadcast email and postcards have just gone out to increase the visibility of the activity. These activities have an enormous impact. For example, a cell phone design activity produced in collaboration with the College of Engineering at Ohio State has involved over 6 million users since June, 2009. The activities motivate young participants to consider careers in science and engineering. There is also a section of the Edheads web site called Career Choices, where participants meet a diverse set of actual professionals.

Explanation: CANPBD has entered into a fruitful collaboration with Edheads, to teach curious minds of all ages about nanotechnology and biotechnology. Edheads (www.edheads.org) creates unique, educational Web experiences designed to make hard-to-teach concepts understandable. The hallmarks of the Flash-based Edheads activities are a focus on real-world applications, and involving the viewer in interactive problem-solving.



The CANPBD center has collaborated with Edheads to produce an Edheads activity based on the research of CANPBD investigator Jessica Winter. The real-world application is the design of fluorescent and magnetic nanoparticles that mark a brain tumor, enabling a surgeon to remove all cancerous tissue while leaving healthy tissue intact. The participant interactively designs nanoparticles that are small enough get past the blood-brain barrier, and are both magnetic and fluorescent for pre-operative magnetic resonance imaging (MRI) and interoperative fluorescence imaging.

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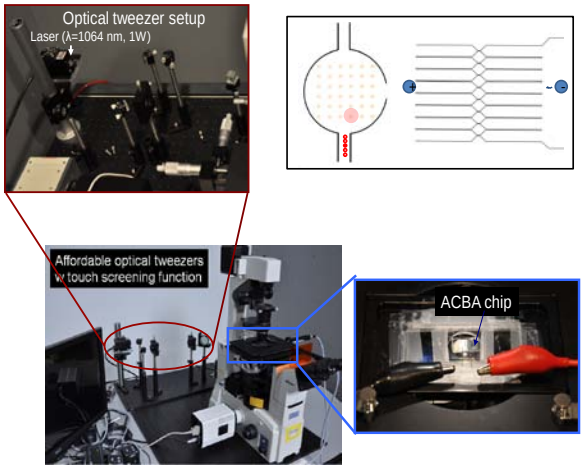
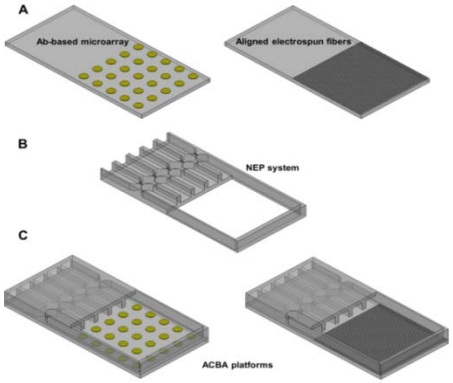
On-chip Capture/Sorting and Characterization of Cancerous Cells

ACBA Platform: *Antibody Microarray - Optical Tweezer - NEP*



Top: Photograph of the Automated Cell to Biomolecule Analysis (ACBA) Platform



Bottom: Design of an ACABA chip: (A) Glass substrate with the antibody-based microarray or aligned electrospun fibers. **(B)** NEP system. **(C)** ACBA platforms

Outcome: Researchers at CANPBD have developed a powerful lab-on-a-chip (LOC) platform, called Automated Cell to Biomolecule Analysis (ACBA) to sort potentially highly metastatic tumor cells from pre-treated human blood samples based on specific cell membrane receptors. Such platform also allows for downstream single cell analysis on the same chip via a newly developed nanochannel electroporation (NEP) technique, which is critical for gaining a better understanding of the behavior and biology of the sorted cancerous cells. The figures show the ACBA Platform and the design of the ACBA chips. The ACBA chip incorporates an improved antibody microarray to capture CTCs in pre-treated human blood samples (i.e. <10 CTCs in ~9,000,000 WBCs in 1 mL blood), and the *in-situ* detection of intra-cellular biomarkers in living CTCs by NEP.

Explanation: Although tumor metastasis is responsible for more than half million deaths in the US each year, there has been little improvement of the cancer death rate in the past 20 years despite tremendous research and clinic efforts in this field. One of the major reasons is the lack of reliable and patient friendly early detection methods. Current methods such as X-ray imaging and tissue biopsy are invasive and expensive. They are also not sensitive in the early stage of cancers. In recent years, capture and characterization of circulating tumor cells (CTCs) has gained a great deal of attention in the medical field because of its potential to detect cancer early in a less invasive manner. Up till now all existing methods have had limited ability to capture highly metastatic CTCs when their abundance is low (3-7 CTCs per mL of blood) as in the early stages of cancer formation, metastasis or re-occurrence. Current methods to characterize CTCs are also time consuming and expensive.

In addition to the sorting of CTCs from human blood samples by the antibody microarray, a simple modification allows the platform to sort and analyze tumor cells from solid biopsies based on their migration/dissemination behavior on a functionalized and aligned nanofiber surface. This was done by incorporating aligned electrospun fibers into the LOC platform to mimic micro/nanostructural cues present in a number of tissues (e.g., blood vessel walls, white matter tracts), which have been known to facilitate and promote rapid tumor cell dissemination and metastasis.

Impact/Benefits: Cells of interest can be transferred from the sorting stage to the analysis stage via optical tweezers. Single cell analysis through our unique NEP technology can be used to inject selected cells with different molecular probes (e.g. molecular beacons) to detect certain messenger RNA and microRNA biomarkers targets, or to inject a specific drug or genes to up-/down-regulate certain pathways of interest. Furthermore, NEP-treated cells remain viable, which facilitates continued cell monitoring and treatment. This type of analysis is critical for gaining a better understanding of the behavior and biology of cancerous cells.

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