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Wavelength shift in a whispering gallery microdisk due to bacterial sensing: A theoretical approach

Hala Ghali[a,*], Pablo Bianucci[b], Yves-Alain Peter[a]

[a] Department of Engineering Physics, Polytechnique Montréal, H3T 1J4, Canada
[b] Physics Department, Concordia University, Montréal H4B 1R6, Canada

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ABSTRACT

Whispering gallery mode microcavities have recently been studied as a means to achieve real-time label-free detection of biological targets such as virus particles, specific DNA sequences, or proteins. Binding of a biomolecule to the surface of a microresonator will increase its path length, leading to a shift in the resonance frequency according to the reactive sensing principle. In this paper, we develop a theoretical expression that will link the reactive shift to the bacteria and microdisk parameters and help quantify the number of bacteria that bind to the surface of a 200μm-diameter silica microdisk.

1. Introduction

Whispering gallery microcavities (WGM) have attracted increased attention in the last decade as tools for label-free biosensing [1–4] and single molecule detection using plasmonic enhancement and optomechanical spring sensing [5–8]. They are structures that can efficiently confine light at the microscale due to total internal reflection of light at the interface between the cavity and its surrounding medium. WGM have a high sensitivity potential due to their high quality factors, as well as being selective when their surface is properly functionalized with the appropriate recognition element, relatively low cost and yield fast response. While the bulk of the research in this field is oriented towards the detection of viruses and small molecules, whispering gallery modes microcavities have a great potential for the detection of bacteria. For instance, *Staphylococcus aureus* (*S. aureus*) is a gram-positive bacterium, which is a common cause of skin and respiratory infections and presents antibiotic-resistant strains such as methicillin-resistant *S. aureus* (MRSA). MRSA is responsible for over 60% of staphylococcal infections in hospitals. It becomes thus crucial to find tools to achieve fast diagnosis and early detection of staphylococcal infections. There exist several techniques that are widely used for this purpose. Bacterial culture is used in most hospitals to identify the presence of *Staphylococcus aureus*. However this kind of tests can take several days to identify the pathogenic bacteria [9]. Real-time PCR is the most widespread FDA-approved test for MRSA [10,11]. However, this technique is time-consuming (about 2 h), requires prior isolation of bacterial DNA, can be very expensive and is impractical for use on-site or in developing countries. Another study shows the detection of *Staphylococcus aureus* using aptamer-conjugated gold nanoparticles [12]. Although this technique is very sensitive, it can take up to 1.5 h to detect the presence of the bacteria, making it unsuitable for real-time detection. Recently, we have shown the detection of *S. aureus* bacteria in Tris-buffered saline (TBS) (10 mM Tris-HCL, 150 mM NaCl, pH 7.5) by measuring the resonance shifts in silica microdisks which were specifically functionalized to react only to *S. aureus* [13].

2. Materials and methods

2.1. Microdisks fabrication

In this study, optical microdisks are used to detect the presence of bacteria. This particular geometry of optical microcavities was chosen because it can be easily integrated with optics and microfluidics components into a lab on a chip for on-site use, contrary to other microcavity geometries. The microdisks fabricated have a diameter of 200 μm and a 800 nm thickness. The relatively large diameter will decrease the radiation losses inside the disk leading to higher Q factors, whereas the 800 nm thickness was chosen to maximize the confinement of the fundamental energy mode inside the cavity. Optical microdisks are fabricated using standard silicon micromachining

* Corresponding author.

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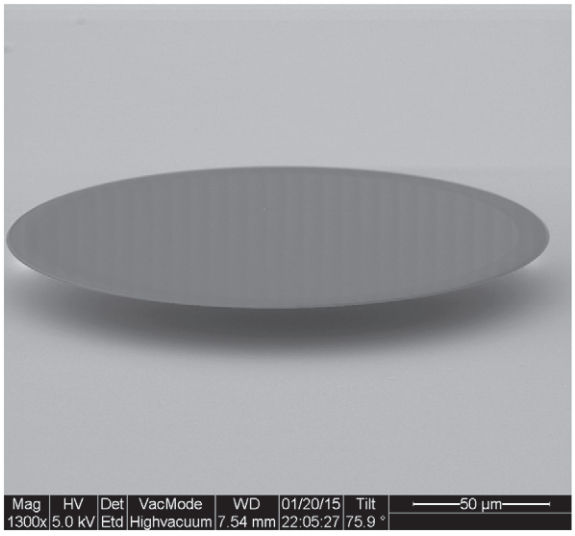

Fig. 1. Scanning electron micrograph of an optical microdisk.

techniques [14]. The substrates used are silicon topped with an 800 nm thermal silicon dioxide layer. The first step performed is UV photolithography to generate photoresist patterns of 200 µm-diameter microdisks with AZ5214 photoresist. Patterns are then transferred to silica using buffered oxide etch (B.O.E.) of silicon dioxide. The last step is isotropic dry etching of silicon with SF_6 gas to obtain microdisks on pedestals. A scanning electron micrograph of a fabricated 200 µm-diameter silica microdisk is shown in Fig. 1.

2.2. Surface functionalization

The functionalization process, needed for the response to be specific to *S. aureus* [15] and shown in Fig. 2 starts by cleaning the microdisks using oxygen plasma to remove organic residues. The samples are then immersed in an ethanol:water (95:5) solution containing 2.5% triethoxysilane-PEG-NH_2 (Nanocs, MW = 3400) for 2 h, then thoroughly washed with ethanol and dionized water. The PEGylated wafers were then immersed in a 2 µM LysK: 2000 µM EDC (1-Ethyl-3-(3-dimethylaminopropyl)carbodiimide) (1:1000) solution in buffer (10 mM Tris.HCl pH 7.5, 150 mM NaCl, 1% glycerol) at 4 °C overnight, then thoroughly washed with PBS and water.

After the functionalization process, the microdisk surface exposes a layer of LysK, an endolysin from the staphylococcal phage K that binds strongly to staphylococci. It contains three domains: an N-terminal cysteine, a histidine-dependent amidohydrolase/peptidase (CHAP) domain, a midprotein amidase-2 domain, and a C-terminal SH3b cell wall-binding domain [16]. This layer causes approaching bacteria to stick to the surface of the microdisk and then starts lysing their cell membranes. Lysing starts immediately after the addition of *Staphylococcus aureus* to LysK.

2.3. Optical characterization setup

The resonance shifts caused by the binding of bacteria were measured with the experimental setup represented in Fig. 3. Light from a tunable laser source was coupled into a tapered optical fiber next to the 200 µm-diameter silica microdisk, and the transmitted light intensity was measured as the laser wavelength was scanned. The resonances appeared as dips in the transmission spectrum. A typical transmission spectrum of a 200 µm-diameter silica microdisk coupled to a 1.2 µm waist diameter tapered optical fiber is shown in Fig. 4. The quality factor of the microdisks used in this study is around 10^4. This value might seem to be low, but it is common for microdisks, especially when working in an aqueous environment. One method to improve the Q factor is to etch silica using RIE to obtain smoother edges.

Quantitative sensing of the bacterial concentration is important, as a simple presence/absence detection scheme might not be efficient enough in a clinical setting. In this article, we develop a detailed model of the silica microdisks as biosensors in order to quantify the number of detected bacteria based on the measured resonance shifts.

3. Biosensing principle

The biodetection principle has been demonstrated and described in several studies [17,18]. When particles bind to the surface of the microresonator, they interact with its evanescent field, hence increasing the optical path length, leading to a shift towards lower frequencies, i.e. longer wavelengths. This is known as the reactive mechanism for biosensing [19]. The wavelength shift is given by:

$$\frac{\Delta\lambda}{\lambda} = \frac{\Delta R}{R} + \frac{\Delta n}{n}, \tag{1}$$

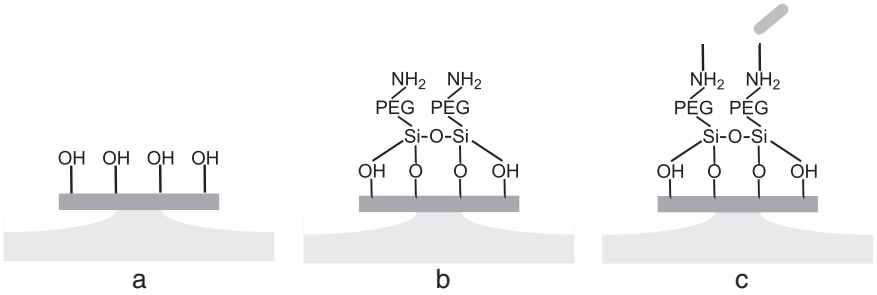

Fig. 2. Functionalization of silica microdisks. (a) The silicon dioxide surface is hydroxylated with oxygen plasma. (b) The disk is immersed in triethoxysilane-PEG-NH_2. (c) The free amines of the PEG-silane are covalently coupled to LysK using carbodiimide coupling.