Sensors and Actuators B xxx (2017) xxx–xxx

Contents lists available at ScienceDirect

Sensors and Actuators B: Chemical

journal homepage: www.elsevier.com/locate/snb

Differentiation of live and heat-killed *E. coli* by microwave impedance spectroscopy

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ARTICLE INFO

Article history:
Received 11 February 2017
Received in revised form 11 August 2017
Accepted 23 August 2017
Available online xxx

Keywords:
Impedance sensing
Bacterial detection
Cell viability
Biosensor
Microfluidic device
Lab on a chip

ABSTRACT

The detection of bacteria cells and their viability in food, water and clinical samples is critical to bioscience research and biomedical practice. In this work, we present a microfluidic device encapsulating a coplanar waveguide for differentiation of live and heat-killed *Escherichia coli* cells suspended in culture media using microwave signals over the frequency range of 0.5–20 GHz. From small populations of ~15 *E. coli* cells, both the transmitted ($|S_{21}|$) and reflected ($|S_{11}|$) microwave signals show a difference between live and dead populations, with the difference especially significant for $|S_{21}|$ below 10 GHz. Analysis based on an equivalent circuit suggests that the difference is due to a reduction of the cytoplasm conductance and permittivity upon cell death. The electrical measurement is confirmed by off-chip biochemical analysis: the conductivity of cell lysate from heat-killed *E. coli* is 8.22% lower than that from viable cells. Furthermore, protein diffusivity increases in the cytoplasm of dead cells, suggesting the loss of cytoplasmic compactness. These changes are results of intact cell membrane of live cells acting as a semipermeable barrier, within which ion concentration and macromolecule species are tightly regulated. On the other hand, the cell membrane of dead cells is compromised, allowing ions and molecules to leak out of the cytoplasm. The loss of cytoplasmic content as well as membrane integrity is measurable by microwave impedance sensors. Since our approach allows detection of bacterial viability in the native growth environment, it is a promising strategy for rapid point-of-care diagnostics of microorganisms as well as sensing biological agents in bioterrorism and food safety threats.

1. Introduction

Detection of bacteria and their viability in food, water and clinical samples is critically important in fields such as bioscience research, medical diagnosis, food screening and environment monitoring [1]. Conventional methods for bacteria detection, albeit sensitive and specific, are often time-consuming, infrastructure dependent, and require skilled technicians [2]. For example, cell growth-induced turbidity in liquid culture or colony formation on solid culture is inexpensive and relatively simple to operate, but both require extensive time to generate detectable signals [3,4]. Additionally, cultivation of bacteria is not always successful under lab conditions [5]. Fluorescence and colorimetric stains, such as SYTO 9 and propidium iodide (PI), provide viability results in a short time, with established protocols to inspect cell staining by optical microscopy, flow cytometry and microliter plate readers, etc. [6]. However, the labeling procedure is tedious and invasive [6–8]. In addition, the size of bacterial cells is at the detection limit of optical microscopy, making high-resolution imaging difficult [9]. Methods based on nucleic acid amplification are highly sensitive and specific by using target-specific primers to amplify DNA or RNA, however they are destructive and provide little information about cell viability [10,11].

Electrical sensing of cells, including bacteria, is attractive since it is label-free, easy to miniaturize, and offers the possibility of real-time results with high throughput [12,13]. Impedance sensing is one of the most popular methods, and has been implemented both for cells adhered to a substrate or in a suspension [14,15]. For example, Coulter counters have been widely adopted for cell counting and sizing in a suspension, based on impedance sens-

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http://dx.doi.org/10.1016/j.snb.2017.08.179
0925-4005/

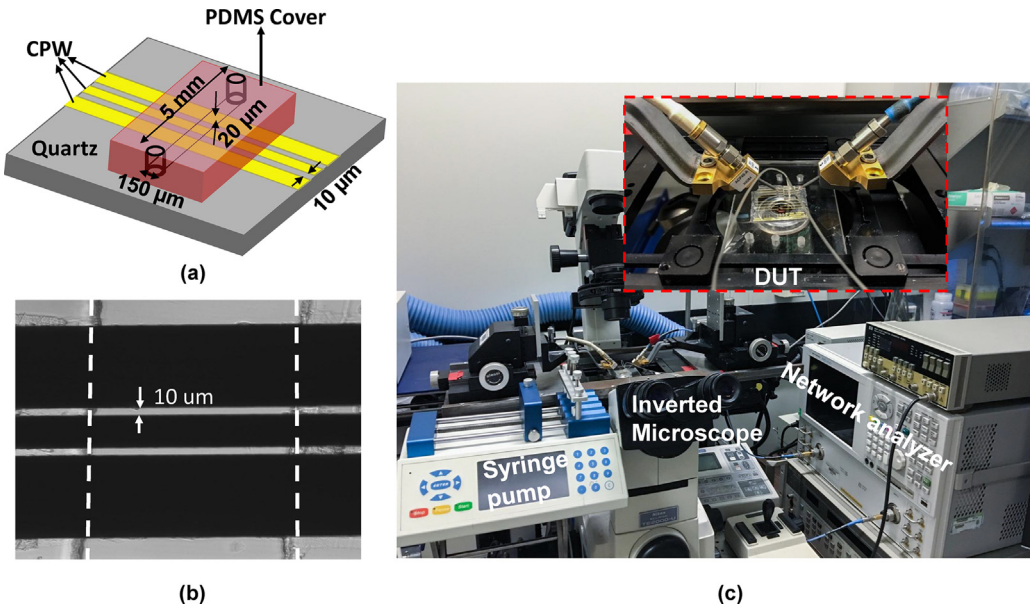

Fig. 1. (a) A schematic of the device based on a CPW sandwiched between a quartz substrate and a PDMS cover. (b) A micrograph of the device observed under the optical microscope. The three black bands are the electrodes. Dash lines on the sides trace side walls of a transparent microfluidic channel. (c) A photograph showing the experimental setup, containing the test devices connected to a PNA and mounted on an optical microscope. Samples were injected into the device through a syringe pump. The inset shows a zoomed-in picture of the device under test (DUT) interrogated by two microwave manipulators on the microscope stage.

ing across a pinhole in the kHz range [16,17]. Microfluidic Coulter counters have been demonstrated by several groups, allowing cell counting and sorting in a portable device [18–21]. Miniaturized impedance sensors using coplanar electrodes have been implemented for monitoring cell proliferation [22], spreading [23,24] and membrane integrity [25], which find applications in basic cell biology research [26] and drug screening [27]. Furthermore, impedance sensing has been used for cell viability detection, including real-time and long-term monitoring of epidermal cell viability [28], cell death induced by viral infection [29,30], chemical toxicity [31] and bacterial metabolism [32,33]. However, most of these impedance sensors are operated using discrete frequencies on the order of MHz or lower, where solution parasitics are severe and the signal is sensitive to the bulk ion concentration. Double layers on the surface of electrodes limit the voltage drop on target cells and reduce detection sensitivity [34]. The dielectric properties have also been found to depend on many parameters of the cells in this frequency range, so it is hard to attribute the signal change to a single biological parameter using discrete frequencies [35]. Another concern is cell damage by electroporation at such frequencies when the voltage is too high [36–39]. Cell sorting by dielectrophoresis followed with impedance sensing [40], albeit effective to separate live vs. dead cells, requires low-conductivity solutions to promote cell polarization, thus preventing cell detection in their native environment.

Microwave impedance sensing mitigates most of the challenges heretofore mentioned. One of the main advantages is that ion conduction in physiological solutions diminishes [41–44] because ions are too slow to respond at gigahertz frequencies. This facilitates direct cell detection in their native growth environment and extraction of their electrical properties without preparing cells in a low conductive solution. Additionally, transparency of the cell membrane to microwave signals minimizes electroporation and allows interrogation of the cytoplasmic properties, providing complementary information to conventional measurements at radio frequencies. In the past few years, microwave impedance sensors have been implemented by several groups for the detection of biological cells [45–47] and proteins [48]. For instance, Nikolic-Jaric's group detected yeast and mammalian cells in microwave frequencies [45]. Blondy's group reported a biosensor design based on microwave impedance measurements to analyze the growth of different types of adherent cells [46]. Using microchip-based dielectric spectroscopy, Grenier's group characterized dielectric properties of different aqueous solutions [49], biological cell suspensions and a population of adherent cells [47,50–52], and related the measured parameters to cell proliferation and pathogenic states [53]. Moutier's group reported using dielectric spectroscopy to detect bacteria proliferation in their native culture environment at a frequency range of 1–3 GHz [54]. Recently single-cell dielectric measurements up to 40 GHz have also been reported [52]. Furthermore, a model to extract the dielectric parameters has been proposed based on Maxwell's mixture equation [55]. However, most of these studies have limited sensing bandwidth and worked with relative large mammalian cells. In addition, measurement reproducibility was not always confirmed [52]. Many technical challenges are also present for microwave impedance sensing, including impedance match and calibration, circuit modeling and analysis, detection sensitivity in an aqueous environment, and selectivity. Here, we demonstrate for the first time the use of a microwave impedance sensor for reproducible detection of *E. coli* viability in their native culture media in the frequency range of 0.5–20 GHz. The microwave coplanar waveguide (CPW) was designed with broadband impedance match and low loss, and integrated with a microfluidic channel for delivering culture media with and without cells [56]. By fitting the measured insertion loss $|S_{21}|$ and return loss $|S_{11}|$ to an equivalent circuit, cytoplasmic electrical properties were extracted for small populations of *E. coli*. The difference between live and dead *E. coli* was confirmed by off-chip measurements of cytoplasm conductivity, permittivity, protein diffusivity and membrane integrity.

2. Material & methods

2.1. Bacteria culture and sample preparation

E. coli strain PHL 628 was cultured overnight in lysogeny broth (LB, Sigma Aldrich, St. Louis, MO) containing 50 μg/mL kanamycin (Sigma Aldrich, St. Louis, MO) in a 37 °C shaking incubator. The *E. coli* suspension was then centrifuged and washed twice in fresh

Please cite this article in press as: H. Li, et al., Differentiation of live and heat-killed *E. coli* by microwave impedance spectroscopy, Sens. Actuators B: Chem. (2017), http://dx.doi.org/10.1016/j.snb.2017.08.179