MICROSCALE IMPEDANCE-BASED DETECTION OF BACTERIAL METABOLISM

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ABSTRACT

Impedance-based detection of bacterial viability relies on measuring changes in the AC impedance of two electrodes immersed in a liquid were the bacteria are cultured, caused by the release of ionic species by metabolizing bacterial cells. Here we present results on the impedance-based detection of the viability of *Listeria innocua* cells incubated in a nanoliter-scale volume in a microfluidic biochip. During incubation the complex impedance of the suspensions is measured over time at several frequencies between 100 Hz and 1 MHz. The shape of admittance *vs.* time curves obtained from live cells agrees very well with the known characteristics of bacterial growth.

Keywords: Detection, impedance, bacteria, metabolism

1. INTRODUCTION

Rapid detection of viability and growth of pathogenic microorganisms is very important in many applications such as food and drug production, health care, and national defense. We present results on the impedance-based detection of the viability of bacterial cells in a microfluidic biochip. Impedance-based detection of bacterial viability relies on measuring changes in the AC impedance of two electrodes immersed in a liquid were the bacteria are cultured, caused by the release of ionic species by metabolizing bacterial cells. Impedance-based detection of bacteria is a well established technique in microbiology, but in its conventional, macro-scale implementation it suffers from low sensitivity and long assay times due to the large volumes of liquid that are used [1,2]. Small initial numbers of bacterial cells (1 to 100) suspended in a large volume (on the order of milliliters) require a long time to multiply and excrete enough ions to be detectable by this method. However, rapid and sensitive detection of a few cells is possible if the cells are confined into a volume on the order of nanoliters, such that a small number of ions released by a few cells produces a large change in the volumetric concentration of ions and thus a large change in the measured impedance.

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Preliminary on-chip measurements performed on bacterial suspensions of different concentrations incubated off-chip demonstrated the feasibility of detecting between 50 and 200 cells in a 5.3 nl volume [3, 4]. Here we report on the first on-chip incubation measurements done to date.

2. EXPERIMENTAL METHOD

Since the fabrication of the biochip is discussed in detail somewhere else [3], here we will only briefly describe the most important features of the device (see Figure 1). The biochip has a series of chambers connected by channels, both etched to a depth of 12 μm onto a silicon substrate. The volumes of the chambers range between approximately 75 pl and 5.3 nl. Platinum electrodes are patterned at the bottom of the chambers to measure the impedance of liquids injected into them. The whole microfluidic system is sealed by a glass slide bonded using spin-on-glass (SOG) as adhesive [5]. Microbore tubes for the injection of liquids into the chip are bonded using biomedical-grade epoxy adhesive into deep trenches etched on the edge of the chip.
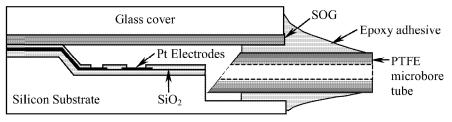

Figure 1. Simplified cross-section of the biochip.

Two different kinds of samples were incubated on-chip: Sterile Luria-Bertani (LB) broth (10 g/L tryptone, 5 g/L yeast extract, 3.3 g/L dextrose; no salt was added to minimize the baseline ionic content of the broth) and live *Listeria innocua* cells at concentrations between 10^6 and 10^8 cfu/ml. *L. innocua* were cultured in LB broth for a minimum of 16 hours at 37°C. After culturing, the cells were harvested and washed by centrifugating and re-suspending in fresh LB broth several times, to eliminate all the metabolic byproducts released by the cells during growth. The final washed suspensions were diluted in LB broth to obtain cell concentrations between 10^6 and 10^8 cfu/ml (estimated by culturing on agar plates and counting the resulting colonies). The sterile LB broth and cell suspensions were injected into sterile biochips using pressurized nitrogen to drive the flow. After injection of the cells, the microbore tubes connected to the chip were irreversibly pinched to stop the flow and to completely isolate the microfluidic path in the chip from the external world. The chip was heated to 38±1°C, to stimulate the growth of the cells, and the temperature was actively controlled to prevent changes larger

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than ± 0.2°C over the whole incubation period. As the cells were incubated for up to 24 hours, impedance measurements were performed in a chamber with a volume of 5.3 nl. Approximately every four minutes, an LCR meter (4284A, Agilent Technologies, Palo Alto, California) measured the impedance of interdigitated electrodes in the chamber, at 52 frequencies logarithmically spaced between 100 Hz and 1 MHz, with a 50 mV$_{pp}$ voltage excitation.

3. RESULTS

Figure 2 shows plots of admittance at 1.06 kHz *vs.* time for two different samples incubated on-chip: Sterile LB broth and *L. innocua* at a concentration equivalent to approximately 160 cfu's in 5.3 nl. As was expected, sterile LB broth exhibits a very small and linear change in admittance over time (1.4% after 14 hours), probably caused by ions leaching out of some of the materials that make up the biochip, especially the spin-on-glass and the epoxy adhesive. The shape of the admittance *vs.* time curve obtained from live *L. innocua* cells agrees very well with the known characteristics of bacterial growth: There is an initial "lag" phase where there is little change in the admittance because no growth occurs and bacterial metabolism is slow; this is followed by a fast increase in admittance as cells start to multiply exponentially and their metabolism releases large numbers of ions; and the process ends with a "stationary" phase where admittance continues to change slowly because of a constant rate of ion release from a relatively constant cell population. On-chip incubation of *L. monocytogenes* suspensions results in very similar impedance/admittance curves over time (unpublished data). It is not yet understood why the admittance sometimes decreases during lag phase. Several samples exhibit a sharp dip in the admittance immediately before the start of exponential growth (unpublished data). One possible explanation for this dip is that the ionic concentration of the suspension decreases temporarily as cells substantially increase their uptake of nutrients (some of them ionic) in preparation for the start of multiplication, but this hypothesis has not been tested yet.

4. CONCLUSIONS

We have successfully monitored the metabolic activity of multiplying bacterial cells incubated in a microfabricated device using an impedance-based method. The shape of admittance *vs.* time curves obtained from live cells incubated on-chip agrees very well with the known characteristics of bacterial growth, while sterile LB broth exhibits very little changes in admittance during incubation.

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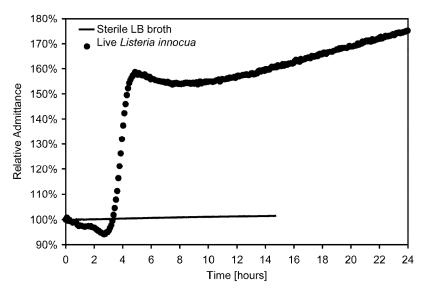October 5–9, 2003, Squaw Valley, California USA

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Figure 2. Admittance at 1.06 kHz, relative to initial value, as a function of time, for sterile LB broth and live *L. innocua* cells at a concentration equivalent to approximately 160 cfu's in the 5.3 nl chamber were impedance was measured.

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