TOWARD BACTERIA DETECTION ON CHIP: A BIOSENSOR BASED ON MAGNETOTACTIC BACTERIA AND IMPEDANCE SPECTROSCOPY

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ABSTRACT

The design, fabrication and preliminary results for a new class of biosensors based on magnetotactic bacteria (MTB), aimed at detecting pathogenic bacteria with significantly shorter detection periods, is presented. The MTB are used to push microbeads coated with phages or antibodies specific to the targeted bacteria. By controlling the swimming directions of the MTB with magnetic fields, controlled sweeps of the samples are achieved. Each microbead, being propelled by the flagella motor of the MTB, is then brought to a microelectrode array for detection by impedance spectroscopy. Finite Element Method (FEM) simulation and preliminary results presented in this paper demonstrate that 10 µm melamine beads can be detected with the proposed biosensor.

KEYWORDS: Biosensor, Magnetotactic bacteria, Impedance Spectroscopy, MicraGEM.

1. INTRODUCTION

Fast pathogen detection is a critical component of clinic diagnosis, disease control and the food industry. The conventional bacteria identification methods currently require hours, or even days, to accurately detect bacteria. This is especially evident at very low concentrations (early stages of bacteria growth), at which point detection is most vital. In previous work, we proposed to exploit magnetotactic bacteria (MTB) [1] as bio-carriers, in order to accelerate the detection procedure by binding the motile MTB to microbeads coated with antibodies or bacterial-phages. The swimming directions of the MTB are controlled by using a directional magnetic field [2], which induces a torque on a chain of embedded magnetosomes inside each MTB, acting like a compass. Under the control of external magnetic fields, the flagellated MTB propel the functional microbeads, effectively sweeping the aqueous sample [3-5] to search for the targeted pathogenic bacteria. Then, the MTB transport the pathogenic bacteria to the microelectrodes array, where the variations in electric impedance are measured. The existence of a pathogen is indicated when the impedance variations increase beyond a certain threshold.

With the proposed approach, this biosensor offers several advantages. First, with the assistance of the MTB, active detection can be realized by transporting the targeted pathogenic bacteria to the microelectrodes, thus avoiding the long waiting time for the diffusion and duplication of bacteria required in current detection methods. This will greatly decrease the detection time required for a given sample and will increase detection sensitivity. Second, the use of the MTB as carriers as opposed to the similar approach of utilizing magnetic beads as carriers allows for much smaller power requirements. The power consumption for this biosensor will be significantly decreased because the minimum magnetic field required to effectively navigate the MTB can be as low as 3 Gauss, which can easily be achieved by small electrical currents flowing through specially designed micro-wires. This feature makes the future integration of the biosensor with the microelectronic sensing circuit and conventional CMOS technology feasible.

In this paper, the design and simulation of an impedance sensing platform based on the MicraGEM processes is discussed. The design and fabrication of planar microelectrodes used to verify the concept of detecting single cells or microparticles by impedance measurement are introduced. Preliminary experimental results indicate that a microbead, 10µm in diameter, can be detected by measuring the variations of impedance between a pair of planar electrodes.

2. METHODOLOGY, SIMULATION AND FABRICATION

The main structure of the microsystem is fabricated using the MicraGEM process provided by Micralyne [6]. MicraGEM uses Pyrex glass as its substrate material. Two different microchannel depths, 10 and 12 µm can be etched into the Pyrex layer. A single crystal silicon layer (10µm) is anodically bonded to the Pyrex substrate to form the structural layer. Metal can be deposited on the Pyrex glass and on top of the single crystal silicon layer, which allows the creation of electrodes and/or electronic connections. In this process, we implement a microchamber (3mm × 3mm) and numerous microchannels (25µm × 10µm) on the Pyrex substrate. Microelectrodes (20µm × 20µm) for impedance measurement are patterned on the bottom of each microchannel. The single crystal silicon layer is used to seal the microchannels and the microchamber, and to form openings which provide inlets and outlets for sample injection. Metal microcoil arrays, used to generate the local magnetic field in the microchamber, are deposited on the top of the single crystal silicon layer. The local magnetic field generated by the microcoils can control the swimming direction of the MTB to realize the function of bacteria mixing and sorting. Since the MTB react to a very small magnetic field, only small DC currents are required when coupled with an insulation layer with a thickness of 10µm. The layout of the whole system is presented in Fig. 1. The fabricated microdevice is shown in Fig. 2.

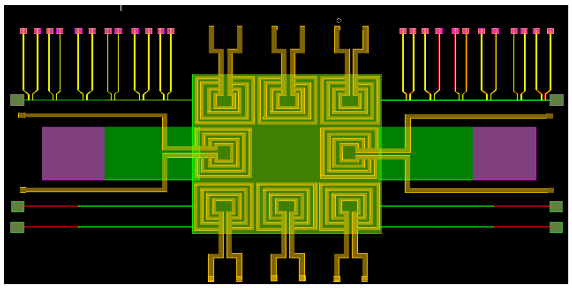

Figure 1. Schematics of the microchip system using the MicraGEM process.

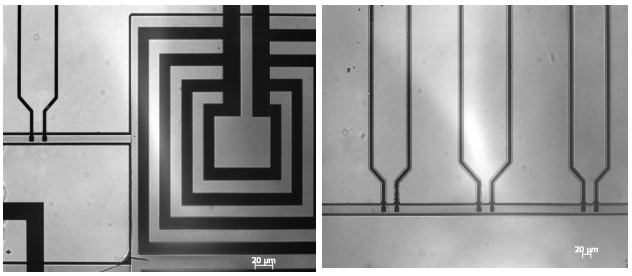

Figure 2. Microscopic pictures of microelectrodes, microchannels and a microcoil

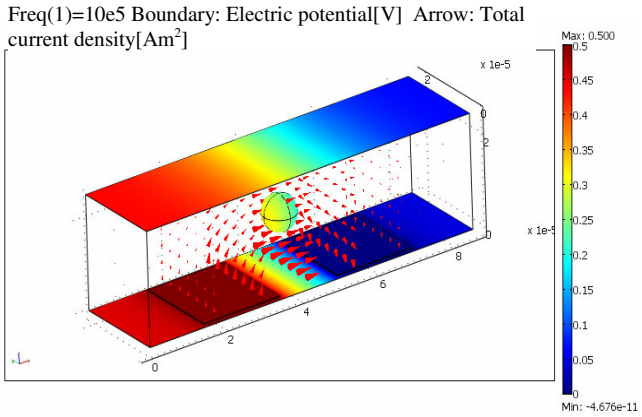

Figure 3. FEA simulation of a microbead between microelectrodes (20µm × 20µm)

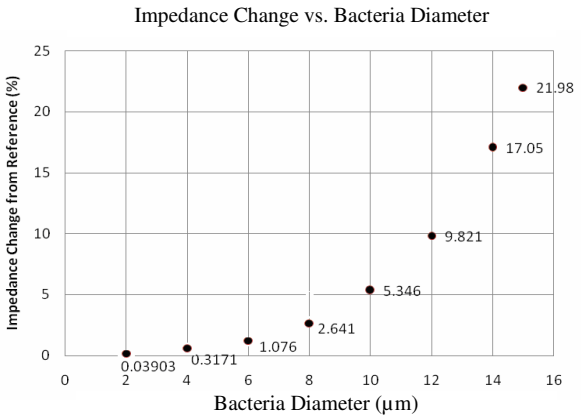

Figure 4. the impedance change from reference vs. bacteria size

FEM simulation shows that detection of a single bacterium or microparticle by measuring impedance variations between the microelectrodes is possible with this device. Fig. 3 illustrates the FEM simulation of a single microparticle passing through a pair of embedded microelectrodes. Fig. 4 shows a graph obtained from the FEM simulation that gives

the increase in impedance from the reference signal as a function of bacteria diameter. This simulation allows investigation of the detection capabilities of our device. It is used to determine the device characteristics (electrode size, orientation, separation, and channel size) that are required to detect certain sizes of bacteria. This is imperative in order to construct a sensor that possesses adequate sensitivity to detect a single bacterium, yet being practical when performing fabrication and integration with an on-chip electronic sensing circuit.

3. EXPERIMENTAL RESULTS

In our preliminary experiments, an impedance analyzer (Agilent 4294A) is used to record impedance variations caused by the presence of bacteria or microparticles between a pair of microelectrodes embedded in a microfluidic device. As depicted in Fig. 5, navigation of MTB (with a microbead being attached to each bacterium) in the microchannel can be achieved by using external magnetic fields. Shown in Fig. 6, the preliminary experimental results indicate that variations of impedance caused by the microbeads can be detected by the dedicated microdevice. Issues such as mixing rate, binding efficiency and packaging will be addressed in more details with respect to the constraints of microfabrication and integration.

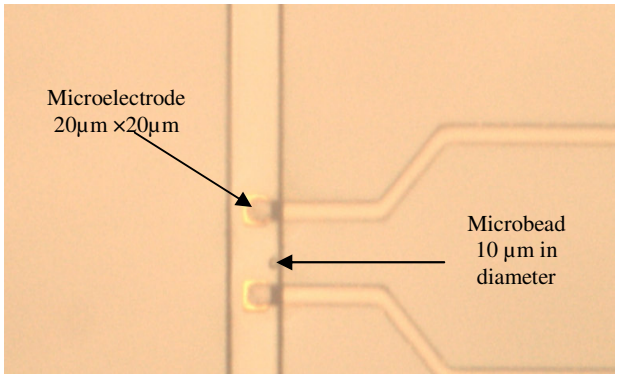

Figure 5. 10 um bead moving between electrodes in the microchannel

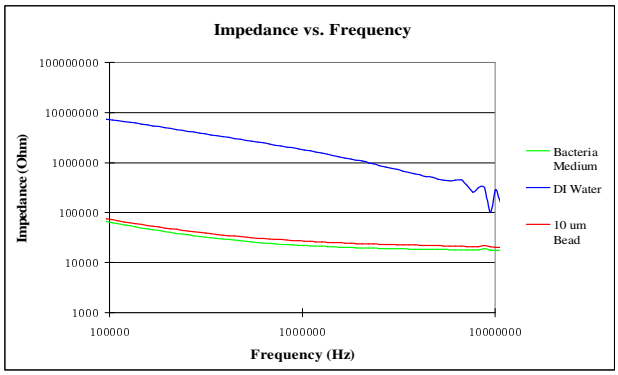

Figure 6. Experimental results showing the impedance signals of de-ionized water, bacteria medium, and a 10 µm bead in the bacteria solution over the signal range 0.1 MHz to 10 MHz.

ACKNOWLEDGEMENT

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