Nanoliter/Picoliter Scale Fluidic Arrays for Rapid Identification of Pathogenic Bacteria

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The development of a rapid and reliable detection method of food-borne pathogenic bacteria is an important step in improving food safety. Currently available methods require at least an overnight enrichment cultivation step. We proposed a novel, nanotechnology-based detection method to enhance food safety by the use of highly sensitive and specific nanofluidic reactor arrays targeting single pathogenic bacterial cells. During the first year of the project, we focused on developing a nanoliter scale fluidic array and designing the specific primer sets and fluorescent probes for the detection of Listeria monocytogenes, a bacterial strain that causes Listeriosis with fetal rate of 20%. As a result, a nanoliter fluidic array with 10,000 nanoliter reactors was designed and fabricated successfully. In addition, a unique gene of *hemolysin (hly)* of *L. monocytogenes* has been chosen as a target gene to distinguish the target bacterium from other bacterial cells. Combinations of primer sets and probes were designed and evaluated by using a conventional fluorescent PCR method. We finally selected one primer set based on the threshold cycle (C_T) analysis and normalized fluorescence intensity analysis. We demonstrated the detection of a single copy of target molecules with purified genomic DNA and heat-inactivated *L. monocytogenes* on nanoliter fluidic reactor arrays without having an overnight enrichment culture. For the next step, we will demonstrate the detection of single or several *L. monocytogenes* by using combinations of primer sets and probe. Indeed, we will try low temperature gene amplification as a new tool for detection of *L. monocytogenes* by using our nanoliter fluidic arrays.

Patent Application: U.S. Provisional Patent Application No. 61/310,566 entitled "Primers and Probes for Listeria Monocytogens," JW Hong, JY Yun, Filing Date 3/4/2010

Conference Presentation: American Society for Microbiology Southeastern Branch Meeting, " Fluidic Arrays for Rapid Identification of Pathogenic Bacteria," JY Yoon, J. Zou, O. Oyarzabal, JW Hong, Nov. 6, 2010, Montgomery, AL.

Bacteria, Biofilms and Foods: A Nanotechnology-Based Approach

John R. Dutcher, University of Guelph and AFMNet

I will describe recent results from our large, multidisciplinary research project on bacteria at surfaces. In particular, I will focus on nanotechnology-based measurements of components of bacterial cells, individual cells, colonies of cells (biofilms), and novel anti-microbial and anti-biofilm strategies using a variety of experimental and computational approaches.

Strategies used to improve the stability of L-5-methyltetrahydrofolate

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Abstract

Stability of free L-5- methyltetrahydrofolate (MTHF) in model systems and specific food products, (e.g. skim milk (SKM) and soy milk (SOY)), was studied and evaluated using biophysical principles (e.g. Ea, and thermal degradation kinetics). Residual MTHF concentration after thermal treatments was measured using reverse phase high pressure liquid chromatography (RP-HPLC). MTHF degradation followed first-order reaction kinetics in the temperature based model systems. SKM and SOY provided protection to MTHF against thermal condition.

The effect of ascorbate on the stability of MTHF in the model systems was also studied. It was found that the thermal stability of MTHF was enhanced in the presence of increasing vitamin concentration. The reducing activity of ascorbate was effective at limiting the initial oxidation products of MTHF following thermal treatment.

Microencapsulation technology was applied to improve the thermal stability of MTHF. Stearic acid, polysaccharide and skim milk powder were used as coating materials. Electron microscope pictures were obtained for different microencapsulated MTHF products. Results so far showed that stearic acid coating has limited potential to improve the thermal stability of MTHF. Studies on the thermal stability of MTHF with other coating materials and a combination of using antioxidants and microencapsulation to improve the stability of MTHF are currently ongoing.

Molecular Tagging for Food Traceability

Amanda Naaum, Allison Ozog, Apichaya Lilavanichakul, Robert Hanner, Andreas Boecker, Jill Hobbs and Nicholas Low

Traceability systems are in place to track materials and manufactured goods throughout production. However, adulteration of food products remains a constant problem, and causes significant economic loss to consumers and businesses in the industry. Current methods rely on external tags on food packaging, which can be easily tampered with or counterfeited, and which make substitution of package contents impossible to detect. This study aims to introduce the use of internal oligomolecular tags to replace external tags currently in use, with the aim of protecting brand integrity by making adulteration more difficult for unscrupulous manufacturers or retailers. These tags are comprised of unique molecular markers that can be added to food products, and recovered with a variety of techniques to confirm the authenticity of the product.

ABSTRACT EPA-G2010-STAR-N2 Food Matrices: Oral delivery of bioactives with polymeric nanoparticles: bioavailability and tissue distribution of delivered active ingredient, nanoparticle translocation, and overall nanoparticle bioreactivity

PI: C. M. Sabliov[1], Co-PIs: T. Morgan[2], J. Blumberg[3], C. Astete[1], and M. Trif [4]

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Objectives of the study: The overriding goal of the project is to assess the bioreactivity (biotoxicity, inflammatory effects and immune-reactivity) of polymeric nanoparticles specifically designed for delivery of biologically active compounds via food, with a focus on quantifying the bioreactivity as a function of nanoparticle properties and dose, type of entrapped active ingredient, and characteristics of the meal with which administered. The following objectives are proposed: (1) Synthesize and characterize polymeric poly (lactic-co-glycolic acid) (PLGA) and PLGA-Chitosan (PLGA/Chi) nanoparticles of controlled properties (size and zeta potential) with entrapped hydrophilic and hydrophobic bioactives; (2) Measure *ex-vivo* and *in-vivo* nanoparticle intestinal uptake and translocation as a function of composition (PLGA and PLGA/Chi) and nanoparticle properties (size, zeta potential); (3) Study the effect of bioactive solubility, type of delivery system, and type of meal with which administered on pharmacokinetic profiles and tissue distribution of flavanol epigallocatechin gallate (EGCG) and xanthophyll lutein (LT); (4) Determine *in-vivo* acute and chronic biotoxicity, inflammatory effects, and immuno-reactivity associated with nanoparticles; (5) Develop collaboration with EU biochemistry laboratory on polymeric nanoparticle *in-vitro* biotoxicity studies.

The experimental approach: Polymeric nanoparticles of well-controlled sizes, surface properties, and material compositions with entrapped hydrophobic (LT) and hydrophilic (EGCG) bioactives will be synthesized out of PLGA and PLGA/Chi by (double) emulsion evaporation method. Pharmacokinetics of entrapped EGCG and LT in 3 forms (purified, PLGA, and PLGA/Chi) provided via 2 types (normal and high fat) meals will be quantified by HPLC, *in-vivo*. *Ex-vivo* and *in-vivo* experiments will be performed to quantify NP bioadhesion and translocation by confocal microscopy. *In-vivo* NP bioreactivity will be measured by clinical pathologic assessment of serum chemistry parameters and histopathologic evaluation of target organs of mice following acute and chronic exposure to PLGA and PLGA/Chi nanoparticles of two sizes.

The expected results of the project: The mechanisms involved in GI delivery of hydrophobic and hydrophilic bioactives with polymeric nanodelivery systems of controlled characteristics (size and zeta potential) will be elucidated and insights on nanoparticle bioreactivity associated with nanoparticle uptake will be provided. The results are critical in building the knowledge base required to design nanoparticulate systems for safe and efficient delivery of bioactives through food applications.

Project title: Nanoscale analysis of chemical residues in foods using whispering-gallery-mode miniature sensors

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Abstract of Presentation:

The spectral shift response of optical whispering gallery modes due to the adsorption and desorption of water molecules into a SiO_2 nano-coating is investigated. The coating of nano-particles onto the transducer (i.e., silica microsphere) is prepared by the electrostatic-self-assembly (ESA) layer by layer method. The coating quality is characterized by the Atomic Force Microscopy (AFM). Optical shift measurements are made at a very low humidity level (<10%). The micro-optical coupling system is incorporated into a sealed vacuum chamber for controlled humidity testing. Experimental observation of the whispering gallery mode spectral shift with refractive index is consistent with a theoretical analysis using Mie theory. A significant hysteresis effect is observed in the spectral shift of individual whispering gallery modes during variable humidity tests. To understand the hysteresis mechanism, tests with step changes in humidity are performed, which reveal complex and dynamic water molecule transport phenomena between the nano-coating and surrounding environment. A change of 1 ppm of H_2O (0.003% RH) is detectable using the current instrument. This study clearly shows the merit of optical resonance methods as a useful measurement technique for determining surface interactions and transport dynamics within thin film coatings for detection of gas-phase analytes of food safety such as water molecules.

Achievements:

Publications in archival journals with acknowledgement of the USDA grant

1. Q.L Ma, L. Huang, Z. Guo and T. Rossmann, 2010, "Spectral shift response of optical whispering gallery modes due to water vapor adsorption and desorption," *Meas. Sci. Technol.,* vol. 21**,** 115206 (7pp).
2. Q.L. Ma, T. Rossmann and Z. Guo, 2010, "Whispering-gallery mode silica microsensors for cryogenic to room temperature measurement," *Meas. Sci. Technol.*, vol. 21, 025310 (7pp).
3. H. Quan and Z. Guo, 2009, "Analyses of whispering-gallery modes in small resonators," *J. Micro/Nanolith. MEMS MOEMS*, vol. 8 (3), 033060 (7pp).

Publications and presentations in conference with acknowledgement of the USDA grant

1. Q. Ma, L. Huang, Z. Guo, and T. Rossmann, "Whispering-Gallery Mode Silica Micro-sensors for Temperature and Gas-phase Concentration Measurements," AIAA 2010-4911(11pp), 27th AIAA Aerodynamic Measurement Technology and Ground Testing Conference, 28 June – 1 July 2010, Chicago, IL.

Impact of the Physicochemical Properties of Engineered Nanomaterials on their Cellular Uptake and Potential Toxicity in the Gastrointestinal Tract Environment

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Abstract

Nanomaterials are expected to be increasingly incorporated into food ingredients and food packaging. However, understanding of the potential risks associated with consumption of these materials currently lags far behind developmental aspects of this technology. To address these issues, we propose to **test the hypothesis that following ingestion, engineered nanoparticles (ENPs) are modified by the environment of the gastro-intestinal (GI) tract, and that the size, composition, surface charge, crystallinity, and surface chemistry/physics of particles thus modified affect their interactions with intestinal epithelial cells, thereby influencing particle toxicity, bioavailability and tissue distribution.** Toward this end, ENPs of ZnO, SiO_2, TiO_2, silver, and lycopene will be synthesized or acquired, dispersed in matrices which simulate conditions under which they are incorporated into food products, and thoroughly characterized with respect to size, physical and structural form, chemical composition, and surface chemistry/physics (Aims 1 and 2, total of 45 nanoparticles). Matrix-modified particles will be subjected to conditions which simulate the environment of the GI tract (digestive enzymes, acidic environment of the stomach, neutral environment of the small bowel) followed by thorough characterization (Aim 3a). Interactions of "digested" particles with intestinal epithelial cells will be assessed, specifically particle internalization, epithelial cell toxicity and inflammatory responses, and the impact of particles upon intercellular adhesion and epithelial barrier function (Aim 3b). Finally, data generated by these studies will be used to develop material descriptors for computational use such as read-across or quantitative structure-activity relationships between the ENP and their biologic activity (Aim 4). The data will be shared with European Union NATOM partners who have submitted a proposal to EU Seventh Framework Programme (FP7). Data generated by these experiments will provide the foundations for the design of animal studies of nanoparticle translocation across the gastrointestinal epithelium and into the vascular system, routes of *in vivo* distribution, sites of accumulation, and routes of elimination.

A soluble nanoscale self-assembling complex from starch, protein and lipid for healthy nutrient delivery

P.I.s: B.R. Hamaker and O.H. Campanella
Researcher: Deepak Bhopatkar, Ph.D. Student
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ABSTRACT

Our laboratory previously reported an interaction between starch, protein and fatty acid that produces a self-assembled nanoscale complex. Novel aspects of the complex are its water solubility and recently found ability to carry small hydrophobic molecules. Typically amylose-lipid complexes have very low iodine-binding ability caused by complete occupation of the hydrophobic inner helical core of amylose with the aliphatic fatty acid tail. With the three-component soluble complex we speculated from earlier work that the approximately one-third to one-half of the interior amylose helix is free and can used for binding valuable insoluble molecules.

In the current study, we investigated the ability of the nanocomplex to solublize small hydrophobic compounds (fourth component), such as a chemotherapeutic drug (5-flurouracil), a dye (fluorescein), and an antimicrobial compound (α-napthol). The nanocomplex was prepared in a diluted system by mixing supernatant from high amylose corn starch, β-lactoglobulin, and linoleic acid (20:2:1). The fourth component was introduced either during the formation of nanocomplex or after the formation of the nanocomplex. Iodine binding ability of the nanocomplex carrying the fourth component and turbidity was used as tool to confirm inclusion and solubilization of fourth component in the nanocomplex. For α-napthol, HPSEC was used to determine its binding to the nanocomplex. It was hypothesized that complete saturation of available sites of amylose helices by the fourth component results in less bound iodine by nanocomplex and increased turbidity.

Initial results indicate that the ternary complex can carry small amount of hydrophobic compound (0.5 mg/ml) without any substantial change in the turbidity and can carry as much as 1 mg/ml without being precipitated out of solution. This notable finding has opened the opportunity for investigation of this nanocomplex to carry and solubilize small hydrophobic compounds such as bioactive nutrients, drugs, and flavors for delivering in a hydrophilic system.

Influence of Nanoparticle Characteristics on Fate, Bioavailability, and Toxicity of Food-Grade Nanoemulsions

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Nanoemulsions have many potential advantages over conventional emulsions for certain applications (optical clarity, increased bioavailability, enhanced physical stability), and so they are being used more frequently within the food industry to encapsulate bioactive components. However, there is increasing concern that creating ultra-fine particles in food emulsions may have adverse effects on human health. Currently, knowledge of the impact of nanoparticle characteristics on the biological fate of encapsulated components within nanoemulsions is limited. Our overall objective is to address this lack of knowledge by systematically examining the impact of specific nanoparticle characteristics (i.e., size, charge, and composition) on the bioavailability and safety of β-carotene encapsulated in nanoemulsions using in vitro and in vivo models. Our central hypothesis is that the bioavailability of highly lipophilic food components can be substantially increased by encapsulation in nanoemulsions, which could potentially cause certain dietary bioactive food components to reach tissues levels where they can exhibit toxicity. β-Carotene was selected as a model bioactive food component because: it is widely used as a colorant and nutraceutical; it normally has a low bioavailability; it may exhibit adverse health effects when consumed at high levels, which could occur when it is encapsulated in nanoemulsions. Our Specific Aims and Approaches are:

Aim 1: Fabrication of nanoemulsions with well-defined particle characteristics. A series of β-carotene oil-in-water nanoemulsions with well-defined particle characteristics will be prepared and characterized based on their particle size, particle charge, and carrier lipid composition.

Aim 2: Quantification of the major factors impacting the bioavailability and distribution of β-carotene-nanoemulsions in simulated gastrointestinal conditions. An in vitro digestion model will be used to systematically determine the impact of particle characteristics on the behavior of nano-droplets within a simulated digestion model, as well as on the release and absorption of encapsulated β-carotene.

Aim 3: Determination of the bioavailability and safety of β-carotene-nanoemulsions in Mongolian gerbils. β-carotene-nanoemulsions will be prepared with particle characteristics shown to increase β-carotene bioavailability in the in vitro study. Feeding studies with Mongolian gerbils will be used to compare the in vivo fate, bioavailability and potential toxicity of β-carotene delivered using nanoemulsions, conventional emulsions, and bulk carrier oils.

This project will provide fundamental knowledge about the impact of specific particle characteristics of nanoemulsions on (i) the mechanisms of digestion of nano-droplets within the GI tract; (ii) the bioavailability of encapsulated β-carotene; and (iii) the potential toxicity of encapsulated β-carotene. This knowledge is critical for the rational design and development of nanotechnology-based food strategies to improve human health. Consequently, food manufacturers can establish the most appropriate levels of carotenes to incorporate into suitable nanoemulsions so that they exhibit beneficial effects, without introducing unwanted side-effects (e.g., potential toxicity). This project will also lead to the development of a highly integrated multidisciplinary approach for testing food nanoparticles, which will provide a useful model for establishing the safety of other types of engineered nanoparticles intended for food and beverage applications, such as those fabricated from proteins, polysaccharides or inorganic materials.

Super-Repellent Antimicrobial Coatings to Ensure Food Safety

Project #: 2011-67022-20036 (12/2010 - 11/2013)

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ABSTRACT

Unsafe food and food poisoning due to insufficient protection from pathogenic bacteria results in human suffering from illness and tremendous financial repercussions. Numerous outbreaks in the U.S. were due to contaminated food processing facility. Therefore, it is of extreme importance to prevent the accumulation of pathogenic bacteria inside food processing facilities. Our objective in this project is to develop super-repellent antimicrobial nanostructured coatings for food processing facilities (both food contact and non-contact surfaces) to eliminate a major external source of bacterial infection for food. The super-repellent antimicrobial coatings, which are topographically structured to mimic the lotus leaf surface (i.e., dual-scale structure), will combine two mechanisms of eliminating bacteria into one system: repelling (due to super-repellency against both water and oil) and killing (due to covalently bonded quaternary ammonium compounds (QACs) at the coating surface). Our preliminary results have demonstrated that polymer coatings with covalently bonded perfluoroalkyl QACs are very effective in killing bacteria. The innovative antimicrobial coatings can be applied to food processing facilities and peripheral surfaces (drains, floors, storage tanks, apparel, etc.), and have the potential to greatly reduce bacterial attachment and biofilm formation, thus reducing foodborne pathogens. Moreover, the technology to be developed may also find applications in healthcare settings, personal hygiene industry, biomedical industry, and other high-touch, high risk environments, making significant contributions to a better and safer society.

Multifunctional Food Grade Nanofibers with Biological Control Agents for Improving Food Safety of Fresh Produce and Cut Fruits

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Abstract:

Despite strict regulations to control presence of human pathogens in our food supply, there is an increasing incidence of disease resulting from contamination of food with human pathogens. Food safety issues with fresh produce and cut fruits are highly significant as these products are minimally processed and undergo multiple handling steps prior to consumption.

To address these challenges we propose to develop a novel and a sustainable solution based on integration of multifunctional food grade nanofibers with bacteriophages and food grade enzymes for controlling specific pathogens and disrupting biofilms on surface of fresh produce.

The specific objectives of the proposed research are: (a): Develop food grade nanofibers with model biocontrol agents; (b): Develop antimicrobial coatings based on encapsulation of phages in edible films and compare their antimicrobial properties with nanofiber-phage complexes; (c): Design and development of food grade nanofibers linked with biofilm extracellular polysaccharide degrading enzymes and to evaluate synergy between enzymes linked nanofibers and nanofiber-phage complexes in treating biofilms on food materials.

The key expected results are: (a) Novel food grade nanomaterials with enhanced antimicrobial properties (specificity for target pathogens, improved performance of biocontrol agents) to maintain safety of minimally processed food for an extended period of time; (b) Synergistic combination of nanomaterials, bacteriophages and enzymes to effectively treat biofilms on surface of food products ; (c) Edible antimicrobial material formulations that can integrate with current packing materials and to develop edible antimicrobial sprays. The results of this research will have a significant impact improving food safety.

Impact, Detection, and Tracking of NP in Agriculture: A Focus on Crops and Rhizosphere Microbes

David Britt, Anne Anderson, Joan McLean, William Johnson, Bruce Gale
Utah State University and the University of Utah

The project goals achieved during year-1 have been disseminated through national and international conference presentations, including Pennsylvania, California, Utah, Washington D.C., and New Zealand. The project directors, post-doctoral associates, graduate students, and undergraduate students working on this project have all presented this work at a wide range of venues, thus addressing a broad audience. The work also gathered media attention and was one of two projects highlighted in an American Chemical Society article addressing the impact of nanoparticles in the environment. This article has been picked up by numerous other sources:

http://www.sciencedaily.com/releases/2009/03/090326162747.htm

http://portal.acs.org/portal/acs/corg/content?_nfpb=true&_pageLabel=PP_ARTICLEMAIN&node_id=222&content_id=WPCP_012400&use_sec=true&sec_url_var=region1&__uuid=e0ccac82-9279-4584-8c37-0c9f79ff6038

The project directors compiled the year-1 findings in a paper published in the Journal of Biological Engineering (Gajjar et al.), which is an open access journal, allowing anyone to have access to the paper. Five additional papers and a book chapter are in preparation for further dissemination of findings.

As per our original project goals we are investigation nanoparticles (NP) of Ag, copper oxide and zinc oxide with regard to food safety and agriculture. Soil microbes that have beneficial properties in nutrient cycling and plant performance could be adversely affected by release of NP into the environment. Crops may be directly or indirectly exposed to these particles.

We have focused our research on the effect of NPs of Ag, Cu oxides and Zn oxides on two Pseudomonas bacterial strains: The beneficial, plant-colonizing soil bacteria, Pseudomonas putida KT2440 and *Pseudomonas chlororaphis* O6. Pseudomonads are common soil microbes that function in plant health and carbon cycling. The KT2440 isolate was engineered as a biosensor by transfer of a plasmid containing *lux*AB genes that endow light production. A toxic response is observed as a loss in light output relative to the control. Toxicity was observed for the NPs of Ag, Cu oxide and Zn oxide and for the free metal ions when exposed at doses between 0.1 to 10 mg/L. The threshold for response to NP Ag and Ag ion was the same at 0.2 mg/l. The cells were more sensitive by a factor of ten to Cu and Zn ions, with threshold concentrations of 0.5 mg/L. Loss in light output correlated with non-culturability for NP-Ag and Cu ions, reduced culturability for nano-CuO and bacteriostasis for nano-

ZnO and Zn ions at a 10 mg/L dose. Of particular interest, toxicity did not correlate with induced cell lysis.

Pseudomonas chlororaphis O6 studies demonstrated that this beneficial microbe has more resilience to the antimicrobial activities of CuO and ZnO nanoparticles compared with the pathogenic *Escherichia coli* strains. When the CuO nanoparticles were included in the growth matrix for wheat seeds, impaired root growth was observed and correlated with increased lipid peroxidation. Elevated levels of Cu in the shoots were detected that were similar to the burden imposed by growth in 10 mg/L Cu ion. Thus, the Cu in the CuO nanoparticles was bioavailable for the wheat seedlings under these growth conditions.

Field flow fraction measurements showed that these commercially prepared CuO and ZnO NPs agglomerated, forming particles with a mean size of 390 nm, although particles of about 5 nm also were present. Removal of the agglomerated particles of CuO by filtration showed that filtrates retained toxicity. Atomic force microscopy analysis of NPs dried on atomically flat mica sheets support the FFF findings by showing NP polydispersity arising from aggregation of monodisperse particles as well as polydispesrsity due to NPs ranging in size. These characterization methods are critical in assessing the toxicity of as manufactured NPs, especially since it is evident that a large fraction of NPs exist in aggregated states, thus the bacterial and plant responses to as manufactured NPs likely grossly overestimate the actual number of nano-sized particulates. We are addressing this through a series of pre-fractionation and collection steps in order to challenge bacteria and plants with NP solutions free of aggregates.

Nanoengineered surfaces for controlling the attachment of pathogenic and biofilm forming bacteria to food contact surfaces

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Contamination of processing equipment and other food contact surfaces with pathogenic bacteria is in many instances at the root of foodborne illness. The goal of this work is to generate an understanding of the rules of attachment of foodborne pathogenic bacteria to nanostructured surfaces, and use these rules to design and develop nanoengineered surfaces able to control the attachment of bacteria to food contact surfaces. As a first step, the effect of nanoscale surface topography on the attachment of *Escherichia coli* and *Pseudomonas fluorescens* will be evaluated. Commercial and lab created nanoporous alumina of different pore sizes will be used as substrates. Bacterial attachment and biofilm formation on these substrates will be evaluated using a combination of microscopy techniques, with the intention of finding correlations between the nanoscale surface topography and bacterial attachment. This work is expected to ultimately provide solutions for minimizing microbial contamination in food processing environments, thus reducing the incidence of foodborne illness.

NANO PUBLIC PERCEPTION

CARL A. BATT, CORNELL UNIVERSITY
ANNA WALDRON, WALDRON EDUCATION CONSULTANTS
DEBORAH BYRD, RYAN BRITTON, JORGE SALAZAR, EARTHSKY

The public has little understanding of the core concepts of nanotechnology yet when pressed will express opinions on safety, appropriateness and value of nanotechnology to society. A coordinated effort to both inform the public and assess their awareness of nanotechnology is being carried out using a radio/video presentation format at a major theme park. A series of six EarthSky radio episodes were created using nanotechnology thought leaders from across the United States and Europe. A distribution of thought leaders was selected to represent the different positions on nanotechnology. Three of these 90 second interviews were selected for an extended presentation on the EarthSky website. The six radio episodes were broadcast in the Spring 2009 and also made available on iTunes and mobile distribution networks. An estimated 90 million impressions were created through these media. The website generated almost 2 million more impressions. Listeners had the opportunity to post comments on the website. The radio episodes were videofied with images and have been shown at a long-term exhibition, *Take a Nanooze Break* that opened at Epcot, Walt Disney World Resorts, Lake Buena Vista, FL in January 2010. An estimated 5 million guests interact with the exhibit each year with the opportunity to listen to these video segments in a circular seating area. Assessment of impact on public awareness is ongoing with plans to complete the assessment in focus groups as well as at the theme park. Preliminary data will be presented from one such effort conducted in early 2010 at Walt Disney World.

STAND-OFF SERS DETECTION USING NANOPARTICLES

CARL A. BATT, ADAM LOWE, DICKSON KIRUI, CORNELL UNIVERSITY
JOHN BRUNO, OPTECH

Surface enhanced Raman scattering (SERS) represents a valuable spectral interrogation platform that combines a robust signal and an informative spectra. We have been developing nanoparticle-based SERS to detect pesticides in the environment. Gold or silver nanoparticles when coupled to an analyte specific binding agent can be used to detect a number of small molecules, including pesticides. A system that uses a cyclodextrin inclusion complex to bind the analyte was designed and tested to bind an analyte. This platform was tested using carbendazim and concentrations as low as 50 μM can be detected. The hydrophobic cavity in the cyclodextrin inclusion complex binds the target analyte with some degree of specificity. The SERS spectrum allows for a more precise identification of the bound analyte.

To increase the diversity and specificity of the system, we have incorporated aptamers to serve as the analyte specific binding agent. A series of aptamers previously screened for binding diazinon were synthesized, using a simple thiol chemistry to link them to gold nanoparticles. CTAB stabilized 10 nm gold nanoparticles were used and thiolated aptamers bound through ligand exchange reaction. These nanoparticle-aptamer conjugates have been tested using diazinon. A strong SERS spectrum was obtained from aptamers that shares characteristic spectral peaks with the pesticide applied to a planar gold surface. A SERS peak that originates from the aptamer provides a good internal reference for standardizing the SERS signal from the analyte. The incorporation of an aptamer component permits a wide range of options in tailoring a nanoparticle SERS system to detect virtually any analyte.

Title: Influence of Nanoparticle Characteristics on Fate, Bioavailability, and Toxicity of Food-Grade Nanoemulsions

PI: Hang Xiao (hangxiao@foodsci.umass.edu); ***Co-PI***: D. Julian McClements, Eric A. Decker (University of Massachusetts Amherst); ***Collaborators***: Qasim Chaudhry (Food and Environment Research Agency, Sand Hutton, York, United Kingdom), Guang-Yu Yang (Dept. of Pathology, Northwestern University, Chicago, IL). *Project period*: 12/15/2010 – 12/14/2013; *Location*: University of Massachusetts Amherst; *Cost*: direct + indirect: $490,000.

Nanoemulsions have many potential advantages over conventional emulsions for certain applications (optical clarity, increased bioavailability, enhanced physical stability), and so they are being used more frequently within the food industry to encapsulate bioactive components. However, there is increasing concern that creating ultra-fine particles in food emulsions may have adverse effects on human health. Currently, knowledge of the impact of nanoparticle characteristics on the biological fate of encapsulated components within nanoemulsions is limited. ***Our overall objective*** is to address this lack of knowledge by systematically examining the impact of specific nanoparticle characteristics (*i.e.*, size, charge, and composition) on the bioavailability and safety of β-carotene encapsulated in nanoemulsions using *in vitro* and *in vivo* models. Our ***central hypothesis*** is that the bioavailability of highly lipophilic food components can be substantially increased by encapsulation in nanoemulsions, which could potentially cause certain dietary bioactive food components to reach tissues levels where they can exhibit toxicity. β-Carotene was selected as a model bioactive food component because: it is widely used as a colorant and nutraceutical; it normally has a low bioavailability; it may exhibit adverse health effects when consumed at high levels, which could occur when it is encapsulated in nanoemulsions. Our ***Specific Aims and Approaches*** are:

Aim 1: *Fabrication of nanoemulsions with well-defined particle characteristics.* A series of β-carotene oil-in-water nanoemulsions with well-defined particle characteristics will be prepared and characterized based on their particle size, particle charge, and carrier lipid composition.

Aim 2**:** *Quantification of the major factors impacting the bioavailability and distribution of β-carotene-nanoemulsions in simulated gastrointestinal conditions.* An *in vitro* digestion model will be used to systematically determine the impact of particle characteristics on the behavior of nano-droplets within a simulated digestion model, as well as on the release and absorption of encapsulated β-carotene.

Aim 3: *Determination of the bioavailability and safety of β-carotene-nanoemulsions in Mongolian gerbils.* β-carotene-nanoemulsions will be prepared with particle characteristics shown to increase β-carotene bioavailability in the *in vitro* study. Feeding studies with Mongolian gerbils will be used to compare the *in vivo* fate, bioavailability and potential toxicity of β-carotene delivered using nanoemulsions, conventional emulsions, and bulk carrier oils.

This project will provide ***fundamental knowledge*** about the impact of specific particle characteristics of nanoemulsions on *(i)* the mechanisms of digestion of nano-droplets within the GI tract; *(ii)* the bioavailability of encapsulated β-carotene; and *(iii)* the potential toxicity of encapsulated β-carotene. This knowledge is ***critical*** for the rational design and development of nanotechnology-based food strategies to improve human health. Consequently, food manufacturers can establish the most appropriate levels of carotenes to incorporate into suitable nanoemulsions so that they exhibit beneficial effects, without introducing unwanted side-effects (e.g., potential toxicity). This project will also lead to the development of ***a highly integrated multidisciplinary approach*** for testing food nanoparticles, which will provide *a useful model* for establishing the safety of other types of engineered nanoparticles intended for food and beverage applications, such as those fabricated from proteins, polysaccharides or inorganic materials.

Supplemental Keywords: metabolism, dose-response, diet, pathology.

Nano-scale colloidal assemblies in foods

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Pickering emulsions are encountered in many fields, including the recovery and separation of crude oil, in cosmetics, in wastewater treatment and in biomedical applications. In the food industry, Pickering-type stabilization is vital for the proper processing of foodstuffs such as margarine and ice cream where lipid crystals originate via surfactant solidification at the interface or the migration of existing surface-active fat crystals towards dispersed droplets. During the past decade, solid lipid nanoparticles (SLNs) have been extensively researched as pharmaceutical drugs carriers. Though progress is being made, there remains surprisingly little evidence of their exploitation in the food arena. Furthermore, SLNs have not been investigated as food emulsion stabilizers, in part due to the limited choice of cost-effective, food-grade compounds. Typical Pickering species are micron-sized, yet a growing body of evidence has shown that nanoparticles (*e.g.*, silver, clay) can act as effective Pickering species. In this presentation, the development and use of food-grade SLNs as Pickering stabilizers is demonstrated. Water-continuous 'nanoreactors' based on microemulsions and nanoemulsions were used to create surface-active SLNs. These particles were then used to generate and kinetically stabilize water-in-oil or oil-in-water emulsions. Depending on the concentration used, SLNs formed monolayers or multilayers around dispersed droplets as well as a continuous phase network that acted as an effective stabilizing agent. At sub-monolayer SLN concentrations, incompletely covered droplets flocculated and coalesced, leading to macroscopic phase separation. As the adsorbed SLNs were primarily wetted by the continuous phase, they sat within a deep energy well that effectively anchored them to the oil-water interface. Furthermore, their outward projection created a physical barrier that prevented the close contact required for dispersed phase coalescence. Finally, as emulsification with this method took place at or below room temperature, it offers possible advantages for the preparation and stabilization of emulsions that contain thermo-labile components (*e.g.*, aromas, bioactive compounds).

Second year report for USDA-NRI 75.0 Nanoscale Science and Engineering for Agriculture and Food Systems award # 2008-01366. Chitosan/PLGA Nanoparticles for increased bioaccessibility and bioavailability of model lipophilic vitamin. 2008-2011.

Cristina Sabliov and Dorel Moldovan, Louisiana State University Agricultural Center

It was *hypothesized* that entrapping lipophilic vitamins (i.e. alpha-tocopherol, or vitamin E) in a mucoadhesive cationic Chitosan/Poly (D,L-Lactide-co-glycolide) (PLGA) nanoparticle system stabilized by lecithin can increase the intracellular/paracellular uptake of the vitamin in the small intestine. Of the proposed four objectives, **Objective 1** was fully executed during the first year. Positively charged (~ +60mV zeta potential) Chi/PLGA(αT) particles measuring 200-350 nm in diameter, depending on the chitosan concentration and negatively charged (~-60mV) PLGA nanoparticles measuring 80-150 nm in diameter were made by emulsion evaporation. Alpha-tocopherol was entrapped in Chi/PLGA(αT) and PLGA(αT) nanoparticles at three different initial loadings, 8, 16, and 24% to study the effect of the initial loading on the drug release profile.

Objective 2, namely to study the degradation and release profile of αT from the Chi/PLGA(αT) nanoparticles in simulated gastric and intestinal environments, as a function of size and charge was partly met in the first year and completed in year 2, as follows. Degradation of the two systems, PLGA(αT), and Chi/PLGA(αT) were followed by TEM and DLS under gastric (pH_{basal} =1.5 and pH_{fed} =5.5) and intestinal (pH_{basal}=6.5 and pH_{fed}=5.5) conditions. Particle stability was pH and system dependent. PLGA particles aggregated and clumped out of solution at pH 1.5, whereas Chi/PLGA particles were well-dispersed at this pH, but aggregated instantaneously at intestinal pHs. *In-vitro* release profiles showed that under both basal and fed gastric conditions the Chi/PLGA particles released approximately half as much as the PLGA particles. This is beneficial to lipophilic bioactive delivery because lipophilic compounds entrapped in Chi/PLGA particles when ingested with or without food would still exhibit a lower gastric drug release compared to PLGA and hence most of the compounds will be released mainly in the small intestine, where they are absorbed.

Objectives 3: To study the uptake of the vitamin at intestinal site using atomistic molecular dynamics (MD) simulations and experimental studies was initiated over the past year. Two PhD students, Kai Xia and Jijun Lao, were hired to work on the simulation aspects of the project. The following progress was made thus far. 1) the pdb structures for Span 80 and Vitamin E were secured. 2) Using the Dundee PRODRG2 program the force field describing the inter-atomic interactions of Span 80 and Vitamin E was generated. 3) Based on these realistic all-atom inter-atomic interaction models we performed large-scale molecular dynamics (MD) simulations of self-assembly of Span80 surfactant in water at concentrations above the critical micelle concentration. Our studies show that at intermediate Span 80 concentration of 0.25 M, in a simulation system containing 125 Span80 molecules, the system evolves into a single spherical micelle in about 10 ns. 4) Additional MD simulations describe the mechanism of passive absorption and entrapping of alpha-tocopherol (vitamin E) into Span80 micelles. Simulation of intestinal transport of micellized alpha-tocopherol will be performed over the next year.

Objective 4: To study the mucoadhesion of the Chi/PLGA(αT) NPs, and the *in vivo* uptake of the vitamin released from Chi/PLGA(αT) using animal models was initiated and will be pursued further during the following year. A research associate was hired to address this objective. An *ex-vivo* study was performed on rabbit intestinal tissue and showed that fluorescent PLGA and Chi/PLGA particles associated with the surface of the intestinal

villi, and penetrated deep in the endothelial lining of rabbit intestinal explants, indicating uptake. *Ex-vivo* and *in-vivo* experiments will be performed to quantify and compare the uptake of PLGA and Chi/PLGA will to understand the effect of size/charge on the nanoparticle uptake.

Presentations:

1. Lin, J., B. Novak, and D. Moldovan. December 2010. Molecular dynamics study of the effect of DMSO on permeation properties of DMPC lipid bilayers. American Chemical Society, SE/SW Regional Meeting, New Orleans, Louisiana.
2. Sabliov, C. M. October 2010. Nano and microdelivery technologies in food systems. Invited speaker representing Food Engineering at the Centennial Celebration of National Autonomous University of Mexico. Mexico City, Mexico. Invited.
3. D. Moldovan. September 2010. Atomistic simulation studies of interfacial phenomena in biomolecular systems. The LSU Center for Computation & Technology, Louisiana State University, Baton Rouge, LA. Invited.
4. Sabliov, C. M. September 2010. Nano- and colloidal delivery systems for improved food quality, food safety, and human health. LSU Science Club. Baton Rouge, LA. Invited.
5. Sabliov, C. M., C. E. Astete, and D. D. Doliver. July 2010. Antioxidant PLGA Nanoparticles: Particles with Entrapped Alpha-tocopherol versus Particles Synthesized with Alpha-tocopherol derivative surfactant. The Eighteenth Annual International Conference on COMPOSITES/NANO ENGINEERING (ICCE - 18). Anchorage, Alaska, USA.
6. Sabliov, C. M. May 2010. Applications of nanotechnology in food ingredients and improving food quality. Short Course on Nanotechnology: Fundamentals, Applications, and Regulations in the Food Industry. Department of Food, Bioprocessing and Nutrition Sciences. North Carolina State University, Raleigh, NC. Invited.
7. Sabliov, C. M. February 2010. Nanodelivery of natural hydrophobic ingredients. 3rd Annual Conference Innovations in Natural Colour. London, UK. Invited.
8. Lao, J, Lin, J., Xia, K., C. M. Sabliov, and D. Moldovan. February 2010. Molecular dynamics simulation of self-assembly of Span80 into micelles and their interaction with vitamin E. Mardi Gras Conference 2010: Computational Materials and Methods, Louisiana State University, Baton Rouge, Louisiana.
9. Sabliov, C. M. November 2009. Nanoparticulates and Carotenoids at Novus International Science Day, focused on Science and Nutrition: Pigments, Poultry, Poisson & People. St. Charles, MO. Invited.
10. Sabliov, C. M. May, 2009. Applications of polymeric nanoparticles in drug delivery. NASA / TIMES – APTEC Annual meeting. Tulane Institute for Macromolecular Engineering and Science (TIMES). New Orleans, LA. Invited.
11. Sabliov, C. M. June 6, 2009. New and Emerging food Applications of Polymeric Nanoparticles for Improved Health. IFT International Food Nanoscience Conference. Anaheim, CA. Invited.
12. Murugeshu, A., C. E. Astete, R. Adams, C. M. Sabliov. 5-8 April, 2009. Release of α-tocopherol from chitosan/PLGA particles in the gastrointestinal tract. CoFE. Ohio State University, Columbus, OH.
13. Murugeshu, A. and C. M. Sabliov. June 2008. Cationic chitosan/poly (D, L-lactide-co-glycolide) particles for improved oral delivery of alpha-tocopherol. 82nd ACS Colloid and Surface Science Symposium. Raleigh, NC.

Publications:

1. Murugeshu, A., C. E. Astete, C. Leonardi, and C. M. Sabliov. Chitosan/PLGA particles for controlled release of alpha-tocopherol in the gastrointestinal tract via oral administration. Nanomedicine. (In press).
2. Lin, J., B. Novak, and D. Moldovan. Molecular dynamics simulation study of the effect of DMSO on permeability of lipid bilayers. Annals of Biomedical Engineering (submitted, Nov. 2010).

USDA Grantees Conference Progress Report: Development of Rapid Detection Technique of Pathogens using SERS with Silver nanorod Array Substrates

PI: Yao-wen Huang, University of Georgia

Co-PI: Yiping Zhao (UGA) and Bosoon Park (USDA)

Surface-enhanced Raman scattering (SERS), as a sensitive and rapid detection method, had long been considered as a potential powerful tool in detection application for its advantage over other conventional methods. Novel silver nanorod array substrates fabricated by oblique angle deposition (OAD) techniques has been used in our lab to detect foodborne pathogens. Procedures of sampling methods such as centrifuge and two-step filtration followed by SERS measurement using such silver nanorod substrates was developed. Centrifuge method use centrifuge force as 7000 rpm for 10 min to distribute and concentrate the bacteria onto the surface of the SERS-active substrate. It conquered the problem of uneven sample distribution and undesired distance between target bacteria and the substrate surface. The whole procedure could be completed within 30 min from sampling to quantization, and increased the limit of detection (LOD) of *Salmonella* Typhimurium to 10^5 CFU/ml. Two-step filtration methods started with conventional vacuum filtration of 250 ml sample solution through filters with pore diameters of 0.22μm to recover the Salmonella cells. Salmonella cells were then removed from the filter by vortexing in 2 ml of DI water, and this sample was then subjected to second step of filtration using

centrifugal filters with pore diameters of 0.22μm for further concentration to 50μl. From sampling to SERS measurement, the whole procedure took 1 hr, and the LOD of *Salmonella typhimurium* reached to 10^5 CFU/ml. In order to reduce the baseline of SERS spectrum, plasma cleaner was developed and tested. The results showed a promising improvement. We will use it for the following experiments.

Developing "Green Nanotechnology" for Eliminating Foodborne Pathogens

Nina Shapley and Karl Matthews, Rutgers University; Anubhav Tripathi, Brown University

Currently, there is a critical need for low energy and mild technologies that can improve microbial safety reliably in minimally processed foods such as fresh produce. Recent outbreaks of pathogenic bacteria contaminating widely consumed produce have been reported in the national media, with *Escherichia coli* O157:H7 and *Salmonella* among the leading pathogenic threats to food safety. Since 1995 FDA has identified eighteen outbreaks caused by *E. coli* O157:H7 associated with lettuce and one outbreak associated with spinach. On May 7, 2010 a new outbreak linked to *E. coli* contaminated lettuce was reported. Other recent outbreaks of foodborne illness have been related to parsley, green onions, basil, cilantro and cabbage[1, 2]. Thermal treatments are not appropriate for fresh produce, and large scale irradiation is limited by cost. A range of permeations for application of chemical sanitizing agents has been evaluated with little change in the 1 to 2 log reduction traditionally reported [3-5].

In response, we envision the development of a nanoparticle wash treatment with the capability of significantly reducing or eliminating pathogenic bacteria associated with fresh or fresh-cut fruits and vegetables. We aim to apply a nanoparticle wash synergistically with a conventional chlorine wash in order to achieve a 3 log reduction or better in the bacteria population. The success of such a treatment depends on improved understanding of nanoparticle-bacteria interactions. The project objectives address the priorites of the program, "Nanoscale Science and Nanotechnology to Ensure Safe Food," focusing on novel nanotechnology mitigation measures in minimally processed foods.

The proposed treatment will employ suspensions of cationic, preservative-loaded, antimicrobial peptide-decorated nanoparticles, where washing or immersion in a nanoparticle suspension, possibly in conjunction with the application of a mild electric field, can flocculate and destroy pathogenic bacteria on fresh produce immediately prior to packaging. Ultra-potent, peptide-enhanced nanoparticles combine the synergistic action of cationic chitosan nanoparticles, encapsulated lysozyme, surface-attached nisin and applied electric fields on the bacterial cell membrane, and consist only of GRAS materials. The novelty of the proposed research centers on the effective delivery of multiple antimicrobials which are required to positively eliminate both Gram-positive and Gram-negative foodborne pathogens.

The proposed system is a "green technology" because it utilizes edible, environmentally benign, and naturally abundant materials. If used in conjunction with conventional antimicrobial treatments, the new method has the potential to reduce the concentrations of chemical preservatives and energy intensity required for pathogen lysis from current levels. New tools such as a high precision microfluidic platform and nanoencapsulation will aid the development of the unique "green technology."

<u>The specific tasks of the proposed program are:</u>

1) Design, synthesis and characterization of ultra-potent chitosan nanoparticles coated by antimicrobial peptides (e.g. nisin) ("green particles").

2) Evaluation of peptide-enhanced nanoparticles as a lysis agent in realistic food processing environments (neutral to acidic pH, low to moderate temperature, salts or lipids present).

3) Development of a post-harvest nanoparticle-electric field treatment for decreasing the bacterial load of fresh cut leafy greens and tomatoes (improving safety and prolonging shelf life).

The primary deliverables of the proposed study include:

1) Development of synthesis methods for antimicrobial peptide-coated chitosan nanoparticles.

2) Determination of the operating conditions under which the nanoparticles are most effective at causing pathogen lysis.

3) Evaluation of the effectiveness of the "green nanoparticle" treatment for decreasing the bacterial load of fresh produce.

Challenges and limitations include low numbers of *E. coli* O157:H7 associated with plant tissue, possibly below detection levels. To ensure detection of low numbers of *E. coli* O157:H7, plant tissue samples will be enriched. Although not quantitative, information will be gained concerning efficacy of the treatment. Another issue that may limit use of the nanoparticle wash technology is the high cost in comparison to chlorine treatment. However, by using multiple antimicrobials we expect a synergistic effect reducing the amount of each antimicrobial agent required. Finally, the utilization of a greater range of conditions (challenge inoculum, pH of chlorine wash, multiple strains of each pathogen) would be ideal. Inclusion of too many variables would be overly ambitious and potentially prevent delivery of a novel technology. This study is intended to demonstrate proof of concept clearly and provide the basis for future experiments.

Our study can provide guidelines for combining mild, biocompatible and energy efficient food safety methods synergistically in the design of a "green" nanoparticle treatment step for improved safety of minimally processed fruits and vegetables.

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FY 06 Project

Research Proposal Title: "Multi-Layered Surround SERS Nanosensor Array System for the Rapid, Specific and Multiplexed Detection of Foodborne Bacteria and Toxins"

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EXTENDED ABSTRACT

Project Relevance to Agriculture and Food Systems

A real-time monitoring and identification system for the multiplexed detection of pathogenic species (e.g., bacteria) in the complex "real-world" environment of food processing, with trace detection limits and negligible false positive rates, is being developed for rapid food screening.

Methodologies

To achieve this goal, coherent surface enhanced Raman scattering (SERS) nano-biosensing arrays, containing 30,000 individual sensing elements, in conjunction with optically amplifying immuno-magnetic capture particles are employed in a sandwich assay format for the multiplexed and redundant detection of the pathogens. The narrow linewidths and spectral signatures of the analyte species, or corresponding label, afforded by SERS allow the analytes of interest to be detected in the presence of many other species and with a high degree of sensitivity and selectivity.

Specific Scientific Challenges/Objectives

The scientific challenges to be addressed include: (1) the optimization and evaluation of controlled diameter nanosensor arrays possessing the desired nano-photonic properties (i.e., uniform SERS enhancement across the surface and tunable plasmon absorption bands) and particle filtering properties; (2) the fabrication of unique SERS labeled immuno-nanosphere capture particles for antigen concentration and signal amplification and (3) development and integration of the nanoarray probes into a chemical imaging platform for evaluation of "real-world" samples.

Key Results

Following fabrication and optimization of the dye labeled immuno-magnetic separating nanoparticles, optimization of the photonic and array deposition properties of the SERS-active nano-arrays has been accomplished. In these efforts, SERS active arrays with 30,000 individual SERS active wells have been created that have enhancement factors of 10^{10} and less than 2% variation reproducibility from well-to-well. Furthermore, studies into the controlled tunability of the SERS plasmon band for 632.8 nm have been performed allowing excitation wavelengths to be employed which limit the auto-fluorescent background of food samples.

Optimization of SERS active dye labeled magnetic separation particles and evaluation of their properties has revealed that separation of bacterial species can be performed in a few minutes with greater than 95% efficiency. Furthermore, the large number of dye molecules associated with each sphere is sufficient enough for single sphere detection and thus single bacteria detection. Blind studies in the past year have demonstrated that simultaneous, multiplexed detection of different pathogenic species using various spectrally distinct dyes is easily achievable. The ability to monitor up to five different species in water, chicken and ground beef samples with accuracies of 99%, 96% and 93% respectively has been demonstrated. This covers the five main pathogenic species specifically set out in this proposal. Additional dyes were also investigated for extended multiplexing. With the optimized spheres and SERS nano-arrays, integration of these pieces into a single multi-spectral imaging platform has been completed and demonstrated and evaluation of the detection capabilities via blind studies have been performed.

Impacts

Fabrication of optically tunable, uniformly enhancing SERS active extended nanowell arrays with enhancements as great 10^{10} along with coupling to unique SERS active immuno-magnetic separation particles has resulted in the first extended SERS platform capable of quantitative/semi-quantitative SERS analyses of as many as 30,000 isolated reaction sites. The separation efficiency as well as the optical amplification of pathogenic species signals via dye labeled nanoparticles provides an ideal platform for trace multiplexed detection of pathogenic species of interest from "real-world" food samples in minutes.

PUBLICATIONS FROM THIS PROJECT

- (C. K. Klutse), and B. M. Cullum*, "Development of SAM-based Multi-layer SERS Substrates for Intracellular Analyses", Proc. SPIE – Soc. Opt. Eng., **7674**, p. 767402-1 – 767402-12 (2010).
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Nanodispersing Lipophilic Antimicrobials to Improve Food Safety

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Abstract

Many generally-recognized-as-safe or natural antimicrobials are used in food systems as intervention strategies. Because many antimicrobials are only marginally water soluble or insoluble in water, this project develops technology and knowledge of nanometer-sized structures that disperse antimicrobials in food systems to enhance their efficacy without negatively impacting or ideally improving the quality of corresponding foods. These nanostructures are based on high-quality food proteins and carbohydrates and have promise to improve healthfulness, safety, and quality of various food products. Specifically, capsules of lipophilic antimicrobials (plant essential oil components) are prepared by spray-drying emulsions with a hexane-oil phase containing antimicrobials and whey protein or its conjugate with maltodextrin as a surfactant. Transparent nanodispersions of 5% thymol-loaded capsules maintained visual clarity after heating at a wide range of solvent conditions and effectively inactivated *Escherichia coli* O157:H7 (8 log CFU/mL) and other foodborne pathogens. Ongoing research is to continue to develop and characterize this class of promising nanoscale antimicrobial delivery systems (NADS) via these objectives: (1) Physicochemical properties of NADS as affected by preparation conditions, (2) In vitro antimicrobial efficacy of NADS against pathogenic bacteria in growth media, and (3) Antimicrobial efficacy of NADS in food matrices and the impacts on quality. Completion of our research objectives will demonstrate the antimicrobial capabilities of this novel group of NADS against *E. coli* O157:H7, *Salmonella*, and *Listeria monocytogenes* in the food matrices of fluid milk, unpasteurized fresh apple cider and ground beef. The project may provide solutions to reduce/eliminate outbreaks of foodborne illness to supply safe, high-quality foods in the US and beyond.