NOVEL THERMAL MICROSENSOR METHOD FOR ONLINE MONITORING OF IN-VITRO BIOFILM FORMATION

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ABSTRACT

A novel sensor principle for continuous monitoring of biofilms is presented. A small heater generates a steady sinusoidal heat signal. The resulting temperature oscillations are measured through a temperature sensor. The amplitude and phase shift of the oscillations are used as sensing parameters. The sensor system successfully detects surface attached bacterial growth within 30 minutes of inoculation. We also show that the sensor enables continuous evaluation of the effect of antibiotics on early stages of biofilm development. Furthermore, the evolution pattern of the signals over time may be useful to differentiate biofilm-forming organisms.

KEYWORDS: biofilm, biofilm monitoring, antibiotic resistance, surface attached growth, growth inhibition test

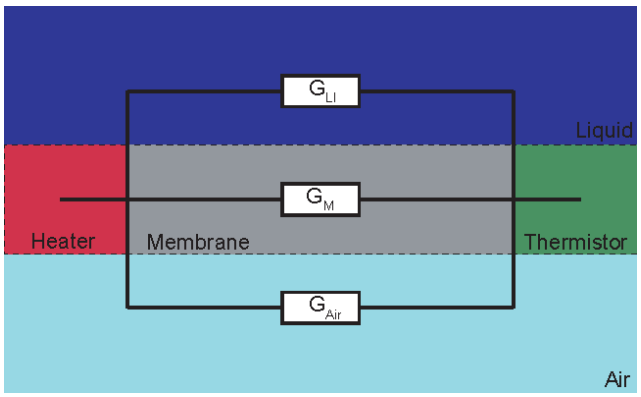
INTRODUCTION

Several methods for biofilm monitoring have been developed in recent years. Some research efforts focus on impedimetric sensors, which monitor the impedance of the biofilm [1–3]. However, these sensors exhibit baseline drift and the flow of electric currents may induce changes in the ionic properties of the growth medium. Other sensors, like thermal calorimeters require a long settling time before measurements [4]. Another approach measures the DC thermal resistance of the biofilm [5,6], but it cannot determine the thickness without prior knowledge of the thermal properties. We propose a novel method based on sinusoidal thermal excitation that might enable the simultaneous determination of thickness and thermal properties of the biofilm as it grows [7,8].

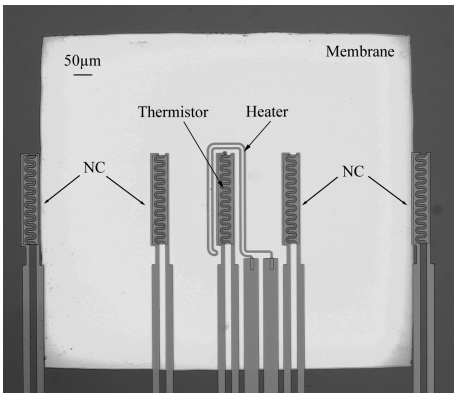
THEORY

Many bacteria can change from a planktonic (free floating) to a surface attached mode of growth. After expression of proteins that facilitate surface attachment of the cell, adjacent bacteria employ quorum sensing to initiate the formation of an extracellular polysaccharide matrix [9]. In addition to mechanical interconnection, the extracellular matrix provides further advantages to the bacteria. The matrix acts as a diffusion barrier that helps to protect the bacteria within from the action of, for example, antibiotic substances and helps to retain nutrients and signal molecules in close proximity. This barrier-like function can also help mask the bacteria from being recognized and attacked by a host organism's immune system.

Thermal systems may be described by equivalent circuits containing complex thermal impedances. Figure 1 (a) gives a simplified thermal equivalent circuit of a small region of the membrane that includes the heater structure and a thermistor. Each of the distinctive layers of the sensor is modeled by a complex thermal impedance G. If a thermal excitation of the form $Q_H(t) = |Q_H| \cdot \sin(\omega_{Th} \cdot t)$ is applied by the heater, the combined impedances G will cause both a reduction in amplitude and a shift in phase of the temperature measured by the temperature sensor. The measured temperature signal will thus be of the form $T_{Therm}(t) = |T_{Therm}| \cdot \sin(\omega_{Th} \cdot t + \theta_{Th})$. Because of their low signal-to-noise ratios, $|T_{Therm}|$ and θ_{Th} are best measured using a lock-in amplifier.



(a)



(b)

Figure 1: (a) Simple equivalent circuit of the central region of a thermal flow sensor. The three layers of the sensor system are modeled by three complex thermal impedances. (b) Close view of the sensor's membrane depicting the heater and the measuring thermistor. Thermistors marked NC are not used in this application.

EXPERIMENTAL

The sensor is fabricated from a silicon wafer, on which a 1.4-µm-thick SiN_x layer is structured. A chromium heater and amorphous germanium thermistors are deposited on top of the SiN_x layer. The wafer is subsequently etched from the backside, resulting in the membrane shown in Figure 1 (b). The heater closely surrounds the thermistor. The average heater-thermistor separation is about 9 µm.

The bottom portion of a microcentrifuge tube is cut off and subsequently glued onto the sensor die to hold the liquid growth medium (see Figure 2).

The initial viable cell count (Colony Forming Units, CFU) at the beginning of each experiment is determined by the standard plate count method on LB-agar.

1.2×10^5 CFUs from an overnight culture of *Enterococcus faecalis* Symbioflor I [10] grown in lysogeny broth (LB) are diluted with fresh M17-medium to yield a concentration of 1.2×10^3 CFU/µl. Fifty microliters of this dilution (6×10^4 CFUs) are inserted into the sensor container. The sensor is connected to the readout circuit and placed inside an incubator set to a temperature of 37°C. After a thermal settling time of 10 minutes, the measurement is started. The heater is electrically excited to produce a steady 40 Hz harmonic heating signal with a magnitude of 0.5 mW. The lock-in amplifier measures both the resulting voltage amplitude and phase shift at the thermistor. The data is continuously read by a LabView program that converts the measured voltage amplitude to temperature amplitude using the Steinhart-Hart model [11].

An antibiotic inhibition experiment is performed when the rate of change of $|T_{\mathrm{Therm}}|$ and θ_{Th} are at their highest. Ampicillin in aqueous solution is added, leading to a final concentration of 200 µg/ml in the growth medium.

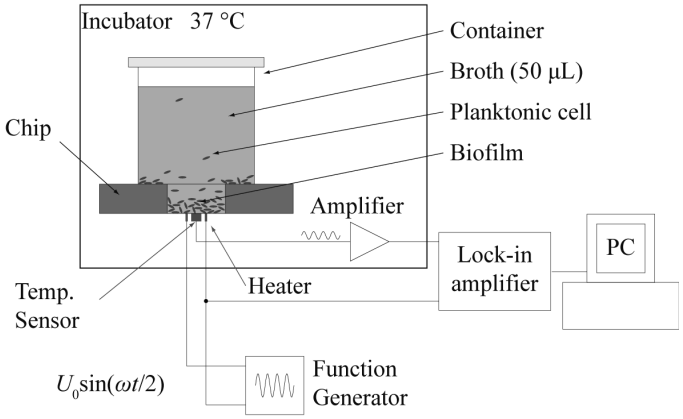


Figure 2: Measurement setup

RESULTS AND DISCUSSION

Figures 3 and 4 respectively show the evolution over time of the phase and amplitude of the temperature oscillations measured by the thermistor. The initial number of CFUs is about 6×10^4 CFU. The no-bacteria baseline for both amplitude and phase (solid) remains stable over the whole measurement. Uninhibited surface attached growth (dotted) is detected after about 30 min. of inoculation. Phase and amplitude change in time due to the increasing thickness of the biofilm. After the addition of ampicillin, the growth pattern (dashed) is clearly distinguishable from the uninhibited pattern.

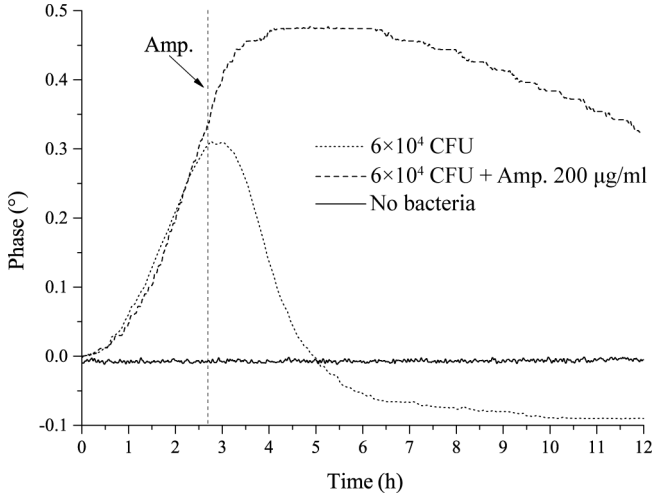


Figure 3: Measured thermal phase for uninhibited (dotted) and ampicillin-inhibited (dashed) surface attached growth and early biofilm development of E. faecalis Symbioflor I in M17 medium. Amp. marks the addition of ampicillin.

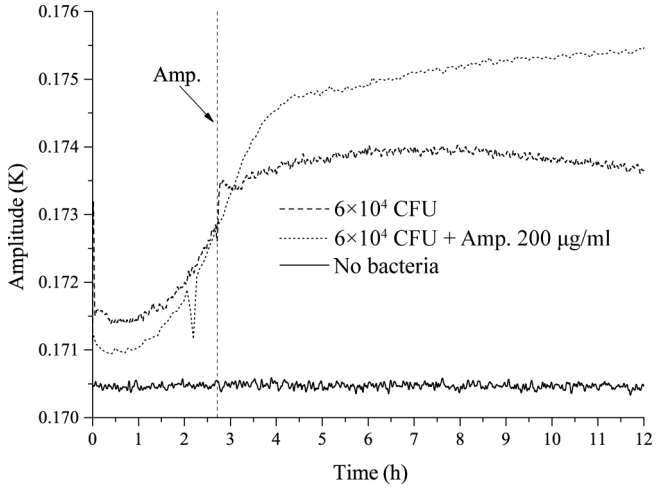


Figure 4: Measured thermal amplitude for uninhibited (dotted) and ampicillin-inhibited (dashed) surface attached growth and early biofilm development of E. faecalis Symbioflor I in M17 medium. Amp. marks the addition of ampicillin.

CONCLUSION

The presented system is shown to be able to monitor both uninhibited and ampicillin-inhibited surface attached growth of *E. faecalis* Symbioflor I. The amplitude and phase information may be used to assess the thickness and thermal properties of the resulting biofilm. Analytical and simulation models will be developed for this purpose.

Though not shown, different subspecies of *E. faecalis* show distinct amplitude and phase evolution over time. This result is in agreement with previous data on biofilm formation by *E. faecalis* [12]. Moreover, the system may allow the elucidation of bacterial resistance to antibiotic substances. After further development, the sensors may be used to test the efficiency of surface modifications on their ability to prevent surface attached bacterial growth.

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