Nanoengineering Biomimetic Nanobrushes for Foodborne Pathogen Sensing Using Stimuli-Responsive Polymers And Metallic Nanostructures

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Abstract

Recent rise in occurrence of food recalls and outbreaks related to Listeria monocytogenes have heightened public concern about food safety and created a greater impetus to improve methods for L. monocytogenes detection. L. monocytogenes is identified as one of the most predominant foodborne pathogens that persistently causes infections with approximately 1600 illnesses and 260 deaths each year in the United States. Aptamer-based biosensors for bacteria detection are emerging as a viable alternative to traditional methods such as total viable counts, aerobic plate counts, enzyme-linked immunosorbent assay, and polymerase chain reaction. Current methods including total viable counts, polymerase chain reaction, and enzyme-linked immunosorbent assays are time-consuming, require laboratory setting and highly trained personnel. Thus, faster techniques are urgently needed to detect L. monocytogenes with the same level of reliability as traditional methods. The goal of this study was to develop rapid, label-free L. monocytogenes biosensors based on composites of pH-responsive polymer (chitosan) nanobrushes and aptamers. Two sensing platforms were designed to achieve this goal: the first was based on the combination of pH-responsive polymer nanobrushes and platinum nanoparticles structures, and the second was based on embedded pH-responsive polymer nanobrushes with platinum nanoparticles. These studies allowed us to determine the optimum arrangement for nanobrush actuation and pathogen capture, while maintaining electrical properties of the sensor platform. Biosensors were characterized electrochemically by electroactive surface area (ESA) and electrochemical impedance spectroscopy analysis, and also by SEM and surface roughness analysis to evaluate chitosan-platinum nanobrush formation and response to stimulus. For the first approach, chitosan nanobrushes electrodeposition was optimized to 0.5% (w/v) low molecular weight chitosan at 2 V for 5 min, increasing the ESA to 0.101 ± 0.004 cm². Actuation tests of chitosan nanobrushes revealed pH 4 and pH 8 were the ideal pHs for capturing and sensing bacteria, respectively. While for the second approach, the ESA values for electrode submitted to the one-step grafting for 240 s was equivalent (p<0.05) to the electrodes obtained with a 3-step deposition (platinum, chitosan, platinum), 0.063 ± 0.004 and 0.065 ± 0.008 cm², respectively. Actuation tests revealed pHs 8 and 4 were the ideal pHs for capturing and sensing bacteria, respectively. Subsequently, DNA aptamer selective to surface protein internalin A of Listeria monocytogenes was covalently attached to the nanobrushes. SEM images and surface roughness measurements demonstrated chitosan-platinum nanostructures for both approaches. The detection range and response time were 10 - 108 CFU/mL and approximately 17 min, respectively; for both sensing platforms. The biosensors were also tested in the presence of equal concentrations of Listeria monocytogenes and Staphylococcus aureus and presented limit of detection (LOD) as low as 31 ± 3 CFU/mL. The biosensors were also tested in vegetable broth showing LODs as low as 36 ± 11 CFU/mL. The aptasensors designed in this study showed to have great potential as a method for foodborne pathogen detection by the food industry.

Dr. Carmen Gomes is currently an Associate Professor in the Mechanical Engineering Department at Iowa State University. Previously she held the same role in the Biological and Agricultural Engineering Department at Texas A&M University where she led a successful research program on the design of novel nanoscale materials using biopolymers for biotechnology and food applications. Dr. Gomes collaborates with scientists and engineers from diverse fields including biological and agricultural, electrical, mechanical, and civil engineers, physicists, food scientists, microbiologists, and plant pathologists to pursue her research projects. Her research has been supported by federal agencies (USDA and NSF) and has been published in 49 peer-reviewed journal articles (31 since 2010). Dr. Gomes received her Ph.D. in Biological and Agricultural Engineering at Texas A&M University. She received her B.S. in Food Engineering at Federal University of Vícosa, Brazil. She has organized several Institute of Food Technologists (IFT) and Conference of Food Engineering (CoFE) sessions on the topic of Biosensing and Nanotechnology applied to Food, and is actively involved with the leadership team of the food engineering division. Through her research and teaching, Dr. Gomes is determined to address the multiple challenges and many opportunities at the interface of food and nanotechnology.

This seminar counts towards the ME 600 seminar requirement for Mechanical Engineering graduate students.

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