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A surface acoustic wave biofilm sensor integrated with a treatment method based on the bioelectric effect

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ABSTRACT

Bacterial biofilms have an extensive impact on quality of life, ranging from severe infections in the clinical field to water facility contamination in environmental science. Biofilms are comprised of diverse bacteria that produce an extracellular matrix which prevents drug diffusion through them. Hence traditional antibiotic therapies require 500–5000 times the concentration used to eliminate non-biofilm-associated infections. Early biofilm detection is critical for effective eradication. Moreover, developing an alternative biofilm treatment method that utilizes low doses of antibiotics is desired. In this paper, a real-time microsystem is shown to detect growth of biofilms as well as their removal through integrated treatment. Detection of biofilms is achieved using a surface acoustic wave (SAW) sensor that monitors the total biomass by measuring the resonant frequency of the system. Biofilm treatment is based on the bioelectric effect (BE), a combination of low-dose antibiotics with application of both alternating and direct current signals. The detection limit of the SAW system is approximately 166 pg, corresponding to a bacterial population on the order of hundreds of bacteria. The system is used to observe an 80% reduction of total biomass when treated by the BE as compared to traditional antibiotics. Through system integration of the BE with the SAW sensor, simultaneous biofilm detection and treatment is achieved. The system consumes 194 μW of power, with the sensor and treatment consuming 100 μW and 94 μW, respectively. The integrated sensing and treatment capabilities of this system advance the development of an innovative biofilm control method.

1. Introduction

Biofilms are complex communities composed of a population of bacteria surrounded by an extracellular matrix [1]. Bacteria in biofilms readily exchange genetic material [2], creating a heterogeneous bacterial composition more likely to be resistant to antimicrobials than a bacterial population in suspension [3,4]. The extracellular matrix is composed of diverse polysaccharides and proteins, among other biological molecules, such as DNA fragments [5]. The matrix maintains the mechanical structure of the biofilm. The physiological function of the matrix is to act as a protective layer against diffusion of antibiotics and prevent recognition by the host immune system [6]. Once biofilms are established, elimination of the biofilm usually requires 500–5000 times the antibiotic inhibitory dose used on suspended bacteria [7]. Hence, detection during the onset of biofilm formation and during the growth stages of the biofilm, before mechanical and physiological structures have completely formed, enables its treatment using low doses of antibiotics [8]. Continuous monitoring of biofilm growth is critical for detecting the onset of biofilm formation [9]. More importantly, developing a new alternative biofilm treatment method that utilizes low doses of antibiotics is required to prevent the formation of mature biofilm infections.

Microfluidics based systems can be ideal platforms for biofilm applications since they require small sample volume, typically nanoliters, and provide precisely controlled conditions including uniform nutrient and electrolyte distribution. Microfluidic systems have been widely utilized in biofilm research to investigate the efficacy of new drugs [10] and also to monitor biofilm growth in real time [11].

In this paper we integrate a microfabricated surface acoustic wave (SAW) [12] sensor for real time biofilm growth monitoring with electric field enhanced treatment of mature biofilms. Previ-

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ously, a microfluidic platform based on optical detection was used for the real time study of this biofilm inhibition method, showing improved treatment efficacy based on the bioelectric effect (BE) [13]. The platform was composed of microfluidic flow reactors for biofilm growth and electrodes patterned along the channel to provide electric fields for the BE. The detection of biofilms was performed by measuring the optical density (OD) using photodiodes. For the biofilm treatment, an electric signal with increased total electrical energy, 0.25 V amplitude sinusoidal signal at 10 MHz with a 0.25 V DC offset, was applied in combination with low doses of the antibiotic gentamicin (10 μg/mL) for the BE. The voltage applied to induce the electric field was maintained at less than 0.82 V (0.25 V for 2 mm spaced electrodes or 1.25 V/cm of electric field) to avoid electrolysis of the surrounding medium [14] and the frequency of the AC component was selected to be 10 MHz based on literature [15]. The antibiotic concentration (10 μg/mL of gentamicin) utilized for the BE was significantly lower than the concentration of antibiotic typically necessary for biofilm treatment (~500–5000 times higher than planktonic *Escherichia coli*, MIC, gentamicin ≈2–4 μg/mL) [16]. Compared to only antibiotic treatment (10 μg/mL of gentamicin), the BE applied to biofilms within the microfluidic device reduced biofilms by about 160%. However, since the photodiodes and the optical source are required to be positioned out-of-plane from the biofilms, systems utilizing optical elements are inherently difficult to integrate into biomedical implants. In order to overcome this shortcoming, an in-plane method for total biofilm detection, such as a piezoelectric sensor is required, that not only detects biofilm growth in real time but can also be used to apply the BE based treatment method in clinical applications including porTable biomedical devices. Piezoelectric sensors also provide the advantage of higher sensitivity to change in biomass as compared to the optical methods.

A SAW sensor was previously developed using zinc oxide (ZnO) based on its high piezoelectric coefficient and biocompatibility [17]. For reliable operation of the sensor, it was coated with an aluminum oxide (Al_2O_3) layer deposited using atomic layer deposition (ALD). This protected the ZnO from degradation caused by the bacterial growth media [18]. As a result of the high quality passivation layer and the crystallized (*c*-axis) low impurities of the ZnO film deposited via pulsed laser deposition (PLD), the SAW sensor demonstrated reliable biofilm detection in real time. Here, the SAW sensor was integrated with a second set of electrodes for biofilm treatment based on the combination of electric field with antibiotics (bioelectric effect). The reliable operation of the SAW sensor in biological media, its high sensitivity for biofilm detection, as well as its two dimensional structure, merits to be a platform for a microsystem for biofilm applications. As discussed below, an integrated device combining the SAW sensor with an additional pair of electrodes was fabricated and tested by establishing and treating biofilms on the system. *E. coli* K-12 W3110 and *Pseudomonas aeruginosa* PAO1 biofilms were chosen as models of clinically relevant biofilms [19]. The device demonstrated successful real-time monitoring of biofilms as well as significantly improved treatment of the biofilms formed by both strains of bacteria.

2. Material and method

The microsystem, shown in Fig. 1, consists of the biofilm detection component using the SAW sensor and a biofilm treatment component using an electrical signal combined with antibiotics. To ensure seamless integration of the sensor with the treatment electrodes, theoretical analyses were conducted for two important factors that could significantly impact the sensitivity of the SAW sensor: (1) interference due to the application of the BE electric field, and (2) effect of shear stress due to the microfluidic flow. Since the acoustic wave is induced by the electrical actuation of the piezoelectric material, the integration of the BE electric field for biofilm treatment can attenuate the SAW propagation, due to the proximity of the BE field [20]. The microfluidic flow can potentially alter SAW propagation, since the flow induces surface stress [21] that may alter SAW velocity, resulting in a decrease in sensitivity.

2.1. Analysis of electric field interference due to the BE

To assess the first mode of potential interference, studies of the electrical signal interference between the BE electric field and the acoustic waves were conducted using the schematic of the device shown in Fig. 2a. The Al_2O_3 film deposited on the ZnO layer was assumed to be separated by an air gap on each side from the treatment electrodes. This assumption was made to investigate the maximum electric field that can be induced in this configuration. Since the fabricated devices were tested in bacterial growth media or deionized (DI) water, the actual voltage drop between the electrode and the Al_2O_3–ZnO layer (from 0 to X_1 in Fig. 1) is smaller than the case of air gap due to the higher relative dielectric constant of water as compared to air ($\varepsilon_{r-air} = 1$, $\varepsilon_{r-water} = 80.1$ at 20 °C).

The induced field intensity on the ZnO layer due to the BE voltage applied (0.25 V DC superimposed on a 0.25 V sinusoidal signal at 10 MHz) was calculated based on electromagnetic theory (Maxwell's equation, Gauss's law) [22] as:

$$E = \frac{Q}{2A\varepsilon_{ZnO}} \approx \frac{C_{total}V_0}{2A(100\times\varepsilon_0)} = \left(\frac{\varepsilon_{total}A}{d}\right)\frac{V_0}{200A\varepsilon_0} = \left(\frac{\varepsilon_{total}}{d}\right)\frac{V_0}{200\varepsilon_0}$$

$$\frac{d}{\varepsilon_{total}} = \frac{2w_{air}}{\varepsilon_0} + \frac{2w_{Al_2O_3}}{9.8\varepsilon_0} + \frac{w_{ZnO}}{100\varepsilon_0} = \frac{10.1\times10^{-4}[m]}{\varepsilon_0} \quad (1)$$

$$E \approx \left(\frac{\varepsilon_{total}}{d}\right)\frac{V_0}{200\varepsilon_0} = \frac{V_0}{200\varepsilon_0}\times\left(\frac{\varepsilon_0}{10.1\times10^{-4}[m]}\right) = 1.238[V/m]$$

where Q is the induced charge due to the applied voltage, A is area of the side electrode, ε_{ZnO} is dielectric constant of ZnO ($\sim100_{\varepsilon_0}$), d is distance between the electrodes (2 mm), $W_{Al_2O_3}$ is the thickness of Al_2O_3 (100 nm), V_0 is the magnitude of the applied voltage (0.25 V), W_{air} is width of air gap (0.3999 mm), W_{ZnO} is width of the ZnO (1.2 mm), ε_0 is the vacuum permittivity, ε_{total} is the total dielectric constant that can be calculated by the electrical capacitance of the system. The relative dielectric constant of each layer indicates the characteristics of the polarity of the medium and directly corresponds to the induced electric field intensity. With device design parameters, the intensity of the electric field was calculated to be 1.238 V/m.

Based on the obtained value, the induced displacement of the ZnO was derived using the general compressed tensor equation [23] and a ZnO piezoelectric coefficient obtained from literature [24].

$$S = d \times E \quad (2)$$

where S is the strain due to the applied electric field ($E = 1.238$ V/m), d is piezoelectric strain coefficient (12.4×10^{-12} C/N). The result of displacement due to the electric effect is approximately 15.4×10^{-15} m. The wavelength of the SAWs on the sensor was previously characterized by our group using the phase velocity of ZnO (4812 m/s) and the distance between the electrodes (interdigitated transducer, 6 μm distance). This design generated a 402 MHz acoustic wave on the ZnO [12]. The resonant frequency is designed based on the suggested frequency range (401–406 MHz, Federal Communication Commission) for biomedical wireless application. The induced attenuation of the SAW due to the application of the BE electric field is nine orders of magnitude smaller than the wavelength of the acoustic waves (12 μm). Therefore, it was concluded that possible electrical interferences between propagation of the SAWs and the electric field for the BE in an integrated scheme were not significant.