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Mapping bacteria on filter membranes, an innovative SERS approach

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ABSTRACT

The existence of pathogenic bacteria in drinking water has been a threat to the safety of human well-being. Traditional methods to detect bacteria are standard plate counts or rapid methods such as ELISA and PCR. However, those methods can be time-consuming or require pre-enrichment when detecting low concentrations of bacteria. Filter membrane has been used for bacteria collection and culturing when monitoring environmental water samples. In this study, we applied surface enhanced Raman spectroscopy (SERS) to rapidly screen bacteria cells on a filter membrane based on the unique signal from an indicator molecule, 4-mercaptophenylboronic acid (4-mpba), that can specifically bind to the surface of bacteria through diol group in its structure and give off consistent SERS signal. With a pore size of 0.22 μm, the filter membrane functions as both a concentrating device and a separation mechanism that eliminates molecules smaller than the pore size. With SERS mapping, 4-mpba gives characteristic signal, indicating the presence of bacteria, whereas no 4-mpba signal was observed in the absence of bacteria. The developed method can detect *Escherichia coli*, *Salmonella enterica*, and *Listeria monocytogenes* on a filter membrane non-selectively in 80 min. Application of the method in pond water was demonstrated. However, this method may not be able to discriminate between live and dead bacterial cells and further development is needed.

1. Introduction

The safety of drinking water is essential to human well-being. However, in some developing countries, people do not have access to clean, sanitized drinking water in which the existence of pathogenic bacteria pose a great threat of bacterial transmitted diseases. According to the world health organization, the mortality of water-associated diseases exceeds 5 million people per year with more than 50% categorized as microbial intestinal infections (Cabral, 2010). Microbial contamination in drinking water is mainly associated with wastewater discharges in freshwater and coastal seawater, which serves as a major source of fecal microorganisms, including pathogens. Some of the pathogens responsible for the main bacterial infections in water are *Salmonella*, *Vibrio* and *Escherichia coli*. The United States Environmental Protection Agency (EPA) has set the standards to monitor fecal indicator bacteria such as *E. coli* to indicate the presence of pathogenic microorganisms caused by recent fecal contamination or unsanitary processing, and the maximum contaminant level goals are zero in most cases (Edition, 2006). However, there are no standard techniques established by the EPA to detect particularly low levels (below 10^1 CFU/ml) of indicator bacteria (U.S. EPA., 2014). Standard plate count has been the most developed and widely used method for pathogen detection, however it usually take days to produce a result, and not able to isolate viable but nonculturable organisms (Gunasekera et al., 2000). New and advanced technologies have been developed for the rapid detection of foodborne pathogens aiming at overcoming disadvantages associated with traditional microbiological detection techniques. Those techniques mainly include spectroscopic methods, e.g. Raman spectroscopy, Infrared spectroscopy (Chu et al., 2008; Rodriguez-Saona et al., 2001), polymerase chain reaction (PCR) (Ashimoto et al., 1996; Josephson et al., 1993) and a variety of sensor-based methods including biosensors (e.g DNA and antibodies) (Baeumner, 2003; Mao et al., 2006), chemical sensors (Su et al., 2013) and impedance sensors (Yang et al., 2004). These methods offer sensitive and specific detection of various microbial targets. Unfortunately when dealing with low concentration of bacteria in large volume of sample, pre-enrichment is often needed before applying these methods (Gracias and McKillip, 2004).

The objective of this study is to develop a rapid method that can directly detect low concentration of bacteria cells without enrichment. This method integrates filtration technique with surface enhanced Raman spectroscopic (SERS) mapping technique to realize rapid screening and quantification of bacteria cells on a filter membrane. A filter membrane can provide a threshold for the dimension of substances to be filtrated, in other words, to eliminate small molecules of interference, as well as to concentrate matter of interest. Filtration

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technique followed by membrane culturing is a conventional approach for separating and detecting coliforms in environmental water samples and liquid food with low particulate matters (Slanetz and Bartley, 1957; Weinbauer et al., 1998). SERS is a rapidly growing analytical technique for detection of low concentration of analytes. The chemical signature of the analyte can be tremendously enhanced by gold and silver nanoparticles. SERS mapping technique is an advanced chemical imaging technique, by which hundreds of SERS spectra can be automatically collected at every pixel of the defined area, and then integrated to generate artificial color images based on the intensity of a designated peak. Previously, we applied SERS mapping to detect and differentiate between several bacteria strains deposited on silver dendrites, as well as using 4-mercaptophenylboronic acid (4-mpba) modified silver dendrites to capture and detect *Salmonella* from skimmed milk (Wang et al., 2016, 2017). 4-mpba can interact bacterial peptidoglycan in cell wall and gives off strong and distinct SERS signals (Su et al., 2013; Wang et al., 2015).

In this study, we used a simple syringe filter to catch bacteria on a filter membrane, then 4-mpba was integrated to interact with bacteria and give off strong SERS signals for the ease of identification. *E. coli* was used as a model bacterium to develop the method. Different membrane types and ways of integrating 4-mpba molecules with bacteria were evaluated. The sensitivity and quantification capability of the method was also determined and compared with the membrane culturing method.

2. Experimental

2.1. Materials

Citrate coated gold nanoparticles (Au NPs) with particle size of 50 nm and 20 mg/l concentration were purchased form Nanopartz (Loveland, CO, USA). 4-mercaptophenylboronic acid and sunset yellow FCF was purchased from Sigma-Aldrich Co. LLC (St. Louis, MO, USA), ammonium bicarbonate and glucose was from Fisher Scientific (Fair Lawn, NJ, U.S.A). Durapore PVDF filter membranes with 0.1 μm pore size were from EMD Millipore Inc. (Billerica, MA, USA). Nitrocellulose membrane filters with 0.2 μm pore size were purchased from Bio-Rad Laboratories, Inc. (Hercules, CA, USA) and polycarbonate Filter Holders from Cole-Parmer Inc. (Vernon Hills, IL, USA) was used in this experiment. *E. coli* 43888 was used in this assay. Minute maid apple juice was purchased from a local grocery store. Pond water was taken from the university pond (Amherst, MA) on the day of analysis.

2.2. Sample preparation

E. coli 0157:H7 ATCC 43888 was inoculated in TSB media for 16 h to obtain an approximately 10^9 CFU/ml concentration. 1 ml bacteria culture was taken from the culture and washed 3 times with 0.9% NaCl at 6.5 ×*g* for 3 min. The washed bacteria were diluted with 0.9% NaCl to make serial dilutions. The concentration of the bacteria is determined by enumeration on tryptic soy agar plate at 37 °C for 24 h. 4-mpba was dissolved in ethanol to make a 50 mM stock solution and diluted with 50 mM ammonium bicarbonate to 100 μM upon detection to maintain a pH of 9. Au NPs were diluted water to make a 10 mg/L solution.

2.3. Filtration and SERS detection of bacteria using 4-mpba

Bacteria solution (1 ml) were filtered through a PVDF or nitrocellulose membrane using a syringe filter, followed by filtering 1 ml water through the membrane to remove small molecule interference in the solution. Then 1 ml of 100 μM 4-mpba solution was slowly (1 drop/2 s) filtered through the membrane and let half of the solution incubate with the membrane containing bacteria cells for 30 min in the filter holder. After incubation, the membrane was washed with 2.5 ml ammonium bicarbonate solution by filtration to wash away any excess 4-mpba molecules that was not bound to the bacteria surface. Finally, 1 ml of Au NPs was filtrated onto the membrane. To perform the control, 1 ml 0.9% NaCl solution was filtrated through the membrane instead of bacteria followed by the same procedure described in the bacteria sample preparation. Sometimes, air bubbles were found trapped in the holder, which could negatively affect the washing process. In this case, transfer the membrane to a clean holder or use a vacuum to suck the air bubble out could help solve the problem.

For SERS detection, a 780-nm laser with 3 mW laser power and 1 s exposure time was used to scan the sample under 20× objective with a 50-nm slit. For each bacteria sample, three 10 × 10 and one 20 × 20 mapping were scanned with 10 μm step size and all spots were randomly selected. For the control with 0.9% NaCl, three 10 × 10 mappings were scanned from three individual samples to study the variation of background signals. SERS mapping images were integrated based on the characteristic peak of 4-mpba at 1072 cm^{-1} using the OMNIC software (Thermo Scientific). SERS spectra were obtained by averaging randomly selected 10 points from a sample.

2.4. Scanning electron microscopic (SEM) sample preparation and instrumentation

The prepared membrane sample with bacteria was incubated in 2.5% glutaraldehyde solution and stored at 4 °C for 28 h. The membrane was washed with 5 ml water and air dried for 12 h. Membrane was coated with approximately 2–5 nm Au layer using a sputter coater and observed under a SEM (FEI Magellan 400 XHR-SEM).

2.5. Culture of the membrane containing bacteria in agar plate

Membranes with bacteria were placed with the side containing bacteria facing up on a tryptic soy agar plate with a thin layer (0.5 ml) of TSB broth on the surface. The membrane was pressed gently against the agar to make the membrane fully moisturized. The agar plate was incubated at 32 °C for 24 h.

3. Results and discussion

3.1. Assay development

3.1.1. Determination of the optimum membrane type

Two types of membrane (PVDF and nitrocellulose) were compared for the performance using the filtration method. To make 4-mpba signals representative of bacteria, there should be no indicator signals in the absence of bacteria, i.e. negative controls. As shown in Fig. 1c and d, SERS spectra indicated that some of the 4-mpba molecules were retained on PVDF membrane even after the washing step, which displayed the characteristic peak at 1072 cm^{-1}. This is possibly due to the hydrophilic coating on the PVDF material that interacts with 4-mpba molecules. Whereas in the case of nitrocellulose membrane, washing with ammonium bicarbonate helps to eliminate the 4-mpba on the membrane surface, and no SERS signals of 4mpba were visible (Fig. 1h). Nitrocellulose membranes were then used in the following study.

3.1.2. Determination of the optimum approach of integrating 4-mpba

The initial approach to integrate 4-mpba was to immerse the membrane with bacteria with 4-mpba to facilitate the interaction. However, only 20% of the image area was detected with bacterial signals with an initial filtration of 10^7 CFU/ml bacteria (Fig. S1 A, B). This may be because that most of the bacteria cells were detached from the membrane surface during the immersion process and the interaction between bacteria and 4-mpba remain unknown.

To retain most of the bacteria cells on the membrane, an alternative approach was tested in which 4-mpba solutions were filtrated onto the membrane instead of immersing bacteria sample in the solution and following by incubation in the filter holder directly. A schematic