Published in final edited form as:

Biosens Bioelectron. 2017 May 15; 91: 32–39. doi:10.1016/j.bios.2016.12.035.

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 bacteria lysate. We evaluated the microchip with spiked samples of PBS with bacteria concentration between 10¹ to 10⁸ CFU/mL. The least diluted bacteria concentration in bacteria-spiked samples with statistically significant impedance change was 10 CFU/ml. We

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Author contributions

H.J.P., M.K.K., and H.S. conceived the method; H.J.P., M.K.K., M.G., and H.S., planned the experiments; H.J.P. fabricated the microsensor; H.J.P., M.K.C., M.K.K., and S.V. performed the experiments; H.J.P., S.V., and M.K.K. analysed the data; M.G. infected the mice and prepared bacteria samples; H.J.P., M.K.K., A. M., M.G., and H.S. wrote the manuscript. All authors have given approval to the final version of the manuscript.

Competing financial interests:

The authors declare no competing financial interests.

further validated our assay by comparing our microchip results with the standard culture-based methods using eye washes obtained from 13 infected mice.

Keywords

Keratitis; Microsensor; Bacteria Lysate; Point-of-Care Detection; Electrical Sensing

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., 1997). 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 herpes simplex viruses 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 (Elissa et al., 2014; Gokhale 2008; Gangopadhyay et al., 2000; American Academy of Ophthalmology 2013; Fintelman 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.

To detect target bacteria in biological samples, several diagnostic approaches based on surface-enhanced Raman spectroscopy (Permasiri 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; Shafiee et al. 2015; Safavieh et al. 2016). 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 μ L of 10% glycerol) to remove the conductive background media. To completely remove the ionic background, we performed four washes (Shafiee et al., 2015). The captured bacteria were lysed in the presence of 5% Triton X-100 (60 μ L). The lysis step breaks the cell membrane and releases charged molecules into the solution, which changes its electrical properties. The bacteria 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 bacteria 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-inch 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 μ m thick oxide. The deposition temperature and rate were 300 $^{\circ}$ C and 0.05 μ m/min respectively, (iii) Chrome/gold (Cr/Au) (0.02 μ m/0.4 μ m) was deposited simultaneously on four oxidized silicon wafers using an e-beam evaporator, (iv) A 0.5 μ m thick layer of positive photoresist (1813 Shipley®) was coated on the wafers deposited with Cr/Au and prebaked at 90 $^{\circ}$ C for 1 min, (v) A photolithography technique was used to pattern interdigitated electrodes (UV exposure (500 W lamp, 18 sec) using EVG® 620, postbake (110 $^{\circ}$ C for 1 min), and photoresist development (MF-321 Developer at 21 $^{\circ}$ C), (vi) The unwanted Cr/Au was etched at 25 $^{\circ}$ C by wet etching (TFA from Transene Company Inc. and CR-7 from KMG Chemicals). The etch rates for Cr and Au were 24 $\text{\AA}$ /sec and 28 $\text{\AA}$ /sec, respectively, (vii) To strip the photoresist, each wafer was dipped inside a beaker containing acetone and placed in an ultrasonic bath for 5 minutes, (viii) To create Poly (methyl methacrylate) (PMMA)/Double Side Adhesive (DSA) wells (2 mm $\times$ 2 mm $\times$ 1.5 mm) VLS 2.3 laser platform (Universal Laser System Inc.) was used. The power, scan speed, and pulse per inch rate were set at 24 W, 5 mm/s, and 1000 pulses/inch respectively. After removing one of the protective layers of the DSA, it was attached to the cut PMMA. PMMA/DSA was cut using a laser cutter to obtain the microwell and the protective layer from the other side of DSA was subsequently peeled off before attaching to each chip to

realize the device. The photograph of a 4-inch oxidized silicon wafer with 30 microchips (magnified view shows the electrodes with 10 μm width and 20 μm spacing) is shown in Figure 2(b). The array of microchips integrated with PMMA wells on a 4-inch oxidized silicon wafer is shown in Figure 2(c). Each microchip is 8 mm $\times$ 8 mm $\times$ 1.5 mm (L $\times$ W $\times$ H) with active area of 2 mm $\times$ 2 mm $\times$ 1.5 mm.

2.2 Sample preparation

2.2.1 Ethics Statement—Animal experiment were carried out adhering to the Nation Institutes of Health guidelines for housing and care of laboratory animals. The experiments were performed in accordance with institutional regulations after protocol review and approval by the Harvard Medical School Animal Care and Use Committee. They were consistent with the Association for Research in Vision and Ophthalmology guidelines for studies in animals (protocol 404R98).

2.2.1 Magnetic bead preparation—Streptavidin-coated magnetic beads (1 μm diameter, Thermo fisher scientific-Pierce™ Streptavidin Magnetic Beads 88816) were washed three times using PBS in a micro-centrifuge tube. All washing steps were done with the help of a magnetic stand (Millipore Magna GriP 20–400). 10% (v/v) solution of biotinylated antibodies (1 mg/mL) against *P. aeruginosa* in PBS was used to re-suspend the magnetic beads. The mixture was incubated overnight on a shaker at 4°C.

Similar procedure was followed for conjugating streptavidin-coated magnetic beads with *S. aureus* antibody (0.5 mg/mL) with 20% (v/v) of biotinylated target antibodies.

2.2.2 Target pathogen capture and conjugation—Magnetic beads were diluted (10%, v/v) in PBS and washed three times. A 60 μL of the magnetic bead solution was placed in a microcentrifuge tube and with the help of the magnetic stand the supernatant was removed and replaced with 100 μL of bacteria-spiked PBS or eye wash samples for enrichment. The sample-bead mixture was placed on a rotator at 15 rpm and incubated at room temperature for 30 minutes. 100 μL of bacteria-free PBS was mixed with magnetic beads to prepare control samples for the experiments. For experiments involving mice eye washes, the eye washes obtained from the uninfected left-eyes were used as negative controls against the respective infected right-eyes.

2.2.3 Washing and lysing—The mixture containing the sample and magnetic beads was washed four times with 10% glycerol to remove any trace of the electrically conductive solution from the sample. After the 4th wash, 10% glycerol solution was replaced with 60 μL of 5% Triton X-100, which can disrupt the phospholipid bilayer of bacteria. The sample was sonicated at room temperature for 10 minutes for complete lysis (Shafiee et al., 2013).

2.2.4 Bacteria sample preparation and growth conditions—*Pseudomonas aeruginosa* (6294 strain) was cultured through overnight incubation in blood agar plates (TSA with 5% Sheep Blood) at room temperature. *Staphylococcus aureus* (LAC) through overnight incubation in blood agar plates (TSA with 5% Sheep Blood) at 37 °C. A swab of bacteria colony was suspended in PBS. *P. aeruginosa* was washed by (ThermoScientific Sorvall Legend XT) centrifuging at 3000 rpm for 15 minutes and re-suspending in PBS. The

washing was performed twice to remove any remnants of noisy media prior to establishing the concentration using the spectrophotometer. *P. aeruginosa* in PBS was diluted to obtain an OD value of 0.45 using a Spectrophotometer (ThermoScientific Nanodrop 1000) at a wavelength of 650 nm and *S. aureus* for an OD value of 0.4 at a wavelength of 450 nm. The concentrations of the samples were pre-established through plate counts as 10^8 CFU/mL for *P. aeruginosa* and *S. aureus*. The bacteria spiked PBS samples were serially diluted to obtain concentrations from 10^8 CFU/mL to 10^1 CFU/mL.

2.3.5 Mice—6–8 week old C57BL/6 mice were purchased from Taconic Farms and maintained in the Channing Laboratory Animal Care Facilities for the duration of the experiments.

2.3.6 Bacteria strains and inocula—Invasive *Pseudomonas aeruginosa* (6294 strain) was used throughout these experiments. The bacteria strain was grown overnight at 37 °C on Tryptic Soy Broth agar plates supplemented with 5% sheep blood. The bacteria suspensions were prepared in saline solution and used for subsequent infection experiments.

2.3.7 Infection model—Infections were carried out as described previously (Gadjeva et al., 2010). Briefly, intraperitoneal ketamine and xylazine injections were used to anesthetize mice. Three 0.5 cm scratches were made with 25G needle tip on the cornea and an inoculum of 5×10^6 CFU of *P. aeruginosa* in 5uL was delivered onto the eye. For approximately 30 mins, the mice remained sedated. An observer unaware of the experimental status of the animals recorded the daily scores for evaluation of corneal pathology. The scoring was done using following scoring system (grade 0 to 4): 4, phthisis bulbi (shrinkage of the globe after inflammatory disease), perforation of the cornea, or both; 3, dense opacity covering the entire anterior segment; 2, dense opacity covering the pupil; 1, faint opacity partially covering the pupil; and 0, eye macroscopically identical to the uninfected contra-lateral control eye. To determine ocular bacteria presence 10 ul PBS was placed onto the corneal tissue and pipetted several times to wash the surface. Ocular washes and corneal bacteria counts at 48h after infection were enumerated serially diluting the homogenates and plating on *P. aeruginosa* selective McConkey agar plates.

2.3.8 Monoclonal antibodies—The monoclonal antibodies (mAbs) were produced in Chinese Hamster Ovary (Cho) cells, transfected with the ORF encoding the heavy and light chains of the F429 (anti-alginate mAb) and 598 (anti-PNAG mAbs) (Zaidi et al., 2008; Zaidi et al., 2010; Bentley et al., 2013)

2.3.9 Impedance measurement—8 μ L of the bacteria lysate was loaded onto a microchip and the impedance magnitude was measured with a LCR meter (LCR8110G, GW Instek, CA). 8 μ L of the bacteria lysate was loaded onto a microchip and the impedance magnitude was measured with a LCR meter (LCR8110G, GW Instek, CA). Impedance magnitude of the samples was measured at 1000 Hz and 1V (Shafiee et al., 2013; Shafiee et al., 2015).

3. Results

3.1 Limit of quantification evaluation

The microchip was first evaluated using bacteria-spiked PBS samples. We have previously observed that the maximum impedance magnitude shift in pathogen lysate samples occurs at 1,000 Hz (Shafiee et al., 2013). Therefore, we performed our measurements and statistical analysis for comparison between bacteria bacteria-spiked and control samples at 1,000 Hz. The impedance magnitudes of bacteria lysate samples at 1,000 Hz and 1V were significantly different than the impedance magnitude of bacteria-free control samples (Fig. 3a and 3b, $p < 0.05$, $n = 3$). The control samples were bacteria-free PBS. We observed that the magnitude of impedance due the bacteria lysate decreased with the increase in the bacteria concentration. Figures 3c and 3d show the linear correlation between the changes in impedance magnitude of the bacteria lysate samples and the bacteria load. The R-squared values for *P. aeruginosa* and *S. aureus* were 0.9568 and 0.9712 (Fig. 3c and 3d). These results show that the least diluted bacteria-spiked PBS sample that showed significantly different impedance magnitude compared to control were samples with bacteria concentration of 10^1 CFU/ml.

3.2 Specificity evaluation

For specific capture of target bacteria in the samples we used in-house antibodies (anti-alginate F429 mAb) (Saidi et al., 2008; Zaidi et al., 2010) and (anti-PNAG mAb) (Maria-Litran et al. 2002; Kelly-Qunitos et al., 2006). PNAG is a surface polysaccharide synthesized by *S. aureus*. The specificity of the anti-PNAG mAb was previously shown by binding to wild-type *S. aureus* (Bentley et al., 2013). Hence, we used anti-PNAG mAb for the specific capture and isolation of *S. aureus*. MAb F429 has been shown to mediate opsonic killing of nonmucoid strains of *P. aeruginosa* by targeting alginate which is an exopolysaccharide (Zaidi et al., 2008; Gadjeva et al., 2010). Cells which did not express alginate were not targeted by the antibody. To evaluate the specificity of our electrical sensing platform, we showed that *P. aeruginosa* could be specifically isolated and detected in PBS samples spiked with both *P. aeruginosa* and *S. aureus* making use of the anti-alginate F429 mAb. A one-way ANOVA analysis showed statistical significant difference between the bacteria-free PBS control and the samples spiked with *P. aeruginosa* (Fig. 4a, $p < 0.05$, $n = 3$). Negative control samples, PBS spiked with *S. aureus* (10^3 CFU/mL and 10^5 CFU/mL), showed no statistically significant difference in impedance magnitude when compared to bacteria-free PBS samples (Fig. 4a, $p > 0.05$, $n = 3$). The impedance magnitude of PBS samples spiked with *P. aeruginosa* with bacteria concentration of 10^3 CFU/mL and PBS spiked with both *P. aeruginosa* and *S. aureus* with concentration of 10^3 CFU/mL were not statistically different (Fig. 4a, $p > 0.05$, $n = 3$). We also observed that the impedance magnitude of samples spiked with *P. aeruginosa* (10^5 CFU/ml) and samples spiked with the mixture of *P. aeruginosa* and *S. aureus* were not significantly different (Fig. 4a, $p > 0.05$, $n = 3$). Similarly, we showed that *S. aureus* could also be captured and detected with high specificity in the presence of *P. aeruginosa* in the samples using anti-PNAG F598 mAb (Fig. 4b). The impedance magnitude of the samples spiked with *P. aeruginosa* was not significantly different than bacteria-free controls (Fig. 4b, $p > 0.05$, $n = 3$), while samples spiked with *S. aureus* showed statistically significant difference compared to bacteria-free controls (Fig. 4b, $p < 0.05$, $n = 3$). These

results showed that the impedance magnitudes of samples spiked with the mixture of *S. aureus* and *P. aeruginosa* (10^3 CFU/mL and 10^5 CFU/mL) were not significantly different than samples spiked with *S. aureus* with the same concentration. These results showed that the microchip was able to detect the target bacteria in the presence of non-target pathogen using magnetic beads conjugated with the antibodies used in this study.

3.3 Evaluating the microchip using infected mice eye wash samples

We evaluated the microchip using mice infected with *P. aeruginosa* (n=13). One eye was infected with *P. aeruginosa* while the other eye acted as a negative, uninfected control. Both the infected and uninfected mice eyes were washed with PBS and eye wash samples were tested using the microchip. A multiple *t*-test corrected for Holm-Sidak statistical analysis was performed for the impedance magnitudes of both the left and right eye wash samples for all 13 mice samples (Fig. 5a). The impedance magnitude of the eye washes from infected and uninfected eyes were significantly different for mice 1, 2, 8–13 (Fig. 5a, $p < 0.05$, $n = 3$). For all other samples (3–7) no significant difference was observed (Fig. 5a, $p > 0.05$, $n = 3$). A sample size of 3 here represents that the sample was tested using three different chips with same dimensions. Figure 5b shows the Daily scores and plate count results, which was done in parallel to the impedance-based testing, for the infected right eye wash samples taken from the mice. Samples obtained from mice 3 through 7 showed no bacteria growth on plate. The plate counts were compared with the microchip output for estimation of the accuracy through a dichotomous test of diagnosis with a positive representing an observable presence of infection and a negative representing a non-observable presence of infection. The Daily scores for samples 1 to 13 were 1, 1, 0, 0, 0, 0, 0, 1, 2, 3, 3, 3, and 2, respectively. Out of the 13 mice samples used in this animal model evaluation, samples 1, 2, 8–13 were positive based on both plate count and microchip results. The raw data obtained from all of the samples used for device performance evaluation using spiked samples as well as eye wash samples from infected mice are shown in Tables S1–S3.

4. Discussion

The current methods for keratitis infection diagnosis are based on visual exam, tissue cytology, and standard microbial culturing. To prescribe appropriate therapy, it is important to distinguish between bacterial, fungal, and viral keratitis, as the treatments are quite different. The common appearance of ulceration as a manifestation of disease does not provide sufficient information about its derivation. Furthermore, 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 (Rodman et al., 1997). Currently, the patients with keratitis are prescribed a variety of topical antibiotic drops as therapy with initial instillation at hourly intervals; day and night for 24–48 h and further treatment is tapered according to the clinical progress (Elissa et al., 2014; Gokhale 2008; Gangopadhyay et al., 2000; Zaidi et al., 2008). Moreover, antibiotic resistance of the bacteria pathogens in ocular diseases makes the identification of the causative pathogen difficult (Fintelman et al., 2011; Leeming et al., 2011; Valardo et al., 2002; Sharma et al., 1999). Thus, an alternative easy-to-use approaches for accurate detection of bacteria or viruses in tear samples of patients with keratitis infection may change the paradigm in the disease

management. The most frequent pathogens that cause the corneal ulcers are *P. aeruginosa* and *S. aureus*. They are found typically in 60% of the keratitis patients (Pharmakakis et al., 2003). Here, we developed a microchip technology for detecting *P. aeruginosa* and *S. aureus* bacteria pathogens through electrical sensing of bacteria lysate.

Electrical sensing is a powerful modality that can be used for the development of microfluidic-based platforms for target cells or pathogen sorting and detection (Shafiee et al. 2009; Shafiee et al. 2010, Shafiee et al. 2015, Safavieh et al. 2016). Electrical sensing-based methods through impedance magnitude measurement of intact bacteria captured on interdigitated electrodes usually requires high concentration of bacteria on the surface of electrodes in order to generate a detectable electrical sensing (Dastider et al., 2015). Bacteria lysis used in our study brings charged molecules into a non-ionic background solution resulting in change of electrical conductivity of the sample. Our method provides a sensitive approach for bacteria detection without the need of nucleic acid amplification. It also eliminates the complexity of signal amplification in electrochemical sensing-based approaches. The use of eye washes as opposed to corneal tissue scrapings that are used commonly in the diagnosis of corneal keratitis, provides a non-invasive and facile approach.

The demonstrated assay is a step towards point-of-care testing for corneal keratitis. It has the potential to be a clinically relevant point-of-care device as the testing is rapid (<1 h) and simple. The assay does not utilize technically challenging methodologies. The required time to be perform the assay in a laboratory setting is estimated to be less than an hour, including 30 minutes for incubation time, 10 minutes washing, 10 minutes for bacteria lysis, and 1 minute for electrical signal analysis and read out. It is both specific and sensitive as demonstrated in this work using the two bacteria strains tested. The least diluted spiked bacteria samples that showed significantly different impedance magnitude compared to control were samples with 10^1 CFU/mL that is clinically relevant for keratitis infection detection (Li et al. 2002). We were able to detect all the positive samples in a pool of infected and uninfected eye wash samples taken from infected mice (n-13). The material cost per test was less than \$5 including \$2 for microelectrodes fabrication, less than 5 cents for DSA and PMMA parts, 80 cents for magnetic beads, and \$1.5 for antibodies. Here, we used lithography to fabricate the electrodes on 4" wafers. The material cost for fabricating the microelectrodes can be significantly reduced by using 12" wafers or screen-printing method. Since the antibodies against *S. aureus* and *P. aeruginosa* used in this work were prepared in-house, we estimated the cost of the antibodies based on commercially available antibodies. The antibodies can be supplied post lyophilisation to help in ease of storage of consumables at satellite testing facilities. We only focused on capture and detection of *S. aureus* and *P. aeruginosa* using on-chip electrical sensing. However, this method can potentially be used for identifying if the source of infection is due to bacteria, fungi or viruses. We envision that such platform using appropriate lysing agents to differentially lyse bacteria, fungi or viruses before target capturing using antibodies may eliminate potential non-specific binding issues due to cross-reactivity between the antibodies and the pathogens (For, example, Chitinase lyses all fungi and not bacteria) (Vega et al., 2012).

Furthermore, we performed target capture off-chip using conjugated magnetic beads. This manual off-chip sample processing required only a 100 μ l of bacteria sample volume for

simple in-laboratory sample processing. We envision that the on-chip sample processing for bacteria capture may eliminate the current restriction on sample volume.

5. Conclusions

Here, we report the development and evaluation of a low-cost, label-free diagnostic assay for detecting bacteria (*S. aureus* and *P. aeruginosa*) on-chip using eye wash samples through electrical sensing of bacteria lysate. The data analyzed in this study has been limited to testing bacteria-spiked samples and eye washes from infected mice. Additional studies are needed using human patient samples with larger sample sizes to make a clear assessment of the device and the presented technology. Though the system requires further validation studies, the inexpensive assay with its rapid, facile and reliable output has taken its first steps towards becoming a highly relevant clinical point-of-care methodology.

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

Acknowledgments

Research reported in this publication was partially supported by the National Institute of Allergy and Infectious Disease of the National Institute of Health under award number R01AI118502; Brigham and Women's Hospital and Harvard Medical School through Bright Futures Prize and Innovation Evergreen Award; and King Abdulaziz University, Saudi Arabia through Scientific WAQF Fund under grant number 17/1436. Mihaela Gadjeva is supported by Public Health Service grant EY022054 from the National Eye Institute and American Association of Immunology Fellowship grant. The content is solely the responsibility of the authors and does not necessarily represent the official views of the National Institutes of Health. We acknowledge the support of Maryland Nanocenter for sensor fabrication. We thank Prof. G. Pier for supplying the antibodies.

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Highlights

- A microchip technology was developed to detect *P. aeruginosa* and *S. aureus* bacteria in tear samples for the diagnosis of keratitis infection.
- Target bacteria was detected through electrical sensing of bacteria lysate on-chip.
- The device was evaluated with spiked PBS samples and eye washes taken from bacteria infected mice.

Figure 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 minutes, (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 bacteria lysate is loaded onto a microchip with interdigitated electrodes for detection through impedance spectroscopy.

Figure 2. Microchip fabrication

(a) Process flow: (i) A 4-inch silicon wafer is used as a substrate, (ii) 500 nm oxide layer is deposited using plasma-enhanced chemical vapor deposition (PECVD), (iii) Chrome/Gold (Cr/Au) (20 nm/400 nm) was deposited using e-beam evaporation, (iv) Positive photoresist (1813 Shipley®) was spun coated at 2000 rpm to obtain 2 μm thick layer and prebaked at 90 °C for 1 min, (v) Standard photolithography technique was used to pattern inter-digitated electrodes (UV exposure, postbake, and photoresist development), (vi) Cr and Au was etched in chrome and gold etchant, respectively, (vii) To remove the photoresist, the chip was placed inside the beaker containing acetone and placed in ultrasonic bath, (viii) Using a VLS 2.3 laser platform PMMA/DSA microwell was fabricated and attached to each chip. **(b)** Photograph of a 4-inch oxidized silicon wafer with 30 microchips (The magnified view shows the electrodes with 10 μm width and 20 μm spacing). **(c)** A fabricated device with PMMA wells.

Figure 3. Electrical sensing of bacteria-spiked PBS samples

(a,b) Impedance magnitude of bacteria lysate in PBS samples spiked with *P. aeruginosa* and *S. aureus* with bacteria concentrations between 10^1 CFU/mL to 10^8 CFU/mL at 1000 Hz and 1 V. (c,d) The impedance magnitude change of bacteria lysate in PBS samples spiked with *P. aeruginosa* and *S. aureus*. The change in impedance is with respect to the bacteria-free control PBS samples. The response of the sensor is linear with R-squared values of 0.9485 and 0.9705 for *P. aeruginosa* and *S. aureus*, respectively. Error bars represent standard error of the mean (n=3).

Figure 4. Specificity evaluation

(a) *P. aeruginosa* was captured and detected in the presence of *S. aureus*. For the capture of the *P. aeruginosa*, magnetic beads conjugated with F429 (anti-alginate mAb) antibody was used. Staph concentrations of 10^3 and 10^5 in PBS were negative controls while concentrations of 10^3 and 10^5 of *P. aeruginosa* in PBS were positive controls (b) Similarly, *S. aureus* was captured and detected in the presence of *P. aeruginosa*. For the capture of the *S. aureus*, magnetic beads conjugated with 598 (anti-PNAG mAb) antibody was used. Staph concentrations of 10^3 and 10^5 in PBS were positive controls while concentrations of 10^3 and 10^5 of *P. aeruginosa* in PBS were negative controls. Control group in both (a) and (b) is bacteria free PBS. Error bars represent standard error of the mean (n=3).

Sample	Plate Count (CFU/mL)	Daily Score
1	5×10^3	1
2	1×10^5	1
3	0	0
4	0	0
5	0	0
6	0	0
7	0	0
8	2.2×10^3	1
9	2.67×10^4	2
10	5.90×10^3	3
11	1.15×10^4	3
12	8.0×10^2	3
13	3.20×10^4	2

Figure 5. Device evaluation using infected mice eye wash samples

Mice right eyes were infected with different concentrations of *P. aeruginosa* and left eyes were left uninfected. **(a)** Impedance magnitude of uninfected and infected mice eye wash sample (n=13). Error bars represent standard error of the mean (n=3), and **(b)** Daily score and concentration of the infected eye wash samples (right eye) measured using MacConkey plates.