NC-1031: Nanotechnology and Biosensors

ANNUAL MEETING

MAY 5-6, 2010
CHAMPAIGN, IL

CHAIR
KAUSTUBH BHALERAO
University of Illinois at Urbana-Champaign

VICE CHAIR
JEONG-YEOL YOON
The University of Arizona

SECRETARY
PAUL TAKISTOV
Rutgers, The State University of New Jersey

ADMINISTRATIVE ADVISOR
VINCE BRALTS
Purdue University

IN ATTENDANCE
Advisor: Vince Bralts
USDA: Hongda Chen
Arkansas: Yanbin Li
Arizona: Jeong-Yeol Yoon
Hawaii: Daniel Jenkins
Illinois: Kaustubh Bhalerao
Indiana: Jenna Rickus & Joseph Irudayaraj
Michigan: Evangeline Alocilja
Nebraska: Milford Hanna
South Carolina: Jeremy Tzeng
Utah: Anhong Zhou

Meeting minutes

May 5th 2010

8:30 Opening remarks by Kaustubh Bhalerao, Vince Bralts and Hongda Chen.
Bralts: Deadline for request for renewal is September 15, 2010. December 1, 2010 is the hard dead line. Continuation begins on October 1, 2011. We need to clearly identify who the stakeholders are, and need to document past accomplishments.
Chen: NIFA update.
Nanotechnology: food safety intervention. Past nanotechnology approaches were primarily on detection. Big grants allow the hiring of a business administrator. What are solid deliverables? Biocatalysts are another possible option (nanotechnology for food safety intervention).
Bhalerao: Info on nano workshop at Illinois (May 6-7).
9:30 AM: Station reports were presented by Daniel Jenkins (HI), Jenna Rickus (IN), Jeremy Tzeng (SC), Yanbin Li (AR), Anhong Zhou (UT), Jeong-Yeol Yoon (AZ), Kaustubh Bhalerao (IL), Milford Hanna (NE), Joseph Irudayaraj (IN) and Evangeline Alocilja (MI).
5:30 PM: Dinner. Discussions over dinner included 2011 meeting location: Michigan State University, tentatively to be held in the first week of May. Officer elections for 2011 conducted.
2011 Chair: Jeong-Yeol Yoon
2011 Vice Chair: Evangeline Alocilja,
2011 Secretary: Dan Jenkins.

May 6th 2010

8:40 AM: Discussion on rewrite strategies: Hanna: More needs to be written on toxicology, education. These elements haven't been effectively dealt with in the last round.
Bralts: How to bring about broader participation?
Yoon: Not feasible due to budget constraints
Alocilja: What grant opportunities exist as a group?
Chen: All members have been successful in getting grants. Each member's salary also partially comes from USDA.
Alocilja: Cohesive and narrow objective.
Yoon: Easy grant from, for example, China MoST, and use as a stepping stone.
Li: Avian flu, food safety at China MoST.
Chen: What is achieved by this group (NC-1031)? What is your product?
Bralts: All members contributed in developing biological engineering courses in their own departments. Can't we include those as our achievements?

IMPORTANT DEADLINES FOR PROPOSAL REWRITE TASKS

The current project expires 9/30/2011. The deadline to submit a request to rewrite proposal is **15th September 2010**. The complete project rewrite deadline is **December 1 2010**. The request for rewrite should include justification for continuing the project as well as consequences of not continuing the project. It should outline the deliverables for the next five years. Additionally, it should provide a justification for existing as a regional project rather than an individual one. It will be good to have a statement on the likely impact as well.

Hanna: The original proposal looks like a collection of individual projects. It won't work for this time.
Rickus: Use nanoHUB (available to anyone, supported by NSF), and create a subset towards food and agriculture.
Alocilja: Cross-validate each other's technology for possible commercialization.
Chen/Bralts: Let us develop a standard. What is the best sensor that works?
Hanna: Developing a standard takes a lot of efforts. Be realistic.
Yoon/Irudayaraj: Cross-validation and/or senior design projects can be an issue for IPs.
Irudayaraj: Why don't we go visiting China in 2010 or 2011, as a group (NC-1031)?
Chen: Future committee meetings at international locations, including China?
Yoon/Irudayaraj: Share class materials. Post on nanoHUB. Go towards a textbook.
Irudayaraj: Can't we get seed money to get preliminary results from our own college?
Yoon/Hanna: May be difficult depending on universities.
Bhalerao: Sensor that can detect multiple species in a complex system. Not a single sensor for a single species.
Yoon/Rickus/Alocilja: Sensor that can detect that we do not know. Expose your sample to cells and see if they die. Pertubation study.
Chen: Create a decision tree out of our detection technologies.
Chen: Sensor that can empower each individual safe. One person may not tolerate low concentration of E. coli. Some people are allergenic to some foods.
Rickus/Yoon: Connect it to cell phone network.
Tzeng: MSG? Some people are allergic to it.

12:01 PM Meeting adjourned.

Meeting Agenda

May 5th 2010:

8:00 AM: Registration and welcome

8:30 - 9:00: Remarks from the Administrative Adviser and Program Manager

9:00 - 10:30: - Station reports (15-20 minutes each, about 5-6 in the first session)

10:30 - 10:45: Break

10:45 - 12:30 PM: Station reports continue (15-20 minutes each, remainder of the reports)

12:30 - 2:00: Lunch (Buffet, served at conference)

2:00 - 3:00: Committee business - approval of minutes, election of officers etc and state of the committee

3:00 - 5:30 - Initial brainstorming on the revision of the proposal: What to keep, what to discard, what to modify, what to add anew? Partitioning of writing tasks (we have some volunteers) How to attract new membership? Where are we lacking as a group? How to do more with the committee?

5:30: - Dinner (Served at conference) and continuation of discussion

May 6th 2010:

8:00 AM: Breakfast (Served at conference)

8:30 - 12:30 PM: Work on rewriting proposal, as well as assemble station reports into a single document

12:30: Adjourn

Station reports

(AR) University of Arkansas
Yanbin Li

Outputs:

By integrating the high efficiency of magnetic nanoparticles-based sample preparation for separation/concentration, the high sensitivity of nanowire/nanoelectrodes, and the high efficacy of flow-through micro/nanofluidics channel into the design, we proposed to develop a nano-biosensor that will meet the required sensitivity, specificity, and speed for screening of pathogenic bacteria and viruses in different food and poultry samples. The specific objectives of this research are: (1) develop an immunoseparation method based on magnetic nanoparticles coated with specific antibodies to separate target bacteria or virus in a food or poultry sample and concentrate them for detection using a biosensor; (2) design and fabricate immuno-nanowire based micro/nanoelectrodes or quantum dots based fluorescent detector to improve detection sensitivity and reduce assay time; and (3) evaluate the biosensor for rapid detection of L. monocytogenes, S. Typhimurium and E. coli O157:H7 in different food samples, and for in-field detection of avian influenza virus in poultry swab samples. The biosensor consists of a sampler, multiple-section microfluidic cartridges, a pumping unit, an impedance detector, a microprocessor, a display, a key panel, and a USB connector. When a food or poultry sample, containing various biological and chemical components with bacteria/virus, is dropped, it is mixed with magnetic nanobeads coated with antibodies for several min to get sufficient immunoreactions to capture target bacteria/virus. Then, the target bacteria/viruses are separated by applying a magnetic field to hold magnetic nanoparticles while washing. During their flowing through a micro/nanofluidics channel, target bacteria/virus are captured by the antibodies immobilized on the nanowire/nanoelectrode/nanochannel. Free nanobeads and others can pass through the channel. The change in impedance, caused by captured target bacteria/virus, is measured and correlated to the concentration of bacteria/virus in a sample. A research prototype of nano-biosensor has been designed, fabricated, and tested. The nano-biosensor will be further optimized, improved and evaluated for its applications in agriculture and foods. In 2009, we have conducted the following research projects on nanotechnology and biosensors: (1) A biosensor for rapid, sensitive and specific detection of avian influenza virus H5N1, sponsored by USDA/NRI; (2) A nanowire switch and nanoelectrode/nanochannel based impedance biosensor for rapid screening of avian influenza, sponsored by USDA/NRI; (3) Aptamer SPR biosensor for rapid detection of avian influenza virus, sponsored by Arkansas Biosciences Institute; (4) Magnetic nanoparticle microfluidics for high efficient capture, separation and concentration of foodborne pathogens, funded by NSF/STTR, in collaboration with Ocean NanoTech LLC; and (5) Development of aptamer-ssDNA intelligent hydrogel materials for magnetoelastic detection of avian influenza virus, funded by NSF/STTR, in collaboration with SenMater Technology LLC.

Outcomes/Impacts:

Contaminated food is estimated to cause 76 million illnesses, 325,000 serious illnesses resulting in hospitalization, and 5,000 deaths in the United States each year (CDC, 1999). The economic impact of foodborne illness has been estimated as high as $10 billion annually (USDA/ERS, 2002). Current practices for preventing foodborne diseases due to microbial contamination of food products rely upon rapid identification and effective control of specific pathogens from farm to fork. However, conventional culture methods are extremely time-consuming, typically requiring at least 24 h and complicated multi-steps to confirm the analysis. Even current rapid methods such as ELISA and PCR still take 4-8 h to generate only qualitative results and require laboratory setup and skilled personnel. Highly pathogenic avian influenza (AI) virus H5N1 has been reported by WHO (2010) in more than 46 countries for animal cases and in 15 countries for human cases with 471 people infected and 282 died since 2003. In the US, a recent outbreak of low pathogenic AI in 2001 and 2002 resulted in the depopulation of over 4.5 million chickens and turkeys and had cost the poultry industry approximately $125 million. World Bank estimated that more than 140 million birds had died or been destroyed due to AI H5N1 and losses to the poultry industry are in excess of $10 billion worldwide. The technology for detection of AI H5N1 is mature, but these tests are complex, some are liable to error, and some can be performed safely only in BSL3 facilities. The nano-biosensors being developed in this project will provide the food industry and poultry in-

dustry with more rapid, specific, sensitive and cost-effective method for the detection of pathogenic bacteria/virus in food/poultry samples. The nanoparticles based fluorescent biosensor is able to detect several cells of L. monocytogenes in a food sample or several hundred cells of Listeria, Salmonella and E. coli O157:H7 simultaneously. The nanowire/nanoelectrode based biosensor can detect several cells of foodborne pathogens. The magnetic nanoparticles and microfluidics based impedance biosensor can detect AI H5N1 and H5N2 at 103 EID50/ml in a swab poultry sample. The biosensors developed in this project are rapid, robust and reliable, and suitable for on-line or in-field use to detect pathogenic bacteria/virus, providing the food and poultry industries with a very needed technology for rapid screening of S. Typhimurium, E. coli O157:H7, L. monocytogenes, AI H5N1 or other subtypes in food and poultry. Therefore, the outcome of this study on nanotechnology-based biosensors for rapid detection of pathogens will assist the poultry and food industries in their efforts to minimize the testing cost, ensure product safety and security, and prevent international trade barriers. In general, this research is leading to the development of a portable biosensor instruments for on-line or in-field rapid detection of foodborne pathogens or avian influenza virus. The biosensor technology can also be applied to other areas such as environmental protection and clinical diagnosis.

Publications:

Kanayeva, D., R. Wang, and Y. Li. 2009. Immunomagnetic separation of Listeria monocytogenes using nanosized beads. Presented at IAFP 2009 96th Annual Meeting, July 12-15, 2009, Grapevine, TX. Poster No. P2-097.

Li, D., R. Wang, Y. Li, Y. Ying and J. Wang. 2009. Detection of E. coli O157:H7 using a piezoelectric immunosensor with gold nanowire modified electrode. Presented at ASABE 2009 Annual Meeting, June 21-24, 2009, Reno, NV. ASABE Paper No. 096631.

Lin, J., and Y. Li. 2009. Bionanobeads-based electromagnetic separator for rapid separation of pathogenic bacteria. Presented at ASABE 2009 Annual Meeting, June 21-24, 2009, Reno, NV. ASABE Paper No. 097079.

Li, Y., H. Lu, T. Huang and C. Ruan. 2009. Nanowire switch and nanoelectrode/nanochannel based impedance biosensor for rapid screening of avian influenza. USDA/CSREES-AFMNet Joint Nanotechnology Grantees Meeting, September 27-28, 2009, Santa Fe, NM.

Lassiter, K., R. Wang, J. Lin, J. Lum, B. Srinivasan, L. Lin, H. Lu, B. Hargis, W. Bottje, S. Tung, L. Berghman, and Y. Li. 2009. Comparison study of an impedance biosensor and rRT-PCR for detection of avian influenza H5N2 from infected chickens. Abstract in the Program Book of PSA 2009 Annual Meeting, Raleigh, NC, July 20-23, 2009. Poster No. 405-P.

Varshney, M., and Y. Li. 2009. Review: Interdigitated array microelectrodes based impedance biosensors for detection of bacterial cells. Biosensors & Bioelectronics 24:2951-2960.

Wang, H., Li, Y., and M. Slavik. 2009. Simultaneous separation and detection of multiple foodbonre pathogens using magnetic nanobeads and quantum dots. Presented at IAFP 2009 96th Annual Meeting, July 12-15, 2009, Grapevine, TX. Poster No. P2-113.

Wang, R., Y. Wang, K. Lassiter, Y. Li, B. Hargis, S. Tung, L. Berghman, and W. Bottje. 2009. Interdigitated array microelectrode based impedance immunosensor for detection of avian influenza virus H5N1. Talanta 79:159-164.

Wang, Y., R. Wang, Y. Li, B. Hargis, S. Tung, L. Berghman, and W. Bottje. 2009. An interdigitated array microelectrode based immunosensor optimized for detection of avian influenza virus H5N1. Analyst (in press).

Wang, Y., R. Wang, Y. Li, B. Srinivasan, S. Tung, M. Slavik, and C. Griffis. 2009. Detection of Escherichia coli O157:H7 using an interdigitated array microelectrode based immunosensor. Biological Engineering. (in press)

Xu, K., J. Huang, Z. Ye, Y. Ying and Y. Li. 2009. Review: Recent development of nano-materials used in DNA biosensors. Sensors 9(7):5534-5557.

Patents:

Li, Y., M. Varshney, and Z. Ye. 2005. Separation System and Efficient Capture of Contaminants Using Magnetic Nanoparticles. US Patent No. 7,699,979 B2, April 20, 2010.

Compadre, C.M., P.J. Breen, H. Salari, E.K. Fifer, D.L. Lattin, M.F. Slavik, Y. Li, T. O'Brien, A.L. Waldroup and T.F. Berg. 2009. Concentrated, Non-foaming Solution of Quaternary Ammonium Compounds and Methods of Use. US Patent No. 7,541,045 B2, June 2, 2009.

(AZ) University of Arizona
Jeong-Yeol Yoon

Outputs

(1) Pathogen detection in lab-on-a-chip: In 2009, we have primarily worked on developing a portable lab-on-a-chip system, through replacing micropositioning stages and optical fibers with on-chip optical waveguides and a miniature spectrometer with an Avalanche photodiode (APD) circuit. External computer was also replaced with a microcontroller board (Arduino) and an LCD display, both powered with two 9-V PP3 batteries. Waveguide chips produced much better reproducibility and sensitivity than our old systems, and successfully tested for the viruses in field samples (nasal swabs from pigs, chicken feces, air samples from swine housing, etc.) with the same impressive detection limits (1 pg/mL avain influenza antigens; 10 TCID50/mL PRRSV). We have also tested E. coli in fresh vegetables with the same system, with detection limit of <100 CFU/mL. (2) Wire-guide droplet microfluidics: This newly conceived method of droplet microfluidics was applied to very quick reverse transcription polymerase chain reaction (RT-PCR). The system is capable of amplifying the 160-bp genes extracted from 2009 H1N1 influenza A from human. 30 RT-PCR cycles were performed for 6 min 50 sec. (3) Protein nanoarray construction: A protein nanoarray made by size-dependent self-assembly (SDSA) successfully detected Octamer-4 (a differentiation factor from human embryonic stem cells) through utilizing FRET as sensing modality. This protein nanoarray is currently being implemented in creating a blood vessel mimic (BVM), by patterning a certain combination of receptors/cytokines/growth factors in a desired nanoarray geometry within a synthetic vascular graft.

Outcomes / Impact

(1) The portable lab-on-a-chip system can be installed in livestock environments to continuously monitor the spread of viral pathogens. This system has a potential to be adapted to human environments, such as a theater, an aircraft cabin or a classroom, to monitor, for example, H1N1 flu. (2) The portable lab-on-a-chip system can also be used routinely in field to monitor contaminations (E. coli and Salmonella) found in leafy vegetables, and potentially other produce as well (ground beef, for example). The wire-guide droplet PCR system can also be made portable to be used for monitoring food safety, which will function as a high-end device (while the waveguide lab-on-a-chip would serve as a low-end device). (3) The protein nanoarray system can become an important tool in designing a better blood vessel mimic, so that tissue-engineered vascular grafts can be used also for small-diameter vessels, hopefully resolving many complications in cardiovascular diseases.

Publications (3/19/2009 - 5/6/2010)

Peer-reviewed journal publications, published or submitted:

Kwon HJ, Lee CH, Choi EJ, Song JY, Heinze BC, Yoon JY. Optofluidic device monitoring and fluid dynamics simulation for the spread of viral pathogens in a livestock environment. Submitted.

Heinze BC, Gamboa JR, Kim K, Song JY, Yoon JY. Optofluidic biosensor for sensitive detection of avian influenza antigens in a real biological matrix. Submitted.

Tran PL, Gamboa JR, You DJ, Yoon JY. FRET detection of octamer-4 on a protein nanoarray made by size-dependent self-assembly. Anal. Bioanal. Chem. Revision requested.

You DJ, Tran PL, Kwon HJ, Patel D, Yoon JY. Very quick reverse transcription polymerase chain reaction for detecting 2009 H1N1 influenza A using wire-guide droplet manipulations. Faraday Discuss. Accepted.

Kwon HJ, Dean ZS, Angus SV, Yoon JY. Lab-on-a-chip for field Escherichia coli assays: long-term stability of reagents and automatic sampling system. J. Assoc. Lab. Automat. 2010, 15(3): 216-223.

Han JH, Kwon HJ, Yoon JY, Kim K, Nam SW, Son JE. Analysis of the thermal environment in a mushroom house using sensible heat balance and 3-D computational fluid dynamics. Biosyst. Eng. 2009, 104(3): 417-424.

Powell TB, Tran PL, Kim K, Yoon JY. Size-dependent self-assembly of submicron/nano beads-protein conjugates for construction of a protein nanoarray. Mater. Sci. Eng. C. 2009, 29(8): 2459-2463.

Yoon JY, Riley MR. Grand challenges for biological engineering. J. Biol. Eng. 2009, 3: 16.

Yoon JY, Han JH, Choi CY, Bui M, Sinclair RG. Real-time detection of Escherichia coli in water pipe using a microfluidic device with one-step latex immunoagglutination assay. Trans. ASABE. 2009, 52(3): 1031-1039.

Han JH, Yoon JY. Reusable, polyethylene glycol-structured microfluidic channel for particle immunoassays. J. Biol. Eng. 2009, 3: 6.

Heinze BC, Song JY, Lee CH, Najam A, Yoon JY. Microfluidic immunosensor for rapid and sensitive detection of bovine viral diarrhea virus. Sens. Actuat. B. 2009, 138(2): 491-496.

Books

Yoon JY, Lucas LJ. Biosensors: From Electric Circuits to Immunosensors. Springer: New York, 2010, ISBN 978-1441960214, in press.

Invention disclosure

Yoon JY, Song JY. Single cell level detection of E. coli in microfluidic device. US Patent, pending.

Yoon JY. Devices and methods for detection of microorganisms. US Patent.

(HI) The University of Hawaii

Daniel M. Jenkins

Outputs:

Research for the project proceeded along roughly three parallel tracks: 1) evaluation and comparison of existing diagnostic protocols to identify R. solanacearum in infected soil throughout the duration of its life cycle; 2) develop sampling technologies to improve the collection efficiency of pathogens from dilute samples of soil, water, or plant tissue and improve the detection limit of downstream detection, and; 3) engineering new tools for the rapid detection of DNA collected from pathogens.

For the first track of research, a long term (5 month) study was conducted with regular screening of soil and drainage water samples from plants infected with R. solanacearum, using a variety of detection methods to determine methods with the greatest efficacy for surveillance of plant and soil materials and agricultural land to prevent the spread of disease.

To develop improved methods of pathogen recovery from agricultural samples, work focused on developing functionalized particles to selectively capture the pathogen. Initial focus was to develop a portable magnetically stabilized fluidized bed (MSFB) to process a continuous flow.

An alternative approach included the use of nanometer scale (to enhance the speed of binding to cells) magnetic anion exchange particles.

A variety of approaches were investigated to develop rapid DNA diagnostics for R. solanacearum. These included:

- Use of a disposable electrochemical strip with immobilized hybridization probe for the direct detection of DNA

- Application of R. solanacearum selective bacteriophages for indirect detection pathogen

- Adaptation of isothermal DNA amplification technologies

- Development of novel fluorescent hybridization probes for the real-time direct detection amplified bacterial DNA

- Development of handheld instruments to enable field detection

Results have been disseminated to scientific communities and to the public through a variety of publications and conference presentations (see publications below) as well as research highlights produced by the University of Hawaii's College of Tropical Agriculture and Human Resources (see videos "Ginger production disease management strategies", and "Saving ginger root in Hawaii" available at http://www.ctahr.hawaii.edu/t-star/TSTARHilitePage.htm; and article "Engineering New Tools to Protect Agriculture in Hawaii", http://www.ctahr.hawaii.edu/ctahr2001/Research/Downloads/ResearchNews/CTAHR_Research_News_November_08.pdf) and publically available articles (see "The business of growing ideas", The Quarterly Journal for Science and Technology in Hawaii, available at http://www.hawaiibusiness.com/pdfs/12-08_Upload_Web_sprd.pdf).

Technologies developed during the course of this project will be demonstrated and disseminated at a workshop at the 2010 meeting of the American Phytopathology Society in Nashville, Tennessee. A business model predicated on dissemination of these technologies won first prize at the University of Hawaii Business Plan competition in 2010, and the participants are actively pursuing commercialization of the technology through funding by investors and SBIR opportunities.

Outcomes/Impacts:

The technologies developed in this research are intended to allow rapid detection of Ralstonia solanacearum, which is a devastating bacterial wilt pathogen infecting a wide variety of crops. The race 3 biovar 2 strains of the pathogen currently are not present in North America though they have cold tolerant characteristics and would be devastating to potato if released on the continent, so that

rapid detection technologies to deploy in imported plant and soil materials is essential for a successful containment program.

Research from this project has helped develop baseline protocols by which testing in soil, plant tissue, and water samples may be implemented in the event of a release of the pathogen into the environment. While rapid disposable immunostrips may provide the first response, they are not selective to individual populations of the pathogen, and so additional confirmation by culturing and subsequent molecular screening is essential. Innovative technologies developed during the course of this project promise to improve the reliability of molecular screening methods so that detection can occur rapidly (within hours) and selectively to ensure that the target organism is correctly identified, thus saving on unnecessary containment actions which are destructive and can result in a high degree of panic to the public which adds additional economic burden on farmers. Our ability to detect pathogens at a very low level has been considerably improved by using a simple and efficient method to concentrate the pathogen targets before subsequent molecular detection. Our research demonstrated the usefulness and versatility of low-cost semi-selective magnetic particles coupled with permanent or electromagnets in achieving such goal.

For DNA detection from specific pathogens, we were able to use bacteriophages to allow the indirect detection of pathogen at levels lower than 10 CFU/ml in raw plant tissue and soil extracts. We have also successfully adapted protocols for Loop-mediated isothermal Amplification (LAMP). Using this process, we have successfully engineered primers for the detection of all R. solanacearum populations, as well as for the selective detection of race 3 biovar 2 strains. Engineering of a novel hybridization probe incorporated into the LAMP amplicon has allowed real time detection of pathogen within 20 minutes, in a process that can be easily controlled and monitored with a simple handheld device. Several provisional patents have been filed for these technologies.

Publications:

Journal Publications:

Paret ML, Kubota R, Jenkins DM, Alvarez AM. (2010). Survival of Ralstonia solanacearum race 4 in drainage water and soil, and detection with immunodiagnostic and DNA-based assays. HortTechnology. 20(3):

Kubota R, Schell MA, Peckham GD, Rue J, Alvarez AM, Allen C, and Jenkins DM. (2010) In Silico Genomic Subtraction Guides Development of Highly Accurate, DNA-Based Diagnostics for Ralstonia solanacearum Race 3 Biovar 2 and Blood Disease Bacterium. European Journal of Plant Pathology (Under Review)

Yang K, Xu NS, Su WW (2010) Co-immobilized enzymes in magnetic chitosan beads for improved hydrolysis of macromolecular substrate under a time-varying magnetic field. Journal of Biotechnology (accepted pending minor revision).

Yang K, Jenkins DM, Su WW (2010) Rapid enrichment of bacteria using submicron magnetic anion exchangers for PCR-based multiplexed pathogen detection (In preparation).

Kubota R, Geisen T, Su W and Jenkins DM (2010). Hand held device with non-contact temperature control and custom fluorometer for sequence specific DNA amplification and detection. (In preparation)

Jenkins D. M., Song, C., Fares, S., Cheng, H., and Barrettino, D. (2009). Disposable thermostated electrode for temperature dependent electrochemical measurements, Sensors and Actuators, B- Chemical. 137(1):222-229.

Kutin, R., Alvarez, A., and Jenkins, D. M. (2009). Detection of Ralstonia solanacearum in natural substrates using phage amplification integrated with real-time PCR assay, Journal of Microbiological Methods. 76(3):241-246.

Abstracts & Conference Papers:

Kubota R, Kawabata NY, Miyamoto AI, Alvarez AM, Schell MA, Allen C, Jenkins DM. (2009). Engineering a Real-Time Disposable Platform for Discrimination of Sub-Populations of Ralstonia solanacearum. American Society for Agricultural and Biological Engineers Annual International Meeting, Reno, NV.

(IA) Iowa State University

Chenxu Yu

Outputs:

The activities during this period included: 1. the development of nitrite sensors using functionalized gold nanorods to detect trace amount of nitrite (as low as 0.25 ppm) in drinking water. Gold nanorods (GNR) were functionalized with 4-aminothiophenol (4-ATP). In the presence of nitrite ions, deamination reaction was induced by heating the 4-ATP modified GNR in ethanol solution, resulted in

the reduction of the GNR surface charges, which led to aggregation of GNRs and a colorimetric response that was quantitatively correlated to the concentration of nitrite ions. This simple assay was rapid (10 minutes) and highly sensitive (< 1 ppm of nitrite), it can be used for rapid monitoring of drinking water quality. 2. nanoSPR enhanced IR spectroscopic biosensing methodology for detection of microorganisms in mixed cultures. In this work, nanoparticle-induced nanoSPR enhanced IR spectroscopy was used in conjunction with a background elimination data processing algorithm to directly identify microorganisms in mixed cultures. It was demonstrated that the microbial composition of mixtures of different E. coli strains could be identified with 100% accuracy. The procedure was also applied to determine the presence or absence of pathogenic microorganisms in a simple but real food matrix (apple juice). Results indicated that microorganisms in a cocktail of up to eight different species suspended in an apple juice matrix could be identified for its presence or absence with 100% accuracy. 3. Continuous work on process characterization of zero flash embossing as well as biosensor characterization in terms of resolution and fidelity. The products from this period included: fundamental understandings of enzymatic oxidation of glucose in the presence of glucose, ethanol and lactate oxidase (GOx).

These findings were presented in several peer reviewed conferences and three journal articles.

Outcomes/Impacts

Impacts included the development of novel techniques for the detection of trace amount of nitrite in drinking water, detection and identification of microorganisms with strain-level differentiation resolution in mixed cultures, and the detection of glucose, ethanol, lactate and oxygen concentration with identical architectures in a lab-on-a CD format. These techniques are highly-accurate, they have the potential to meet the needs in various disciplines for high-accuracy target sensing. And yet these techniques, built upon applications of nanoparticles and/or nanostructures, are user-friendly and easy-to-implement; all of them are envisioned to be low-cost and disposable, allowing high frequency testing without the risk of cross contamination, a characteristic that is critical for many applications, such as testing for pathogens in food processing. In addition, because of the laws of scaling, these devices will function rapidly and allow nearly immediate diagnosis, being a critical feature in detecting possible pathogen-based terrorist threats.

Publications

Qi Wang, Nan Xiao, Chenxu Yu, Detection and identification of microorganisms in mixed cultures by nanoparticle-induced nanoSPR enhanced FTIR spectroscopy and chemometrics, ASABE Annual International Meeting, 2009.

Nan Xiao and Chenxu Yu, Colorimetric Nitrite Sensor Using 4-Aminothiophenol Modified Gold Nanorods, 44th ACS Midwest Regional Meeting, 2009

Chenxu Yu, New Frontier in Biosensing and Biological Imaging using Nanosensors, Donghua University, China, Invited Lecture, 2009

Qi Wang, Nan Xiao, Chenxu Yu, Detection and identification of Foodborne Pathogenic Microorganisms in mixed cultures by nanoparticle-induced nanoSPR enhanced FTIR spectroscopy and chemometrics, Transaction of ASABE, in press

Nan Xiao and Chenxu Yu, Rapid Colorimetric Nitrite Sensing Using 4-Aminothiophenol Functionalized Gold Nanorods, Analytical Chemistry, 2010, 82 (9), pp 3659–3663

Srikanth Vengasandra, Greg Harmon, David Grewell, Zero Flash Ultrasonic Micro Embossing on Foamed Polymer Substrates A Proof of Concept, Polymer Engineering and Science, 49(11): 2204-2211, 2009

Srikanth Vengasandra, Yuankun Cai, David Grewell, Joseph Shinar, Ruth Shinar, Polypropylene CD-organic light-emitting diode biosensing platform, Lab Chip, 2010, DOI: 10.1039/b923689a

Participants

Chenxu Yu, Assistant Professor, Iowa State University, Department of Agricultural and Biosystems Engineering

Nan Xiao, Graduate Student, Iowa State University, Department of Agricultural and Biosystems Engineering

Qi Wang, Graduate Student, Iowa State University, Department of Agricultural and Biosystems Engineering

David Grewell, Assistant Professor, Iowa State University, Department of Agricultural and Biosystems Engineering

Srikanth Vengasandra, Graduate Student, Iowa State University, Department of Agricultural and Biosystems Engineering

Yuankun Cai, Graduate Student, Iowa State University, Department of Physics

Ruth Shinar, Professor, Iowa State University, Department of Physics

Joseph Shinar, Professor, Iowa State University, Department of Physics

Target Audience

These works were presented in two peer reviewed conferences. The audience included engineers and scientists developing novel nanomaterials and sensing platform/technologies for various applications in agriculture, biological and biomedical engineering, chemistry, environmental engineering. The two conferences are also open to industrial personnel as well as general public. Our target audience also include people who are interested in identifying novel technologies to address their needs for monitoring chemical contaminations as well as biological hazards in water, food and environments.

Project Modification

A new group (Yu group) has joined the project in 2009 to expand the scope of the work on nanotechnology and biosensing to include new approaches (nanoparticles enhanced spectroscopy), in addition to the previous efforts led by Dr. Grewell.

(IL) University of Illinois

Kaustubh Bhalerao

Environmental Impact of Nanotechnology

Whether particulates are quantum dots, soot particles, metal oxides, or carbon nanotubes, to anticipate their impact on biological systems in the environment, we must have the capacity to analyze their effects over multiple scales by tracking and monitoring these particles and their impact on the living entities that they encounter. Imagine some CdSe quantum dots (QD) or a single walled Carbon Nanotubes (CNT) being aerosolized due to the lack of proper quality control in the nano-manufacturing processes. These nanoparticles, engulfed in the aerosol moisture droplet, could find their way to the environment and then be inhaled by animals or humans. What would be the interaction of the moisture-laden nanoparticle entity with biological cells, especially the cells that mimic the entry way into humans, i.e. breathing through the lungs. It has been shown that nanoparticles in with diameter less than a 100 nm in fluid can penetrate through biological cell membranes and can have detrimental cytotoxic effects on the cells, however, the impact of aerosolized nanoscale entities on biological material is much less understood. Furthermore, what is the impact of nanotechnology on the lowest strata of our ecosystem, namely, the photosynthetic cyanobacteria? Few studies have explored these systems vis a vis nanotechnological impacts, yet we believe these are the most important systems to study because of the potential of bioaccumulative and biomagnification phenomena that can occur as the particles travel up the food chain. We are looking at the impact of quantum dots and carbon nanotubes on the growth rates, metabolic impact and phenotypic changes in a freshwater cyanobacterium, *Synnechococcus elongatus* PCC 7942.

Synthetic biology and the development of single cell biosensors

Whole cell biosensors are unicellular living organisms designed to detect and report various analytes of interest in their immediate surroundings. Whole cell biosensors could have a significant impact in environmental monitoring, as sentinel organisms in microbial colonies, as detectors of other pathogens and contaminants and in life sciences research. A major drawback with these systems is their unpredictable sensitivity to the analytes of interest. We have been using principles of synthetic biology to develop "signal amplifiers" within whole cell biosensors that can amplify small, transient, biochemical signals produced in response to environmental analytes. In addition to amplification, the synthetic circuits also show "memory-like" behavior: the whole cell biosensor continues to respond to the stimulus long after the stimulus is removed.

Publications:

Journal articles

K. Bhalerao, (2009), Programmable gene networks: The next wave in biotechnology? Trends in Biotechnology. Vol. 27, No. 6, pp 368-373. (Invited Review)

G. Nistala, K. Wu, K.D. Bhalerao and C.V. Rao (2010), A modular positive feedback-based gene amplifier. Journal of Biological Engineering. Vol. 4, No. 4

K. Bansal, K. Yang, R. Gennis, and K.D. Bhalerao (2010), A positive feedback-based gene circuit to increase the production of a membrane protein. Journal of Biological Engineering. Vol. 4, No. 6.

R. Balachandran, V. Parekatt, P. Poisson, G. Nistala and K. Bhalerao (2010) Stress hardened bacterial cells as chasses for synthetic biology. Journal of Biological Engineering. (submitted)

Conference presentations

G.J. Nistala, and K.D. Bhalerao (2009) Using a PF synthetic gene motif to develop a single cell biosensor and to improve protein production, March 19-22, 2009, Santa Clara, CA.

K. Bansal and K.D. Bhalerao (2009) Increasing membrane protein production using a positive feedback-based 'gene amplifier', March 19-22, 2009, Santa Clara, CA.

S.M. Kim, R. Bashir and K. Bhalerao, DIMER - a framework to study environmental toxicity of nanoparticles, IBE Conference Proceedings, March 4-6, 2010, Boston, MA.

K. Bhalerao, G.J. Nistala and R. Balachandran, Metabolic burden in synthetic gene circuits, IBE Conference Proceedings, March 4-6, 2010, Boston, MA.

Book Chapters:

K.D. Bhalerao and G. Nistala (2007) Nanoscale Biology: Engineering Applications. Encyclopedia of Agricultural, Food and Environmental Engineering. Editor: Dennis R. Heldman. Commissioned by Taylor & Francis Group. Accepted. Publish date 9/2010.

K. D. Bhalerao and G.J. Nistala (2010) Fundamentals of Biorecognition: Biomolecular Components of a Sensor. Biomedical Nanosensors. Editor: Joseph Irudayaraj. Accepted.

(NE) University of Nebraska

Yixiang Xu and Milford Hanna

The successful preparation of bovine serum albumin (BSA)-loaded polylactic acid (PLA) using a two-phase coaxial jet electrospray technique demonstrated the feasibility of a two-phase coaxial jet electrospray technique to encapsulate bioactive ingredients (Xu and Hanna, 2008), as well as triggered our interest in extending its application to foods. Currently, we are working on encapsulating vitamin C with zein to generate micro/nano capsules using two-phase coaxial jet electrospraying. The effects of electric field, physical properties and flow rates of the two immiscible solutions on capsule size, morphology, and physiochemical properties of the resulting particles will be investigated. The properties to be measured include apparent characteristics, surface characteristics, core-shell structure, yield, encapsulation efficiency, and controlled release.

Pubications:

Xu, Y.X., and Hanna, M.A. 2008. Morphological and structural properties of two-phase coaxial jet electrosprayed BSA-PLA capsules. Journal of Microencapsulation 25(7):469 - 477.

(NY) Cornell University

Antje Baeumner

Outputs

Pathogen detection in lab on a chip systems: We have worked on the development of a number of different platforms over the course of the last year. This includes microfluidic channels for the isolation and amplification of RNA molecules of the protozoan parasite Cryptosporidium parvum; transfer of the technology to a commercial microfluidic system in collaboration with Rheonix, Inc., development of purely polymer-based electrochemical lab-on-a-chips by making interdigitated ultramicroelectrode arrays directly on polymethyl methacrylate; and by investigating the possibility of using biofunctional electrospun nanofibers directly in microfluidic channels.

Outcome/Impact

We were able to demonstrate the detection of 1 viable C. parvum oocyst with our devices, tested C. parvum directly in environmental water samples (100 L and more) at levels below 50 oocyst per sample. We have applied our electrochemical microfluidic biosensor to the detection of cholera toxin in real samples.

Over the past decade, rapid point-of-care (POC) tests have emerged as one of the largest and fastest growing segments of the human in vitro diagnostic testing market and can also be found in food safety applications. Due to their relative ease, most such tests rely upon immunologic methods. A common testing format employs specific antibodies that recognize and interact with the analytes of interest in a manner that will also induce the generation of a discernable color within a lateral flow device. Such tests are frequently designed to yield qualitative (i.e., yes or no) results rather than quantitative tests. Despite the added information that can be obtained via molecular detection methods, similar rapid tests employing gene detection methods have not yet found widespread use, which is pri-

marily due to the more complex and time-consuming nature of sample preparation methods as well as the more technically complicated gene amplification techniques.

The approach developed by us will greatly ameliorate the situation since it combines a quantitative approach with the highly selective nucleic acid recognition of pathogenic organisms and simplifies the amplification techniques. Thus, pathogens will be detectable and quantifiable at significantly lower costs, and tests will be more reliable since fewer assay steps are needed.

Publications (2009/2010)

Goddard, J., Mandal, S., Nugen, S., Baeumner, A., Erickson, D. "Patterning of Nucleic Acid Probes in Optical Nanocavities", Colloids and Surfaces B: Biointerfaces vol. 76, pp. 375–380, doi:10.1016/j.colsurfb.2009.10.041)

Kumanan, V., Nugen, S.R. Baeumner, A.J. Chang, Y-F "A biosensor assay for the detection of Mycobacterium avium subsp. paratuberculosis in fecal samples" J. of Veterinary Science, vol. 10(1), pp. 35 - 42, (2009)

Nugen, S.R., Asiello, P.J., Connelly, J.T., Baeumner, A.J. "PMMA biosensor for nucleic acids with integrated mixer and electrochemical detection" Biosensors and Bioelectronics, vol. 24, pp. 2428 – 2433 (2009)

Bunyakul, N., Edwards, K.A., Promptmas, C., Baeumner, A.J. "Cholera toxin subunit B detection in microfluidic devices" Analytical and Bioanalytical Chemistry, vol. 393(1), p. 177 – 186, Special anniversary issue. (2009)

Nugen, S.R., Asiello, P., Baeumner, A.J. „Design and fabrication of a microfluidic device for near-single cell mRNA isolation using a copper hot embossing master" Microsystem Technology vol. 15(3), pp. 477 – 483 (2009)

(SC) Clemson University

Jeremy Tzeng

My research team has participated in the Objective 3 of the proposed project developing nanoscale devices and systems. My tasks are to develop alternative microbial capturing agents for use in biosensor applications, and to develop microfluidic devices for manipulation of bacterial particle movement. We have evaluated adhesin-specific nanomechnical cantilever biosensors for detection of microorganisms. In this work, carbohydrate receptor molecules recognized by specific bacterial adhesins were covalently attached to surfaces of gold cantilevers. Binding of bacteria to the sensor surface mediated by adhesion-carbohydrate interaction is determined by measuring changes in oscillation frequency with MSA-400 Micro System Analyzer. We have also fabricated AC/DC driven microfluidic devices for focusing, concentrating, trapping, followed by an in-line RF sensor for detection, enumeration, and differentiation of viable and non-viable targets in low number. Two manuscripts and six conference proceedings have been published during this project year.

Publications

Peer-Reviewed Journal Articles:

Church, C., J. Zhu, G. Wang, T.-R. J. Tzeng and X. Xuan, 2009 Electrokinetic focusing and filtration of cells in a serpentine microchannel. Biomicrofluidics 3: 044109-044110.

Zhu, J., T.-R. Tzeng, G. Hu and X. Xuan, 2009 DC dielectrophoretic focusing of particles in a serpentine microchannel. Microfluidics and Nanofluidics 7: 751-756.

Peer-Reviewed Conference Proceedings:

Adhesin-Specific Nanomechnical Cantilever Biosensors for Detection of Microorganisms, ASME 2009 Micro/Nnoscale Heat and Mass Transfer International Conference, Shanghai, China

Tzuen-Rong, J. Tzeng, Yunyan R. Cheng, Reza Saeidpourazar, Siddharth S. Aphale, Nader Jalili

Broadband Dielectric Properties Characterization of Biological Cells, ASME 2009 Micro/Nnoscale Heat and Mass Transfer International Conference, Shanghai, China

Yang Yang, Rahul Mitchell Jairaj, Gaoyan Wang, Jeremy Tzeng, Xiangchun Xuan, Kama Huang, and Pingshan Wang

Gentle Dielectrophoretic Focusing of Yeast Cells in Curved Microchannels, ASME 2009 Micro/Nnoscale Heat and Mass Transfer International Conference, Shanghai, China

Church Christopher, Junjie Zhu, Tzuen-Rong, J. Tzeng, Xiangchun Xuan, Gaoyan Wang

Dielectrophoretic Focusing of Microparticles in Curved Microchannels, In International Mechanical Engineering Congress & Exposition, 2009, Lake Buena Vista, Florida.

Zhu, J., Tzeng, T.-R. J., and Xuan, X. Dielectrophoretic Separation of Microparticles in Curved Microchannels, In International Mechanical Engineering Congress & Exposition, 2009, Lake Buena Vista, Florida.

Zhu, J., Tzeng, T.-R. J., and Xuan, X. Electric Trapping and Lysing of Cells in a Microchannel Constriction, In International Mechanical Engineering Congress & Exposition, 2009, Lake Buena Vista, Florida.

Church, C., Zhu, J., Huang, G. G., Wang, G., Tzeng, T. R. J., and Xuan, X.

(WI) University of Wisconsin at Madison

Sundaram Gunasekaran

Outputs

Biosensor for Detection of Toxins in Foods: Work is under way to detect botulinum neurotoxin type A (BoNT/A), one of the most poisonous substances known to humans. Its lethal toxicity makes it potentially suitable as a biological warfare and/or bioterrorism agent. We will develop quartz crystal microbalance (QCM)-based biosensor for detection of BoNT/A in food systems and improve its usefulness by enhancing detection sensitivity by employing nanomaterials (e.g., gold nanoparticles, carbon nanotubes) in the fabrication of crystal surface. In this study, the established immunoassay and the QCM with dissipation monitoring (QCMD) will be utilized. The effects of different crystal fabrication techniques and the variable food matrices properties on the detection sensitivity will be investigated. Besides, the kinetics of interaction between BoNT/A and its specific antibodies and the adsorption isotherm of BoNT/A will also be explored. The QCM results will also be compared with other characterization techniques (e.g., atomic force microscopy (AFM), attenuated total reflectance-Fourier transform infrared (ATR-FTIR)).

Whey Protein-based Nanocomposites: Nanocrystalline zinc oxide (ZnO) particles coated with whey protein isolate (WPI) were fabricated in the weak basic aqueous solution condition at near room temperature. The X-ray diffraction and transmission electron microscopy measurements confirmed the nano-scaled composite structure of ZnO-WPI. The average composite granules size was about 300 nm and the embedded ZnO nanoparticles were uniform and monodisperse with an average diameter of 65 nm.In addition, pectin-ZnO nanocomposite was prepared in the aqueous solution condition at room temperature. The Fourier transform infrared, X-ray diffraction, and transmission electron microscope (TEM) measurements confirmed the nano-scaled structure of pectin-ZnO composite.

According to the TEM observation, the average composite granules size was about 150 nm and the embedded ZnO nanoparticles were uniform with an average diameter of 70 nm.

Publications

Ko S, S Gunasekaran, J Yu. 2010. Self-indicating nanobiosensor for detection of 2,4-dinitrophenol. Food Control 21(2):155-161. (doi:10.1016/j.foodcont.2009.05.006).

Zhou J, S Gunasekaran. 2009. Preparation and Characterization of Whey Protein Film Incorporated with TiO2 Nanoparticles. J. Food Sci 74(7):N50-N56. (doi: 10.1111/j.1750-3841.2009.01270.x).

Shi L, S Gunasekaran. 2008. Preparation of pectin-ZnO nanocomposite. Nanoscale Research Letters 3(12):491-495.

Shi L, J Zhou, S Gunasekaran. 2008. Low temperature fabrication of ZnO-whey protein isolate nanocomposite. Materials Letters 62(28):4383-4385.