FIBEROPTICS SERS BIOSENSORS FOR SALMONELLA SENSING

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ABSTRACT

This study presents the design and development of Surface Enhanced Raman Spectroscopy (SERS) sensors for the detection of Salmonella typhimurium in raw chicken samples. Microsphere photolithography was used to pattern the nanoantenna arrays on a side-polished optical fiber, enhancing the SERS signal and generating and concentrating the electric field between the disk arrays. A 3D-printed mold was used to facilitate polishing the fiber. Utilizing Low-cost. The sensor demonstrated sensitivity of 1-3 cells/ml and a detection time of 10 minutes.

KEYWORDS

SERS sensor, Microsphere Photolithography; side-polished multimode optical fiber; SERS; Raman, rapid detection.

INTRODUCTION

Salmonella, a gram-negative bacterium, poses a significant threat, causing gastroenteritis, severe systemic infections, diarrhea, abdominal cramps, and fever [1]. Annually, there are approximately 1.35 million reported cases of infections, leading to 26,500 hospitalizations and 420 deaths in the United States alone [2,3]. To prevent foodborne diseases, the poultry industry implements stringent control measures, including testing for process control and verification. These measures aim to improve the operational efficiency of processing plants and involve pre-release screening of ready-to-eat (RTE) products to enhance safety and prevent foodborne illnesses.

While the traditional microbiological culture methods are reliable, they are time-consuming, requiring 3-5 days because they rely on enrichment and selective cultures and subsequent colony counting [4,5]. Nucleic acid-based assays such as polymerase chain reaction (PCR) amplify specific DNA sequences of *Salmonella*, enabling highly sensitive detection [6,7] and reducing the detection time to 24 hours. However, the turnaround time remains high due to the need for enrichment culture, sample preparation, and nucleic acid extraction [8]. Immunological methods such as ELISA can rapidly detect pathogens only after an enrichment culture step is completed [9]. To achieve rapid sensing, various detection techniques have been investigated over the last two decades such as electrochemical [10,11], impedance [12-15], optical [16,17], and lateral flow assay [18] methods. For example, impedance-based biosensors have achieved LOD of 7-10 CFU/ml for *Salmonella* serotypes in raw chicken and ready-to-eat turkey samples [14,15]. Similarly, potentiometric biosensors based on carbon nanotubes have detected E. coli in apple juice with a LOD of 26 CFU/ml[19]. Optical biosensors utilizing surface plasmon resonance (SPR) have achieved an LOD of 14 CFU/mL for *Salmonella* [20]. Among these advancements, Surface-Enhanced Raman Spectroscopy (SERS) stands out as a promising technology for pathogen detection.

Surface-Enhanced Raman Spectroscopy (SERS) holds paramount importance in various scientific and technological fields due to its sensitivity, specificity, and versatility. One of its primary strengths lies in the ability to detect molecules at ultra-low concentrations, making it invaluable in medical diagnostics, environmental monitoring, and food safety. In medical applications, SERS enables the detection of biomarkers for diseases like cancer, and infectious agents with unprecedented accuracy, aiding in early diagnosis and personalized medicine. Environmental scientists utilize SERS to analyze pollutants, toxins, and contaminants in water, air, and soil, contributing to sustainable environmental management. Additionally, in food safety, SERS can detect pathogens, allergens, and adulterants, ensuring the quality and safety of food products. The rapid development of portable SERS devices further enhances its accessibility and usability in field applications, empowering researchers, healthcare professionals, and regulatory agencies worldwide.

Raman spectroscopy is a potent analytical tool used for molecule identification based on their composition. SERS is highly sensitive, and label-free [21,22]. However, Raman scattering is generally weak due to low concentrations of the target molecules in the sample. To address this issue and detect analytes at low concentrations, SERS surfaces are utilized. These surfaces are purposefully roughened to amplify Raman scattering signals by several orders of magnitude [23]. SERS sensors are fabricated using various methods such as photolithography, chemical synthesis, or nanosphere lithography on flat substrate or fiber tips. Photolithography necessitates expensive tools such as Focused Ion Beam (FIB) [24]. Chemical synthesis involves metal nanoparticles, such as silver Ag [25] with diverse shapes, sizes, and aggregates such as flowers [26], and nano-dome [27]. Although nanoparticles result in signal enhancement, achieving a repeatable or periodic distribution is challenging, leading to uncontrollable hotspots.

Optical fiber-based SERS sensors offer portability, compactness, and high stability, making them ideal for practical applications in food safety. The excitation light is delivered through the fiber, and the scattered Raman signal is collected through the same fiber using a bidirectional coupler. To enhance the Raman signal, various optical fibers have been utilized, including hollow photonic crystal fiber [28], D-shaped fiber [29], tapered fiber [30], or fiber tip. The unique fingerprint Raman spectra obtained from these samples contain distinct peaks representing the molecular composition of *Salmonella*, including proteins, lipids, nucleic acids, and cell wall components. By providing timely and accurate results, this fiberoptic SERS sensor holds promise as a valuable tool for enhancing food safety in poultry processing plants and beyond.

This paper investigates the SERS sensor patterned on a side polished multimode optical fiber. Raman spectra of the measured samples contain specific peaks that represent the molecular composition of *Salmonella*.

MATERIAL AND METHODS

Biosensor Design

Surface Enhanced Raman Spectroscopy (SERS) sensor combines metal nanoantenna arrays with a side-polished multimode optical

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fiber core, embedded within a 3D printed plastic mold. The SERS sensor along with the experimental testing setup are shown in Figure 1. This integration enables precise and swift detection of *Salmonella*, achieving exceptional sensitivity in under 10 minutes. The sensor operates by capturing the distinct fingerprint Raman spectra of *Salmonella*, which are primarily dictated by the molecular constituents of the bacteria such as proteins, lipids, nucleic acids, and cell wall components.

The 3D printed mold includes a groove for side polishing on its top surface, facilitating the side polishing process of the optical fiber core using alumina polishing sheets to achieve a flat and smooth surface essential for nanodisk array patterning. Glue is utilized to secure the optical fiber within the ports and trench. Highly uniform arrays of gold nanodisks are patterned on the side-polished fiber core. These arrays significantly enhance the Raman signal by creating SERS hotspots through laser excitation between the disk arrays, amplifying Raman signals from nearby *Salmonella* or other pathogens.

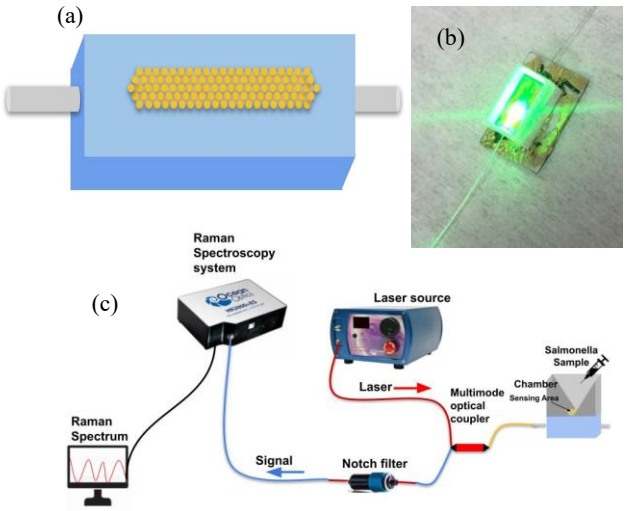

Figure 1. SERS sensor (a) 3D view, (b) Sensor (c) setup. SERs sensing is used in the backscattered configuration.

Sensor Fabrication

The sensor's fabrication utilized microsphere photolithography (MPL) to pattern nanoantenna arrays with precise 2D periodicity. Initially, a multimode optical fiber was prepared by removing its jacket over lengths of 3 mm and 7 mm at the middle portion, exposing the cladding for subsequent polishing. A 3D mold was printed using a Prusa i3 Mk3+ 3D printer with PLA Pro material (Overture). The optical fiber was carefully inserted into the mold and secured using standard glue. Precision polishing was then carried out using carbide polishing paper with varying grit sizes ranging from 5- 0.05 µm to achieve a flat surface. Subsequently, the 3D mold was affixed to a glass slide to facilitate securement on a spin coater. The MPL process was initiated to pattern nanoantenna arrays on the polished fiber surface. This involved spin-coating a 400 nm thick layer of Shipley 1805 photoresist onto the fiber, followed by immersion in a water-filled beaker containing a dispersion of dry silica microspheres in butanol. The microspheres are self-assembled into a close-packed lattice on the water surface, which is then transferred onto the photoresist-covered fiber surface. UV light exposure for 0.8-1.3 seconds was applied using an MA6 mask-aligner, followed by the development, washing, and drying of the fiber/microstructure. Subsequent sputter-deposition and lift-off processes resulted in well-defined Au nanodisk arrays with specific dimensions ranging from 600-800 nm in diameters with 1 µm periodicity, and 60 nm in height), as confirmed by SEM images in Figure 2. Additionally, a 3D plastic chamber was oriented and affixed around the sensing area. Optical images demonstrating the fabricated SERS sensor are provided in Figure 1.

Sample Preparation

The BHI broth was inoculated with Salmonella typhimurium (ATCC 14028) and incubated at 37°C for 24 hours. After the incubation, 0.1ml of Salmonella was spread plated on five BHI agar plates and incubated at 37°C for 24 hours to form bacterial lawns. The Salmonella lawns were harvested twice using 1 ml of 0.1% peptone solution, and cells were dislodged using sterile L-spreaders. The harvested cells were mixed with 9 ml of double filtered rinsate and sterile glycerol to prepare 10% stock solution to use for fiberoptics SERS based analysis. The stock solutions were serially diluted and plated in duplicate on XLD agar incubated at 37°C for 24 hours to determine the Salmonella concentrations.

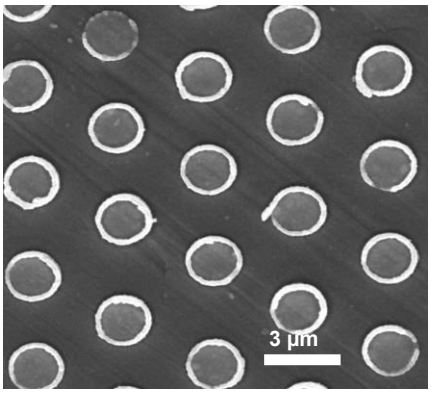

Figure 2. SEMs of the nanoantenna arrays

RESULTS AND ANALYSIS

The results of our study are demonstrated in Figure 3, which shows the ability of the SERS sensor to detect salmonella at concentrations as low as 1-3 cells/ml. This level of sensitivity is a significant advancement in pathogen detection. additionally, one of the key strengths of our sensor is its rapid detection capability, providing accurate results in just 10 minutes. This quick turnaround time is crucial for real-time monitoring and decision-making, allowing for prompt actions to mitigate potential risks associated with pathogen contamination. Additionally, our results showed a direct correlation between sensitivity and signal strength, highlighting the sensor's ability to produce robust and distinguishable signals even at extremely low pathogen concentrations. this characteristic is vital for ensuring accurate and reliable detection.

The performance of the SERS sensors was measured for two different gold nanodisk diameters, specifically 615 nm and 770 nm, in detecting *Salmonella* at a concentration of 205 cells/ml using our fiber optic sensor with SERS technology. The results revealed a notable difference in sensitivity and signal strength between the two diameters, with the larger 770 nm diameter nanodisks outperforming the 615 nm diameter counterparts as shown in Figure 4. The rationale behind this superiority lies in the relationship between nanodisk diameter and the distance between the disks. As the diameter increases, the gap between adjacent nanodisks decreases, leading to a more confined and intensified electric field between the disks. This phenomenon creates enhanced "hotspots" where the SERS effect is significantly amplified, resulting in stronger Raman signals and improved detection sensitivity. This

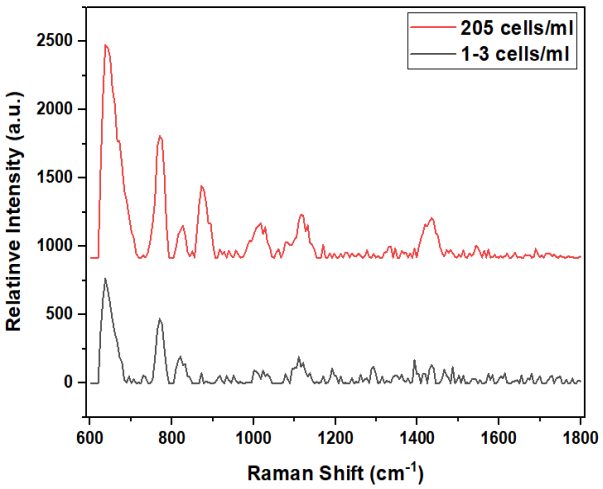

Figure 3: Spectra of Salmonella in raw poultry rinsate samples for 1-3 cells/ml and 205 cells/ml. the fiber has a diameter of 770 nm and a sensing area of 4mm.

outcome underscores the importance of nanodisk geometry in optimizing SERS-based detection, with larger diameters facilitating stronger electric field confinement and heightened signal amplification.

CONCLUSION

This paper outlines an innovative approach to designing, fabricating, and testing a low-cost Surface Enhanced Raman Spectroscopy

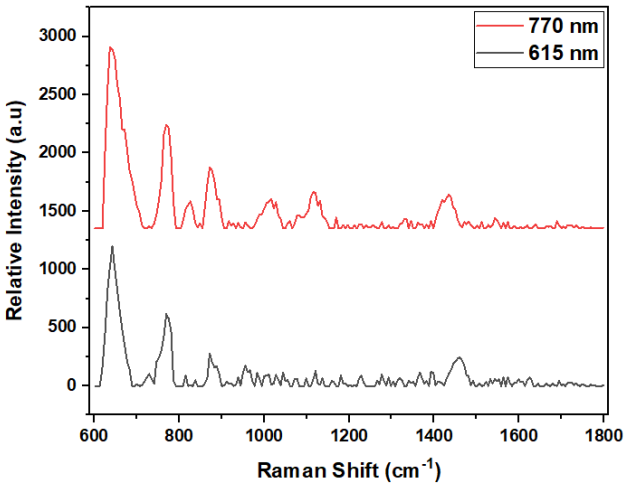

Figure 4: Effect of changing the diameter of the SERS sensor on the signal intensity. A concentration of 205 cells/ml at diameters of 615 nm and 770 nm and a sensing area of 4mm.

(SERS) sensor tailored for the rapid detection of bacterial pathogens. The fabrication of nanoantenna arrays on a side-polished optical fiber core, a key feature, significantly amplifies the signal-to-noise ratio. The sensor has achieved a sensitivity of 1-3 cells/ml for *Salmonella* typhimurium with a detection time of 10 minutes

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