BACTERIAL SENSING USING PHAGE-FUNCTIONALIZED WHISPERING GALLERY MICROCAVITIES

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ABSTRACT

In this work, we demonstrate the specificity of functionalized optical microcavities as bacteria sensors. We used endolysin LysK as a specific binding molecule to *Staphylococcus aureus (S. aureus)*. Wavelength resonance shifts are observed after each functionalization step and biodetection, confirming the label-free detection of bacteria on the surface of the microresonator. Further experiments conducted using *Escherichia coli* (*E. coli)* bacteria with the same endolysin validate the specificity of our biosensor.

KEYWORDS

Optical microdisk, label-free bacteria detection, resonance shifts, specificity

INTRODUCTION

Whispering gallery optical microcavities are structures which can efficiently confine light at the micro scale [1]. This confinement is based on total internal reflection of light at the interface between the cavity and the surrounding medium. Since the field evanescently extends into the medium, the optical properties of the microcavity are extremely sensitive to changes in its surroundings. In this work, we describe a biosensing application of these optical microcavities for the label-free detection of bacteria.

EXPERIMENT

Silica microdisks were fabricated using conventional microfabrication techniques [2]. The initial substrate was a silicon wafer with an 800 nm layer of thermal silicon dioxide. First we used UV photolithography to define the pattern of disks on the wafer, which is then transferred to the oxide using hydrofluoric acid. This is followed by an isotropic reactive ion etching of the silicon to form a silica disk on a pedestal. A scanning electron micrograph of a microfabricated optical microcavity is shown on Fig.1.

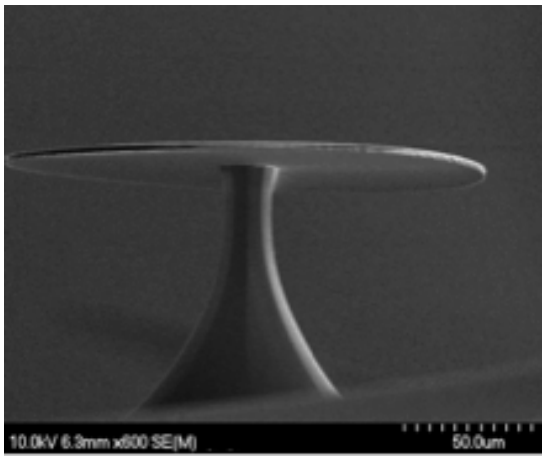

Figure 1: Scanning electron micrograph of an optical microcavity

An as-fabricated microcavity sensor will show a change in its optical response under any alteration of its environment. In order for the sensor to be specific to a particular species of bacteria, we need to properly functionalize its surface so that only that kind of bacteria will result in detectable changes. In our work, we are interested to detect the presence of *Staphylococcus aureus* (*S. aureus*). The microcavity surface was first functionalized using PEGylated aminosilane (PEG-Si). We then introduced phage-derived proteins (the *endolysin LysK*) specific to *S. aureus*. The binding between the bacteria and the phage proteins creates a perturbation in the electromagnetic environment of the microresonator which is observed as a shift in the resonance wavelengths present in the transmission spectrum [3].

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We used a tapered optical fiber to couple the light from a tunable red laser (635 nm) into the resonator, and measured the transmission spectra. Resonances appear as dips in the transmission. The microdisk was functionalized using PEG-Si and LysK protein. S. aureus cells were then introduced. Figure 2 represents a resonant mode of this microresonator (shown on Fig. 3 (c)) after microfabrication and each functionalization step, up to biodetection. It can be seen that the mode wavelength (i.e. the wavelength at the center of the dip) changes after each step. The as-fabricated microcavity shows a resonant mode at 635.4 nm. After adding the PEG-Si, the mode shifts to 636.3 nm. We would expect a shift towards longer wavelength after each functionalization step, but the microdisk covered with PEG-Si and LysK protein resonates at 635.1 nm. This blue shift is most likely due to the difficulty of coupling to the same resonant mode when using off-line functionalization (where the microdisk is removed from the setup for each step of the process). When the bacteria attach to the surface of the microdisk, the resonant mode is observed at 637 nm. It should be noted that the quality factor did not undergo a drastic change. It decreased from 2.54×10^4 for a clean microdisk, to 1.59×10^4 after biodetection.

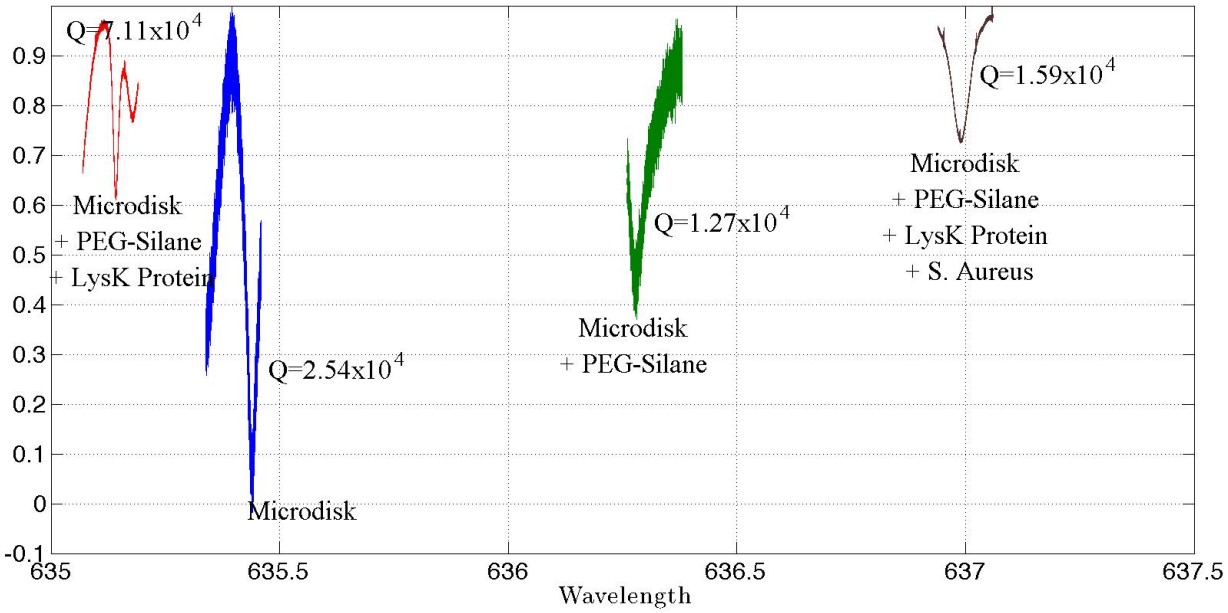
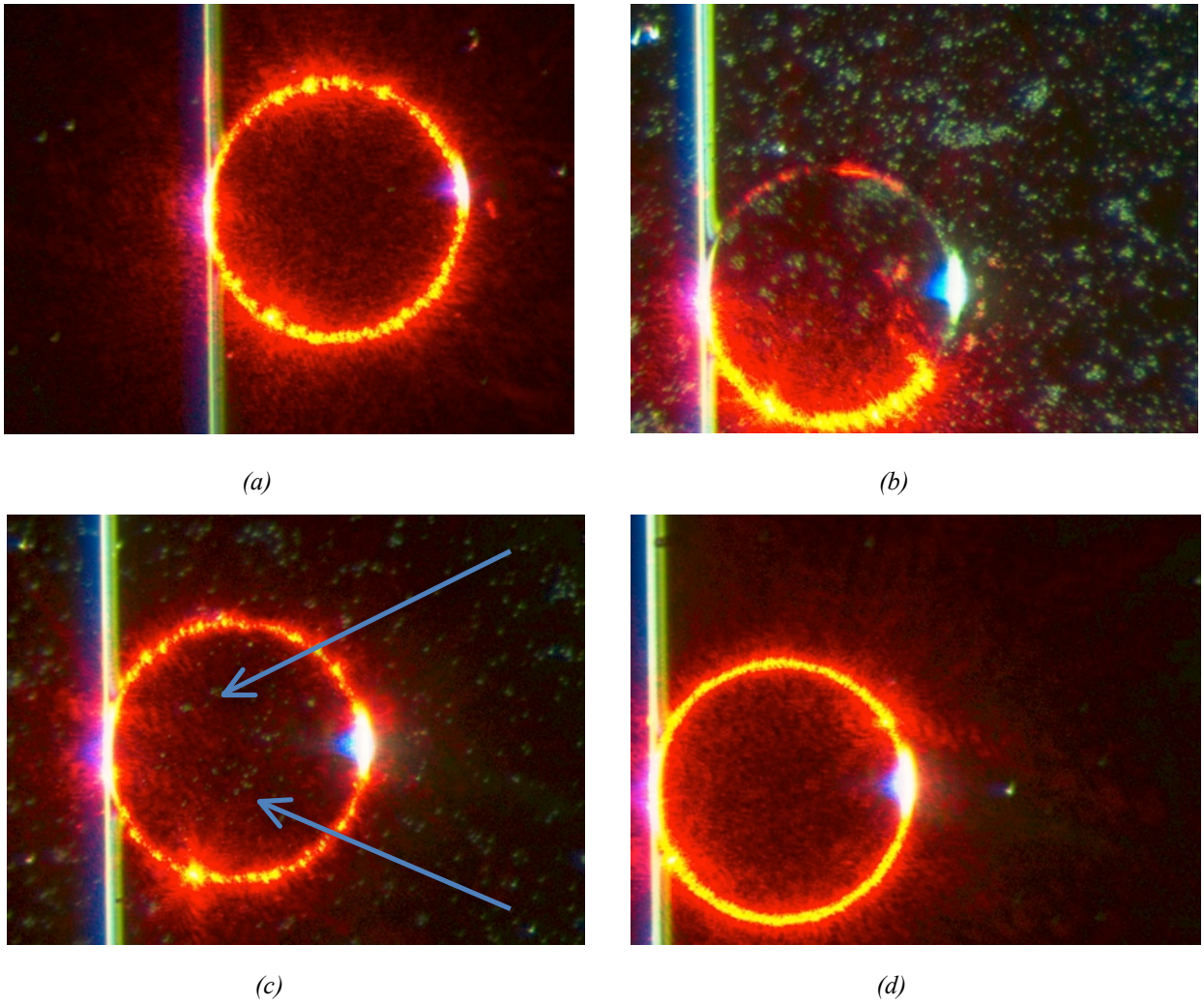


Figure 2: Resonances of the microcavity after each functionalization step and biodetection

To prove the specificity of our microresonator, we tested its surface functionalization. In the absence of PEG-Si and LysK protein, *S. aureus* did not attach on the surface of the microresonator as can be seen on Fig. 3 (a). In microdisks treated only with LysK (Fig. 3 (b)), *S. aureus* cells attached randomly on the surface. When the full treatment with PEG-Si and LysK was performed on the microdisk, a lower density of *S. aureus* attachment was noted, as can be seen on Fig. 3 (c), with a somewhat larger density of attachment on the resonator. Finally, Fig. 3 (d) shows a microdisk treated with PEG-Si, LysK protein and *E. coli* bacteria where no bacteria attached, demonstrating the specificity of the functionalization.

In conclusion, we proved that, with the proper functionalization, we obtained a specific microresonator able to capture *S. aureus* cells on its surface. Optical characterization using a tapered optical fiber showed the response of the sensor after biodetection. We observed a shift of the resonant mode after each functionalization step and biodetection due to a perturbation of the electromagnetic environment. Work is in progress to achieve on-line functionalization and real-time detection of bacteria in order to couple the same resonant mode into the biosensor.



(a) *(b)*

(c) *(d)*

Figure 3 : Microdisk with (a) S. aureus (b) LysK protein and S. aureus (c) PEG-Si, LysK and S. aureus (d) PEG-Si, LysK protein and E. coli

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