United States Department of Agriculture
Project Initiation

Project Data for Accession Number 1034606

Project Title

Development of Novel Nanopillar Metasurfaces for the Detection of Antimicrobial Resistance Signatures in Food Samples

Project Details

Sponsoring Institution National Institute of Food and Agriculture	**Project Status** ACTIVE
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Project Director

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Recipient Organization

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Non Technical Summary

Presently, the effectiveness of many popular and common antibiotics is rapidly decreasing as they are often administered in situations where they are unnecessary or improper. This has led to numerous issues as new antibiotic treatments are sparsely discovered and the rise of highly resistant bacteria strands becomes more common. One key area affected by this is food safety, where the inability to treat livestock and their by-products can lead to food poisoning or worse in consumers. The necessity for a device capable of identifying these resistant bacterial cells is apparent to limit the harm to human health as food production continues to grow. This project aims to provide an alternative detection pathway diagnose when common antibiotics would be ineffective or delay proper treatment of food products. This novel approach would look to provide results in real time without sacrificing the sensitivity seen in popular methods already available.The work outlined in this proposal aims to achieve rapid detection of antibiotic

resistance by taking advantage of the pathway that popular antibiotics, like penicillin, use to inactive and disrupt bacterial infections. This strategy will be the baseline in diagnosing multiple techniques bacteria cells employ to prevent the antibiotic attack pathway. Data of this process will be collected using a silicon surface with nano-scale features that manipulate light to absorb only specific colors. Through monitoring of what colors are absorbed by the silicon device, we may monitor shifts in the color wavelengths following the administering of our sample to the surface. Confirming the experiment's progression will be done at numerous checkpoints along the process in the form of monitoring fluorescent signal output and the concentration of the various components in solutions at key points in the experiment. This project ultimately aims to achieve a simplistic system to operate while maintaining the sensitivity and effectiveness of detection. This proposal also looks to establish a rapid testing strategy to eliminate as much delay as possible in administering the proper medication to livestock products and other crops. This project is expected to further food safety research and human health through the categorizing of microbial contaminants and determining their level of resistance before antibiotics may be misused on food products and other agriculture instances.

Animal Health Component

Animal Health Component: 90%

Research Effort Categories

Basic	75%
Applied	25%
Developmental	0%

Classification

Knowledge Area (KA)	Subject of Investigation (SOI)	Field of Science (FOS)	Percent
712	4010	2020	100%

Knowledge Area

[712] Protect Food from Contamination by Pathogenic Microorganisms, Parasites, and Naturally Occurring Toxins

Subject Of Investigation

[4010] Bacteria

Field Of Science

[2020] Engineering

Keywords

Metasurfaces; Antimicrobial Resistance; Localized Surface Plasmon Resonance; Biosensing; Food Safety

Goals / Objectives

The goal of this project is to develop a novel sensing platform incorporating metasurfaces and plasmonic resonance towards the identification of antimicrobial resistance (AMR) biomarkers. Rapid and sensitive detection of AMR aids in improving human health to combat microbial contaminants, especially in relation to food safety where superbugs, or

bacteria resistant to antibiotics can spread between livestock and crops and into humans. Early AMR detection can also prove useful in healthcare cases such as in sepsispatients where long delays in treatment can prove fatal. Throughout the course of this project, we aim to develop new assay systems that may be customized for the detection of common and popular resistance strategies that bacteria cells employ to fight antibiotics. This work in particular will focus on resistance to beta-lactam antibiotics, one of the most widely administered categories that includes penicillin, amoxicillin, cephalexin, and many others. To achieve this broad goal, numerous objectives will be set with the intent to observe progress of the device and assay. This includes:Fabrication of a silicon nanopillar metasurface with sharp reflectance peaks/valleys at desired wavelengths. This includes control of pillar diameter, period, and height to move the sensing regime between the visible and near-IR wavelengths depending on sensing application. The sensing platform described here will be continually improved and optimized over the duration of the project.Establishing an off-chip assay for the detection of beta-lactamse, an enzyme produced in many gram-positive and gram-negative bacteria cells. This enzyme breaks down the beta-lactam ring in beta-lactam antibiotics, changing the conformational structure to disable them before disrupting cell functions. The objective will be achieved by penicillin-binding proteins on magnetic beads that will bind to antibiotics on gold nanoparticles in the absence of the enzyme. The supernatant will then be added to the metasurface described in the first objective.An on-chip variation of the enzyme detection assay will be investigated, seeing penicillin-binding proteins directly bound to the surface of the silicon nanopillar substrate for capture of intact antibiotic molecules on gold nanoparticles in the absence of the enzyme.Developing an assay for the identification of gene-mutations for the purpose of antimicrobial resistance. The mecA gene in MRSA cells will be amplified by recombinase polymerase amplification and induce trans-cleavage in CRIPSR Cas12a proteins to cleave reporter probes on gold nanoparticles. The gold nanoparticles will then be added to the silicon metasurface.Multiplex detection using a similar nanopillar metasurface that exhibits multiple reflectance peaks for identification of different AMR situations. The nanopillar metasurface may be customized to contain multiple parameter sets of pillar height, diameter, and period. This would create separate reflectance peaks that can be monitored individually while both assays described above may be run across the same metasurface but in different regions.

Project Methods

The silicon nanopillar metasurface will be fabricated using commonplace microfabrication techniques. This includes the use of a deep-UV lithography machine for patterning of the 2D pillar structures on a photosensitive material that is precoated on the flat silicon wafer. The unexposed regions of the photosensitive layer will then be removed, while the remaining components act as a mask, protecting the underlying silicon as the pillars are developed. To grow the height of the pillars, a reactive ion etching procedure will be specially developed to control the etching profile and ensure the sidewall angle of the pillars remains greater than 80 degrees as they grow. Success of this objective will be observed via reflectance spectroscopy and SEM imaging of the silicon nano-features. This will provide the desired audience with a platform capable of real-time sensing.The off-chip assay designed for observing enzymatic activity of the beta-lactamase enzyme will see penicillin-binding proteins loaded on to magnetic beads via a his-tag to NTA-Cobalt bond. Additionally, gold nanoparticles will be coated with beta-lactam antibiotics through EDC/NHS chemistry. These two components will then be incubated together, where in the absence of the enzyme, the antibiotics will bind to the proteins effectively linking the nanoparticles to the magnetic beads and removing them from solution. In contrast, when the beta-lactamase enzyme is present in the sample, the antibiotic will undergo cleavage and no longer bind to the protein-labeled magnetic beads. Addition of the supernatant to the nanopillar metasurface following mixing of the nanoparticles with magnetic beads will see a shift in the reflectance spectrum peaks only if the gold nanoparticles are free floating after the enzyme degrades the antibiotic. Confirmation of proper protein binding to the magnetic beads without destroying the protein binding domains will be determined based on capture of an unbound penicillin analogue with a fluorescent label. Further, antibiotic loading to the surface of the gold nanoparticles will be confirmed through absorbance intensity of unbound antibiotic molecules remaining in solution following incubation procedures. Completion of this assay will provide the basis for a rapid detection strategy of a common form of antibiotic resistance in purified or simplistic media. The aim of this is to provide a framework that future research projects can extend to use complex and real-world samples resulting from patients.Further extensions of the enzyme activity monitoring assay will experiment directly on the metasurface. Different linking systems will be required for this

goal to isolate the proteins from their his-tag using a similar NTA chelation strategy. The same antibiotic-labeled gold nanoparticles will be applied in this assay, but the detection of the enzyme will be opposite to that of the off-chip case. In the on-chip assay, the addition of the beta-lactamase will prevent the gold nanoparticles from being isolated on the metasurface as the antibiotic linker will no longer be able to bind to the protein domains that are now adhered to the silicon nanopillars. This objective will be confirmed by SEM imaging of gold nanoparticles on the silicon substrate and monitoring of reflectance peak wavelength shifts in the presence and absence of the enzyme in solution. The alternative on-chip variation of the enzyme assay may provide a more straightforward operation to achieve positive detection but at the cost of sensitivity and an increase in complexity of the protein binding structure.Towards expanding the application of the novel metasurface, detection of gene-mutations in bacteria cells provides another antimicrobial resistance pathway to explore. Specifically, the mecA gene in MRSA cells will be the targeted mutation. This sequence will be amplified via recombinase polymerase amplification (RPA) to increase the number of DNA copies in solution. CRISPR Cas12a proteins will be employed simultaneously in a one-pot reaction with the RPA components to identify the amplified target gene. Proper application of this assay will be confirmed by monitoring the kinetics of the Cas12a trans-cleavage activity through release of a fluorophore from degraded ssDNA probes. The Cas12a will only be activated in the case that the target mecA sequence is detected. This RPA/CRISPR assay will be further combined with gold nanoparticles, as in the enzymatic activity assay, which can then be applied to the silicon nanopillar metasurface. The gold nanoparticles will be coated in ssDNA reporter probes, replacing the fluorophore used to confirm the initial kinetics. Final confirmation of MRSA present in solution will be observed by reflectance spectrum monitoring for shifts in peak wavelength locations. The completion of this assay in conjunction with the enzyme activity monitoring expands the usefulness of the proposed silicon metasurface and expands the capability of the device for identifying resistance to beta-lactam antibiotics in a bacterial infection.Applying dual parameter sets of nanopillars onto a single metasurface substrate allows for two regions that may produce unique reflectance spectra that can be used for separate detection assays. Fabrication of these pillars will follow a similar process to that outline previously where deep-UV lithography and reactive ion etching will be incorporated to realize the pillars. Proper construction of this multiplexed surface will be validated through SEM imaging of the two regions and collecting reflectance spectra for the various individual sections. Expanding the applicability of the system is key as well as making a compact system capable of numerous detection strategies simultaneously.