NC 1194 Termination Report

Project/Activity Number: NC-1194
Project/Activity Title: Nanotechnology and Biosensors
Period Covered: October 1, 2016 to September 30, 2021
Date of This Report: March, 2022

Accomplishments:
Over five years participants made numerous contributions to achieve all six *objectives* delineated in our original proposal.

1. *Develop new technologies for characterizing fundamental nanoscale processes*

During this multistate collaborative project members have developed and characterized various nanoscale materials that were used for development of novel nano-biosensors. Among the selected biosensing materials, enzymes, antibodies, aptamers, or DNA probes were coupled to electrochemical, optical, or acoustic (e.g., quartz crystal microbalance (QCM)) transducing methods. Thus, numerous advances have been made in nanomaterial-enabled biosensor technologies across a spectrum of applications for improvement safety and security of the food supply chain. Among notable advances are (i) innovative nanoparticle morphologies such as coupling cellulose nanofiber (CNF) to Surface Enhanced Raman Scattering (SERS), for the rapid detection of pesticide residues (paraquat) in vegetables (MO); (ii) assessment of metabolite distributions in plant and animal cells (IA, UT); (iii) development of strategies to stabilize enzymes for the fabrication of glucose and alcohol biosensors with long operational life (GA); and (iv) enhancement of the performance of a variety of other biosensing modalities for rapid detection of pathogens, prions, viruses, contaminants (e.g., heavy metals), pesticides, and antibiotics residues in water and agricultural and food products (AR, AZ, FL, HI, IA, MI, WI).

The research focused on sustainable development of nanotechnology via molecular-level understanding of the interaction of nanomaterials with biological interfaces, both to design applications that interface with biological systems and to evaluate the potential risks posed by the release of nanoscale materials into the environment. Several groups have made significant progress in characterizing and understanding environmental health toxicity and toxicity mechanisms of a wide array of commonly used nanomaterials before and after environmental transformations in simple and complex exposure scenarios (KY, WI and SC). Participants deepened into multidisciplinary collaborations to address antimicrobial resistance, advancing affordable technologies to enhance the speed and sensitivity of the recovery and detection of pathogenic bacteria (AR, MI) as well as for the rapid clinical identification of antimicrobial resistance in pathogenic bacteria (MI). Researchers developed novel approaches with functionalized nanomaterials to prevent and treat infections with pathogenic organisms that were successfully demonstrated in animal models (SC). Members have also engineered new nanomaterials for targeted drug delivery (AR, IA) or vaccines against animal viruses (VA). Edible films with novel antimicrobial and nutritional properties were developed (NJ). Nanomaterials were also developed to improve efficiency / rates for value-added bioprocessing (AR, WI).

We developed electrochemical analyses, using impedance spectroscopy, differential pulse voltammetry of biomolecule interactions such as DNA chains hybridization, antibody-antigens interaction in nano-scale (SD).

2. *Construct and characterize self-assembled nanostructures*

A significant progress has been made in development, characterization and optimization of self-assembled nanobrush/aptamer hybrid nanostructures (FL)) and nickel oxide nanoparticles on three-dimensional carbonized eggshell membrane (SD) for sensing, nano-vaccines (IA and VA), and DNA biosensors (UT). For example, the construction of DNA biosensor includes multiple self-assembled steps to form a highly specific DNA structure on the sensor surface for recognizing the target DNA sequence specific to Cryptosporidium in water samples (UT). For nano-vaccine development, a chimeric purified protein, based on constructed Hepatitis B core antigen, which is self-assembled into virus-like particles has been studied as a potential vaccine against a swine disease (VA).

3. *Develop devices and systems incorporating microfabrication and nanotechnology*

The incorporation of smartphones for the rapid analysis biosensing signals has further been improved, with sophisticated image processing algorithms and the coupling of a fluorescent microscope for detecting pathogens and environmental toxicants (AZ). Fluorescence detection has also been attempted to improve the assay reliability and reproducibility, and successfully demonstrated for detecting cancer markers from blood as well as nucleic acid amplification. A new concept was demonstrated utilizing the capillary flow as a sensing mechanism for detecting *E. coli* and Zika virus (AZ). A user-friendly prototype has been designed and fabricated, and tested for field water and human saliva samples. The sensitivity and specificity of this method were tested for the detection of norovirus from wastewater, COVID-19 virus from aerosols, and COVID-19 virus from human saliva samples (AZ).
Researchers in FL and SC developed value-added nanotechnology products from agricultural waste for food packaging, solar cells, sensors, as well as new sensor systems for studying signaling in plant/mammalian systems. Enhanced electrocatalytic performance of NiO/C electrode toward urea oxidation and urea detection in alkaline solution was developed (SD). Submicron fibers with surface properties suitable to capture and concentrate target chemicals, pathogens (*E. coli*) or biological molecules in microfluidic were fabricated (NY). A novel Raspberry Pi based optics device along with support vector machine (SVM)-based machine learning algorithm has been developed, fabricated, and tested for classifying the oil types from oil spill samples from open sea water with > 90% accuracy (AZ).

4. *Develop a framework for economic, environmental and health risk assessment for nanotechnologies applied to food, agriculture and biological systems*

Complementary to the work on discrete biosensing technologies for wide distribution in the environment, several participants of this project have been working on development of networking approaches and artificial intelligence to aggregate data and provide meaningful decision support to improve food quality, safety, and the cost-effectiveness of agriculture and bioprocessing (AR, AZ, FL, SC, MI, NJ).

5. *Develop/improve education and outreach materials on nanofabrication, sensing, systems integration and application risk assessment*

Project members are highly active in instruction, developing and sharing teaching / training materials related to nanotechnology and biosensors. This includes leading two NSF-REU programs (IA) where students contributed to research and development of wearable graphene-based stress sensors. The participants conducted workshops and disseminated training manuals with high school teachers in the USA (FL, MD) and also abroad (Colombia, China) for creating flexible graphene circuits (FL). During GARD symposium the members organized and taught short courses on Nanotechnology (NJ, KY), Biosensors (SC) and Entrepreneurship (MI)

6. *Improve academic-industry partnership to help move the developed technologies to commercialization phase*

Several groups partnered in various initiatives to strengthen ties to industry, including identification and execution of new research needs, and to strengthen research capacity globally to develop practical, cost-effective biosensing technologies. Academia-industry projects are with cybersecurity for food safety monitoring, and also with BRIDG for establishing a work group to create a draft national nanotechnology roadmap for smart Ag/food (FL). There are also partnerships with the Global Alliance for Rapid Diagnostics (GARD) to establish centers of excellence (COEs) around the world, and agreements with food companies to validate and license technologies for rapid pathogen extraction / detection (MI). Walmart foundation awarded $3.5 M to a group led by a project member from Arkansas for research on biosensors for food safety. A consortium of University groups including participants in this project (FL, IA) are actively involved in a funded planning grant under NSF-IUCRC (Industry-University Cooperative Research Center) to use nano-enabled sensory tools to study soil dynamics, and technologies developed by members of our group have successfully been used by government and industry, such as handheld biosensors for bacteria in drinking water used by Tucson Water (AZ). Participants at FL are working with SBIR funding to develop new mobile device APIs to facilitate risk analysis in water quality. To support commercialization of new technologies participants contributed to the development of open source smart-phone based approaches for portable analysis (AR, AZ, HI), scalable disposable sensors including using paper microfluidics (AZ), scalable manufactured graphene (FL, IA), as well as stabilization of immobilized biomaterials (GA).

Outputs of this 5-year project include 224 peer-reviewed publications in high impact journals, numerous presentations, workshops, two REU programs. In addition, research has been regularly highlighted as the feature article in their respective issues, and / or covered in the scientific press (i.e. NSF Research News and CEP Magazine) (AZ). The members collaborated on review papers on antimicrobial resistance, biosensors in food, agriculture, and the environment. Participants have notably made publicly available several research tools to help identify relevant research (SENSEE; FL) and share protocols for research related to biosensors and antimicrobial resistance (IA, MI). The results were disseminated to the industry and scientific communities at professional conferences such as IFT, ASABE, ACS, SETAC, GRC, and IAFP.

Impacts:

Project supported training of new scientists researching innovative nanomaterials and biosensors (numerous PhD and MS students) and enhanced global networking of researchers with work related to nanotechnology and biosensors.

Numerous technologies developed by project participants have enable broad distribution of biosensors in a wide variety of applications, including speed, efficiency, ease of use, portability, scalability, manufacturability, sensitivity, selectivity, and data management and analysis. Alternative methods and devices have been proposed and successfully demonstrated for smartphone-based paper microfluidic devices. These demonstrations can significantly improve the applicability of our biosensor methods and devices, which can be used by a lay person in many of environmental conditions.

In addition, research has resulted in a variety of innovative use of biological and nanoscale materials for vaccine development, drug delivery and therapies against pathogenic organisms. Toxicological data on widely used nanomaterials can inform policy decisions related to manufacturing and exposure to these materials to safeguard public and environmental health. Secured new research grants from various funding agencies including USDA, NSF, NIH, and DOE as well as UArizona Health Sciences (smartphone detection of COVID-19), Tech Launch Arizona (smartphone microscope development), and ABOR-TRIF. Walmart foundation awarded $3.5 M to a group led by a project member from Arkansas (Yanbin Li) for research on biosensors for food safety.

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