7. Highlights

University Name: The Ohio State University (OSU)
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Science Highlights:

- Highlight Title: Tethered Nanoparticle Arrays for Exosomal miRNA Detection in Cancer
  Author Names: Michael Paulaitis

- Highlight Title: Automated Cell to Biomolecule Analysis: High Precision Single Cell Lysis
  Author Name: R. Sooryakumar

Education Highlight:

- Highlight Title: Interactive Edheads Activities on Nanotechnology and Biotechnology
  Engage Many Thousands of Young Scientists
  Author Name: Sherwin Singer

- Highlight Title: Hands on! Teachers and Students Learn About Nanotechnology by Doing
  Author Name: Sherwin Singer

Shared Facilities Highlight:

- Highlight Title: Single Cell and Single Molecule Fluorescence for Integrated Nanotechnology
  Author Names: Carlos Castro
Tethered Nanoparticle Arrays for Exosomal miRNA Detection in Cancer

**Outcome:** MicroRNAs (miRNAs) are small non-coding RNAs recognized as important epigenetic regulators of gene expression. Observed changes in miRNA expression associated with cancer tumors have motivated efforts to develop technologies for the early detection of many human cancers based on miRNA profiling. The vast majority of these profiles are obtained from tumor biopsies. However, microvesicles released by cancer tumors also contain miRNA, and identifying cancer-related miRNA signatures in these microvesicles offers the attractive possibility of discovering minimally invasive biomarkers for cancer.

Recent evidence also points to microvesicle-mediated intercellular miRNA transfer as a mechanism for sculpting the tumor microenvironment. This mechanism depends on the ability of microvesicles to interact with specific target cells, and to transmit differentiation-regulating signals that can effectively trigger the reprogramming of the target cells. We have taken advantage of these targeting mechanisms to develop a novel assay for isolating and concentrating the exosome subpopulation of cell-secreted microvesicles for miRNA profiling, while simultaneously characterizing their intrinsic targeting mechanisms. The assay involves isolating the exosomes by size using asymmetric flow field flow fractionation, and then capturing the exosomes by binding and fusion with tethered cationic lipoplex nanoparticles for the in situ detection of exosomal miRNA using miRNA-specific molecular beacons.

**Impact/Benefits:** Cancer biomarkers that are minimally invasive, straightforward to implement, and accurate in identifying the disease at an early stage of progression are needed if better treatments are to be found. Current assays for analyzing miRNA expression profiles – Northern blotting, microarray assays, and RT-PCR amplification – are time-consuming, semi-quantitative, and prohibitively expensive for routine clinical applications. The novel assay technology developed here can accommodate small sample sizes and enable quantitative detection at low miRNA concentrations, independent of nucleotide sequence, which are clearly needed before accurate, reliable cancer-related exosomal miRNA profiling can be realized.

**Explanation:** The functional characterization of exosomes released by cancer tumors based on their intrinsic targeting mechanisms as a complement to their miRNA profiles will provide unique insights into role exosomal miRNA transfer plays in cell-cell communications that could not be obtained from either characterization alone. Broader application of this assay technology has the potential to advance new clinical practice paradigms for early diagnosis, progression, and response to treatment of many cancers that would greatly reduce the worldwide health burden of this disease.

NSF Award Number: 0914790
Institution Name: The Ohio State University
Center for Affordable Nanoengineering of Polymeric Biomedical Devices (CANPBD)
Automated Cell to Biomolecule Analysis: High Precision Single Cell Lysis

Outcomes:
Analysis of cell-to-cell variation can further the understanding of intracellular processes and the role of individual cell function within a larger cell population. The ability to precisely lyse single cells can be used to release cellular components to resolve cellular heterogeneity that might be obscured when whole cell populations are examined. Much progress has been made through the Automated Cell to Biomolecule Analysis (ACBA) platform, accomplishments that were based on rational design to position and lyse individual cells on silicon nanowire and nanoribbon biological field effect transistors through collaborations amongst NSEC members at OSU and the University of Illinois.

The results of this particular research have been presented at national conferences and led to a Technical Innovation paper in Lab-on-a-Chip. We have established strategic collaborations with physician scientists and applied for a NIH R21 grant.

Needs:
Analysis of cellular content, such as protein and nucleic acid molecules typically requires a priori cell lysis by rupture of the cell membrane. This can be achieved by chemical, electrical, enzymatic, mechanical or thermal means. However, each technique presents unique advantages and disadvantages that must be considered to ensure compatibility with downstream applications. One major issue is a bulk and non-specific approach leads to breakdown of cell membranes and cell compartments that can cause denaturation of biomolecules. In the approach presented here, we combine magnetic manipulation techniques to precisely locate a cell atop nanowire based field effect transistor to enable gentle and specific cell lysis with the potential for field effect sensing of the released cellular components.

Impact/Benefits:
This work has the potential to enable many applications that require specific, localized cell lysis on a biosensing platform. The simple method developed reduces the impact on molecular stability from thermal, chemical or mechanical degradation. Label-free and trap-less magnetic manipulation of cells especially overcomes the need for: transparent substrates in optical tweezers techniques; microfluidic trapping techniques; high voltages and low media conductivity in dielectrophoresis-based trapping of cells. This methodology can be applied to a variety of biological studies that focus on single cell vs cell population analysis.
Interactive Edheads Activities on Nanotechnology and Biotechnology Engage Many Thousands of Young Scientists

**Outcome:** Many thousands of potential future scientists have both entertained and challenged themselves to understand the benefits of nanotechnology using two on-line interactive activities developed collaboratively by the NSF-supported CANPBD Center at Ohio State and Edheads (www.edheads.org). Edheads is an award-winning, fun, distinctive and highly successful 501(3)(c) educational web development company. The response to the CANPBD/Edheads activities has been overwhelming.

**Impact/Benefits:** The *Nanoparticles and Brain Tumors* activity was launched on December 16, 2011. Since the launch, 577,618 different viewers participated in the activity by designing and testing different nanoparticles for their effectiveness in imaging brain tumors. A second CANPBD/Edheads activity, *Sickle Cell DNA*, just went live on March 8, 2013. Within 19 days, and before any advertisement reached middle and high school teachers, 11,778 young scientists learned about the molecular basis of sickle cell disease. They constructed a complementary strand of DNA, guided the folding of a protein, and saw the benefits that nanotechnology may bring to those who suffer from this disease. These activities have an enormous impact. The activities also 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 through text and pictures.

**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.

In *Sickle Cell DNA*, CANPBD researcher Andre Palmer explains how nanotechnology leads to effective red blood cell replacements.
Hands on! Teachers and Students Learn About Nanotechnology by Doing

**Outcome:** Though a series of outreach activities offered by the NSF-supported CANPBD Center at Ohio State, middle and high school students and teachers have got their hands on nanotechnology. These activities include NanoDay at COSI, our local science museum, Breakfast of Science Champions and YES (Young Engineers and Scientists) Days for middle school students, and a Teacher Workshop for middle and high school teachers.

**Impact/Benefits:** Whether elementary school students, college students, or teachers, knowledge and enthusiasm are most persistent when received *actively*, not passively. Therefore, CANPBD engages students and teachers across the learning spectrum in hands-on nanotechnology activities. Every effort is made to insure that we reach out to underserved urban and rural communities. Many teachers are provided with nanotechnology kits, some components obtained commercially and some assembled by the CANPBD team, so they are prepared to introduce nanotechnology experiments in their classroom. Surveys at the conclusion of the workshop for middle and high school teacher indicate that 100% of participants agree that the workshop was well-organized, gave them useful ideas, and helped them understand nanotechnology. At the end of the activity, 95% of our Breakfast of Science Champions students indicated that they will be going to college, 45% indicated they would pursue a STEM career, while another 15% intended to pursue a medically oriented career.

**Explanation:** CANPBD researchers have assembled a repertoire of hands-on activities appropriate for non-experts. Some are well-known, such as ferromagnetics. We have developed other activities locally. These include comparing how stains rinse off of regular and nanofiber treated fabric, popular among young participants. In another locally developed activity suitable for older students, laser diffraction spots from a CD and DVD are interpreted using interactive computer modeling to determine the track spacing.

← Clockwise from top left: NanoDay at COSI, Breakfast of Science Champions, middle and high school teacher are introduced to computational chemistry software they can use in their classroom, and teachers visiting the nanofabrication clean room executing a gesture known to all Ohio State fans.

NSF Award Number: 0914790, Center for Affordable Nanoengineering of Polymeric Biomedical Devices
**Outcome:** Researchers at CANPBD have recently established a facility to perform single cell and single molecule level fluorescence microscopy. This facility enables efficient real time visualization of nanotechnologies developed through the CANPBD in live cell and/or single molecule experimental assays. This facility is currently being used in several CANPBD studies. For example, single cell fluorescence assays have been developed to efficiently characterize the function of tethered cationic lipoplex nanoparticles for the characterization of miRNA signatures of cancer. This facility is also being used to develop an experimental assay to support the development of multi-functional nanofiber materials. Specifically, researchers at the CANPBD are developing nanoscale force sensors using DNA nanotechnology with a fluorescence-based force readout. These fluorescently labeled force sensors have been integrated onto electrospun nanofibers to make a force-sensitive sensing fibrous matrix for cell migration experiments. Researchers at the CANPBD are currently in the process of proof-of-principle experiments with these force-sensitive fibrous materials. A third CANPBD project that utilizes this facility seeks to visualize the dynamics DNA inside nanochannels to quantify the use of channelgeometries for size-based DNA sorting and nanoelectroporation.

The shared facility is was established this year and has already led to a publication submitted to a high quality journal and two more in preparation. The results of this research have been presented at national conferences, and the results obtained at this facility have also led to several joint NSF and NIH proposals from NSEC faculty. We have also established strategic collaborations with physician scientists working on leukemias, lung cancer, and liver cancer, which has led to additional NIH R01 and R21 grants.

**Explanation:** The CANPBD single cell and single molecule level fluorescence imaging facility at Ohio State University allows real-time characterization of nanotechnology in live cell experimental assays. This facility is based on a Nikon total internal reflection fluorescence (TIRF) microscope. This microscope is capable of multi-color fluorescence excitation with low light detection capabilities for single molecule studies in combination with phase contrast microscopy which is ideal for imaging of cell morphology and nanofabricated microfluidic systems. The imaging system is designed for simultaneous multi-color imaging for fluorescence resonance energy transfer studies. The microscope is fully compatible with several of the other technologies developed in the CANPBD.

**Impact/Benefits:** This facility has been establish to allow for functional imaging of CANPBD technologies in real time at the single cell scale. The microscope is capable of automated switching between distinct imaging modalities and automated rastering of samples, thus enabling efficient imaging of experimental assays over long times and large viewing areas while still maintaining single molecule resolution. Currently, an ongoing collaboration at the CANPBD is developing a platform to integrate magnetic tweezers onto the fluorescence microscope. This collaboration will further allow active local manipulation of samples as well as calibrated force application for measurement of physical behavior and properties of systems.