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Label-free electrical sensing of bacteria in eye wash samples: A step towards point-of-care detection of pathogens in patients with infectious keratitis

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ABSTRACT

The diagnosis of keratitis is based on visual exam, tissue cytology, and standard microbial culturing to determine the type of the infectious pathogen. To prescribe appropriate therapy, it is important to distinguish between bacterial, fungal, and viral keratitis, as the treatments are quite different. Diagnosis of the causative organism has a substantial prognostic importance. Further, timely knowledge of the nature of the pathogen is also critical to adapt therapy in patients unresponsive to empiric treatment options, which occurs in 10% of all cases. Currently, the identification of the nature of the pathogen that causes keratitis is achieved via microbial culture screening, which is laboratory-based, expensive, and time-consuming. The most frequent pathogens that cause the corneal ulcers are *P. aeruginosa* and *S. aureus*. Here, we report a microchip for rapid (< 1 h) detection of *P. aeruginosa* (*6294*), *S. aureus*(LAC), through on-chip electrical sensing of bacterial lysate. We evaluated the microchip with spiked samples of PBS with bacteria concentration between 10^1 to 10^8 CFU/mL. The least diluted bacteria concentration in bacteria-spiked samples with statistically significant impedance change was 10 CFU/mL. We further validated our assay by comparing our microchip results with the standard culture-based methods using eye washes obtained from 13 infected mice.

1. Introduction

Corneal ulceration is a dominant cause of global visual impairment and blindness. Ulcers can be treated, but late diagnosis may lead to severe scars to the cornea, opacification, and eventual loss of vision. Unfortunately, deferred diagnosis typically occurs due to the lack of suspicion in cases of corneal ulcers (Tsai et al., 1992). Corneal ulcer is an epithelial defect followed by inflammation and necrosis of the underlying epithelial tissue caused by the invasion of infectious pathogens. Corneal ulcers are chiefly induced by bacteria but other microbes including fungi (Fusarium and Candida), parasites (Acanthamoeba), and viruses (herpes simplex virus)can also lead to the development of ulcers (Klotz et al., 2000). Traditionally, the most common groups responsible for bacteria-induced corneal ulcers (most often due to the extended usage of the contact lenses) include *Pseudomonas* and *Staphylococcus*, which are typically found in 60% of the keratitis patients (Pharmakakis et al., 2003). A prolonged exposure of these infectious pathogens to the corneal tissue can lead to the destruction of cell components, scarring, and complete vision loss. The contact lens wearers were at a higher risk (40–50%) of developing corneal ulcers as per studies conducted in Paris and Taiwan (Fong et al., 2004; Bourcier et al., 2003). Over 31 million users in the United States use contact lenses that put forth a high risk of developing corneal ulcer (Farandos et al., 2015). Until culture tests results are available, a topical broad range of antibiotics is used for therapy. Some of the disadvantages of using fortified antibiotics include limited availability, short shelf-life, high cost, contamination risks, and the need for refrigeration (Gokhale, 2008; Gangopadhyay et al., 2000; American Academy of Ophthalmology, 2013; Fintelmann et al., 2011; Leeming, 1999; Varaldo, 2002). The efficacy of the treatment involving antimicrobials and frequent administration of antibiotic eye drops (tobramycin, piperacillin, ticarcillin) is tedious both for the patient and the care taker (Zaidi et al., 2008). Therefore, there is a clear and unmet clinical need for alternative easy-to-use approaches for rapid, accurate, and sensitive point-of-care detection of bacteria or viruses in tear samples of patients with keratitis infection.

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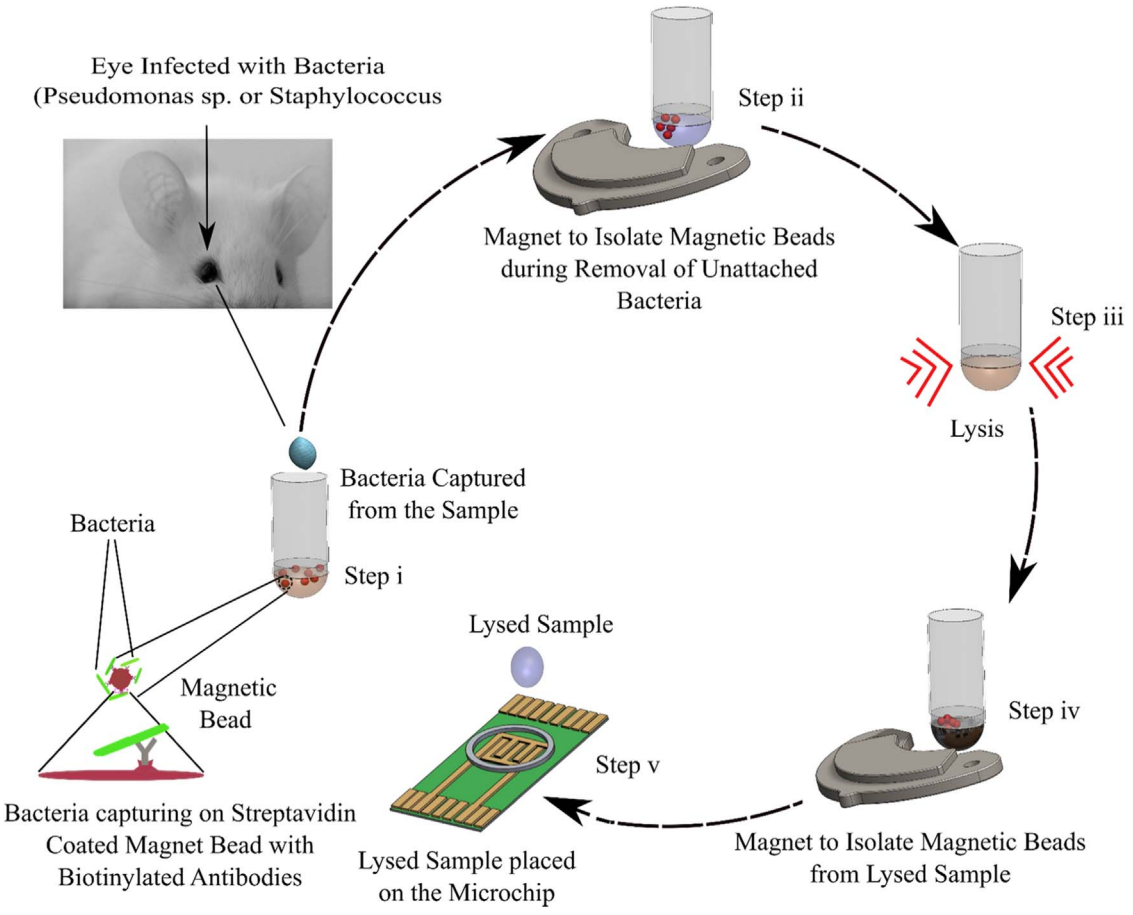

Fig. 1. 3D Schematic of the presented mechanism for bacteria detection using electrical sensing of pathogen lysate. Process flow for bacteria capture and detection: (i) Sample containing target pathogen is suspended with magnetic beads coated with streptavidin and conjugated with biotinylated antibodies and incubated for 30 min, (ii) The conjugated beads are isolated using a magnetic stand, (iii) The beads are washed using 10% glycerol in DI water 4 times to remove unbound bacteria and electrically conductive solution. The captured bacteria are then lysed using 5% Triton X-100 solution and sonication, (iv) Beads are isolated using magnetic stand, (v) The bacterial lysate is loaded onto a microchip with interdigitated electrodes for detection through impedance spectroscopy.

To detect target bacteria in biological samples, several diagnostic approaches based on surface-enhanced Raman spectroscopy (Premasiri et al., 2005), quartz crystal microbalance sensing (Bao et al., 1996), cantilever sensing (Ndieyira et al., 2008; Burg et al., 2007), impedance-based sensing (Mannoor et al., 2010), and electrochemical detection have been developed (Liao et al., 2007). Among the various sensing modalities used in the development of biosensors for bacteria detection, impedance spectroscopy has shown great promise due to its simplicity, low-cost, portability, and adaptability to multiplexing (Boehm et al., 2007; Gibson et al., 1992). It has been used in developing biosensors for virus detection as well (Shafiee et al., 2013, 2015a, 2015b, 2015c; Safavieh et al., 2016a, 2016b). The impedance-based sensing mechanisms developed by others are based on the detection of signal changes due to binding target bacteria on the surface of a functionalized electrode. Such sensing mechanism requires relatively high concentrations of bacteria to significantly change the impedance magnitude in the dielectric property of the electrode surface. Here, we detect the bulk impedance change of the sample due to bacteria lysis and the release of intracellular charged molecules of bacteria into a non-ionic background solution (Fig. 1). *Pseudomonas aeruginosa (6294)* and *Staphylococcus aureus* (LAC) were first captured and isolated using streptavidin-coated magnetic beads conjugated with biotinylated antibodies and captured bacteria were washed using a low electrically conductive solution (with 100 µL of 10% glycerol) to remove the conductive background media. To completely remove the ionic background, we performed four washes (Shafiee et al., 2015a, 2015b, 2015c). The captured bacteria were lysed in the presence of 5% Triton X-100 (60 µL). The lysis step breaks the cell membrane and releases charged molecules into the solution, which changes its electrical properties. The bacterial lysate was separated from magnetic beads using a magnetic stand off-chip and detected through on-chip electrical sensing. The change in impedance magnitude of the bacterial lysate samples was then measured using an impedance meter. We evaluated our microchip with bacteria-spiked samples and eye washes from infected mice (n=13) and compared our results with the bacteria culture-based standard method.

2. Materials and methods

2.1. MEMS-based sensor fabrication

The microsensor was fabricated by combining the microtechnology process (oxidation, e-beam evaporation, photolithography, and wet etching) and additive manufacturing (Laser Cutting, VLS2.30 from Universal Laser System). The process flow for fabricating micro-electro-mechanical systems (MEMS)-based sensor is shown in Fig. 2(a). (i) A 4-in. silicon wafer < 100 > from Semiconductor Wafer, Inc. was used as a substrate, (ii) Plasma-enhanced chemical vapor deposition (PECVD) (Plasmalab System 100 from Oxford Instruments) was used to deposit 0.5 µm thick oxide. The deposition temperature and rate were 300 °C and 0.05 µm/min respectively, (iii) Chrome/gold (Cr/Au) (0.02 µm/0.4 µm) was deposited simultaneously on four oxidized silicon wafers using an e-beam evaporator, (iv) A 0.5 µm thick layer of positive photoresist (1813 Shipley®) was coated