Preliminary design of a biosensor based on MC-1 Magnetotactic Bacteria

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Abstract: A novel biosensor is proposed for detecting pathogenic bacteria. The specificity of the detection is realized by the bacteriophage. The detecting sensitivity and efficiency can be greatly improved by introducing magnetotacticle bacteria (MTB) which can be controlled by an external magnetic field of a few Gauss. The high controllability and swimming speed make the MTB a good candidate for a micro-carrier. After being attached with microbeads coated with specific bacteriophage, a group of MTB can push microbeads actively sweeping in a chamber containing potential pathogenic bacteria. Upon the completion of the sweeping process, microbeads and potentially attached pathogenic bacteria are pushed by MTB to an area where a dedicated micro-electrode array connected to a microelectronic circuit is used to detect and record the number of the pathogenic bacteria by measuring variations of the impedance between the electrodes.

Key Words: Magnetotactic bacteria, biosensor, pathogenic bacteria, micro-electrode.

Introduction:

Rapid and on-site pathogenic bacteria detection is an important issue. Diseases caused from pathogenic bacteria are a major cause of deaths. Worst, the human diseases caused by pathogens have not decreased. In fact, the public Health Laboratory service in the UK has indicated that in 2001, food poisoning notifications increased 600% from 1982. Only in the United States, each year, approximately 14 million illness caused by pathogens [1] such as Salmonella typhimurium, Escherichia Coli, Staphilococcus aureus, and Campylobacter jejuni [1]. For salmonella only, which is a very dangerous foodborne pathogen, approximately 5 million analytical tests are performed annually, which cost $1 billion [2]. E.Coli O157:H7 is a rare strain of E.Coli that is considered to be one of the most dangerous foodborne pathogens. It causes 20 000 illness and 500 deaths per year in the USA [3]. However, generally the pathogenic bacteria are present at very low concentration. For example, the infectious dosage of E.Coli O157:H7 or Salmonella is as low as 10 cells and the existing standard for E.Coli in the water is 4 cells/100ml.

The conventional bacteria identification methods can detect a single bacterium, but the amplification is required, which generally includes four steps [4, 5]: 1) pre-amplification: to reproduce all of the micro-organisms; 2) selective enrichment: to grow the targeted micro-organisms population to the detectable level; 3) isolation; 4) confirmation: serological and bio-chemical analysis to confirm the presence of the targeted pathogenic bacteria. Typically, the whole procedure may require at least 16 hours to several days. In these cases, by the time the pathogen or undesired microorganisms are identified, the products would probably have been fabricated or shipped to customers. Furthermore, detection of a few pathogens in the clinic samples, food, water or cosmetics requires long periods of work involving high professionally skilled laboratory personnel.

During the last decade, considerable efforts were directed towards more automated, rapid and sensitive detection approaches. Currently, the most sensitive technology is the DNA analysis which uses polymerase chain reaction (PCR) to amplify small quantities of genetic material to determine the presence of bacteria. Optical biosensor, especially the bioluminescence sensors show extremely high specificity and can distinguish viable from non viable bacteria. Blasco et al. [6] reported a method to detect Salmonella Newport and E.Coli by measuring the ATP bioluminescence. The sensitivity can reach 10^4 cells/ml. However, both technologies usually take hours to get results.

One common automated bacterial detection technology relies on the changes of electrical characteristics of a medium where the bacterial are cultivated. Electrodes generally were immersed in the aqueous medium and connected with an AC power source. The presence of the bacteria is indicated when the measured impedance changes beyond a certain threshold. Stephen et al. [7] presented a microelectrode array biosensor for detecting E.Coli O157:H7 with a detection limit of 10^3 CFU/ml. In their design, the sensor surface was coated with antibodies...
to ensure specificity. Bacteria suspended in solution were immobilized by antibodies on the electrodes. Then the variance of impedance between the electrodes was measured by an impedance analyzer. Several commercially available instruments based on the impedance measurement can also be found on the market.

Unfortunately for all these reports in literature dealing with the impedance detection techniques, the total detection time depends on the diffusion rate of the target bacteria or the ionic metabolic the bacteria released in the medium (usually requires 12 hours to 7 days). Generally, most of the bacteria are not motile. Furthermore, the diffusion rate of the bacteria and their metabolic are very slow, especially under the condition of low-Reynolds number laminar fluidics [16]. If the target sample only contains a few bacteria, it takes long incubation time for the bacteria to reach the detectable level. Moreover, the sensitivity and specificity are not guaranteed.

Here, we propose a novel biosensor which uses magnetotactic bacteria [8, 9, 10, 12] as a micro-carrier to actively search the targeted pathogenic bacteria in liquid samples. Instead of waiting the targeted bacteria or micro-organisms to move to the detection area or to reproduce to the detectable level, a swarm of MTB pushing microbeads loaded with bacteriophage [13, 14] to actively search under computer control the targeted micro-organisms is used.

Once a pathogenic bacterium is captured by the bacteriophage at the surface of the bead being pushed by a single MTB, it is transported by the MTB to the microelectrode array for impedance measurement.

In this paper we primarily focus on the feasibility of this new approach and preliminary system design. After a brief introduction of MTB, the working principle of this biosensor is given, followed by the design constraints and the main modules, the preliminary simulation and experiments results.

**Methodology:**

### A. Magnetotactic Bacteria (MTB)

MTB were first discovered in 1975 [8]. These kinds of bacteria can synthesize a magnetic chain, which commonly consists of nanometer scale permanent magnetic particles called magnetosomes [9, 12]. Functioning like a compass, the magnetic chain guides the MTB to orient and migrate along geomagnetic field lines. According to their orientation to the magnetic field lines, they can be divided into two groups, axial or polar MTB [10, 11]. Generally, the axial MTB orient and swim to either poles of the magnetic field and they change their swimming direction randomly; whereas for the polar MTB, the magnetic field lines provide an axis and direction to lead MTB to swim towards only one direction [11]. For instance, the polar MTB named *MC*-1 found on the Northern hemisphere predominately swim to the North Pole, so they are referred to as north-seeking MTB.

Most of the MTB discovered are motile [10]. Some of them swim very fast as compared with other kinds of bacteria. Figure 1 shows a swarm of polar *MC*-1 bacteria swimming at the average speed up to 200 μm/s under the control of external electromagnetic field.

### B. Microbead

Directly attaching the MTB with the functional material, in our case bacteriophage, is a very difficult task. Due to biocompatibility and the small surface area of MTB (diameter of 1~2 μm), the number of bacteriophage that can be immobilized on the MTB will be very limited and as such, will have a direct effect on the attachment efficiency. To overcome this problem, microbead is chosen as a bridge to connect the MTB and bacteriophage and target bacteria together. Microbeads are widely used in the cell sorting, biomarker and bio-/chemical analysis [17]. Not only they provide large surface area to load the specific bacteriophage for detecting the targeted bacteria, microbeads also establish a suitable platform to be propelled by the MTB. As shown in Figure 2, our preliminary experiments demonstrated that *MC*-1 bacteria can push a fluorescent microbead (2 μm in diameter).

Figure 2: A single *MC*-1 bacterium pushing a microbead (2 μm in diameter) in a fluid medium. By reversing the orientation of an external magnetic field, a single *MC*-1 bacterium executes a U-turn while pushing a microbead.

### C. Protocol and Fabrication
In our system, MTB are attached with microspheres (several microns in diameter) coated with bacteriophage. Then, they are injected or navigated into the detecting chamber where microcoils and current carrier conductive wires control the swimming direction of the MTB to sweep the fluid sample that may contain the targeted pathogenic bacteria. Then, the MTB with microbeads are guided down to the detection area. A microelectrode array is implemented on the bottom of the detecting chamber to provide an impedance measurement platform. An integrated chip based on standard CMOS technology is bonded with the micro-electrode array to measure and collect the impedance signal simultaneously from each pair of micro-electrodes for detecting the existence of pathogenic bacteria and to record the total number. After each measurement routine, the whole chamber can be washed with de-ionized water prior to the next measurement. A very simplified system diagram is depicted in Figure 3. It includes four modules: 1) microfluidic device, 2) micro-electromagnet array, 3) micro-electrode array and 4) microelectronic circuit. Considering the difficulties in the microfabrication procedure, especially the high density of the microelectrodes, the microfluidic and micro-electromagnet parts will be fabricated on the same substrate, while the micro-electrode array will be implemented on a piece of silicon wafer. The standard CMOS process is chosen for the fabrication of the microelectronic circuit. These three parts will be bonded together to become a Lab-on-Chip system.

Figure 3: System diagram

Preliminary Design and Simulation:

A. Microfluidic Device and Micro-electromagnets

The microfluidic device provides inlet/outlet and a chamber for detection. The current-carrying conductors and coils are implemented on the top of the chamber to generate the local micro-electromagnetic field in the chamber. The first prototype will have a rectangle chamber. The volume of chamber can be arranged from micro-liter to milliliter according to the required sample volume. Another important aspect for defining the size of the chamber depends on the characteristics of the MTB. The analysis given below mainly focuses on the MC-1 bacteria.

The swimming speed of MC-1 bacteria in their media can reach a maximum velocity of 200 μm/s. According to the Stoke’s law [15], after being attached with a microbead, such as a 3 μm in diameter bead in an unbounded medium, the swimming speed is typically decreased to approximately 100 μm/s. Assuming that the depth of the detecting chamber is 1mm, it would take 10 seconds for the MTB to swim from bottom to surface of the detecting chamber. With 1ml of liquid sample, the detecting chamber should have an area of 3.16 cm × 3.16 cm with a depth of 1mm. In our cultures, the density of MC-1 bacteria is approximately $10^6$ to $10^7$ cells/ml without concentration. Assuming that all the MTB averagely spread on the same surface without overlap, the unit area that each MTB and microbead should cover is at least 31.6 μm × 31.6 μm with a density of $10^6$ cells/ml and 10 μm × 10 μm with the density of $10^7$ cells/ml respectively. Shown in Figure 4, although the above analysis is based on ideal conditions, compared with the conventional biosensors, the time to find the targeted pathogenic bacteria should be greatly decreased.

In order to navigate the MC-1 bacteria in the chamber, a layer of conductive material will be patterned on the top of the micro-chamber by the standard photolithography process, by controlling the direction and current density in the current-carrying conductors; and hence electromagnetic field will be generated in the micro-chamber.

Figure 4: Assuming that each time, the MTB swim back and forth by different routes within the defined square. a) Unit area of 31.6 μm × 31.6 μm. A single MTB need to sweep at least 72 times to cover all the volume and in 12 minutes. b) Unit area of 10 μm × 10 μm. A single MTB should sweep at least 9 times to cover all the volume in 90 seconds

B. Micro-electrode Array

After sweeping the sample for several minutes, the MTB would push the microbeads with bacteriophage and possibly with the attached targeted bacteria to the microelectrode array by being guided by the electromagnetic field generated on the top of the chamber. The density, material and structure of the microelectrodes all have direct effects on the sensitivity of this biosensor. Based on the analysis in Figure 4, to detect the $10^6$ cells/ml at the same time, it will need approximately 3K × 3K electrodes; with the concentration of $10^7$ cells/ml, 1K × 1K electrodes need to be fabricated.

We propose two approaches to implement microelectrodes. The first approach is to guide the MTB to the planar electrodes implemented at the bottom of chamber shown in Figure 5a. A layer of silicon oxide is deposited on the surface of silicon substrate. The diameter of the cylinder on the silicon oxide is smaller than that of the microbead. So, when the MTB will push the microbeads down to the electrodes, the microbead will be...
seated between a pair of micro-electrodes where the impedance can be measured. The other approach, illustrated in Figure 5b, allows MTB to pass through between two electrodes. The size of the microchannel is designed to allow MTB to push the microbeads going through one by one. The variance of impedance between the electrodes will be continuously monitored. When the changes occur beyond the pre-defined threshold (based on the impedance of different bacteria), the related circuit will indicate the presence the targeted bacteria and record the total number of the targeted bacteria.

Figure 5: a) The planar electrode array for measuring the impedance when the microbead is pushed by the MC-1 bacterium. b) MC-1 bacterium pushing a microbead and pathogenic bacteria pass between two electrodes.

Compared with these two techniques, the first one makes the microfabrication process easier but lacks flexibility and sensitivity in the case that there are several microbeads stacked together. The second offers precise measurement and can report the total number of targeted bacteria. However to fabricate this structure, especially to implement the electrodes on the inner side of microchannel, will be a challenge.

C. Microelectronic Circuit

Figure 6: The impedance between the electrodes when the pathogenic bacteria attach on the microbead. $R_m$, $R_p$, $R_w$, and $R_m$ is the resistance of microbead, phage, media and cell membrane respectively. $R_c$ is the resistance of the cytoplasm. $C_m$ and $C_w$ represent the capacitance of the cell’s membrane and medium between the electrodes.

The microelectronic circuits are responsible for identifying the presence of the pathogenic bacteria. In our detecting approach, there are four different impedances between a pair of electrodes: 1) pure medium, 2) MC-1 with microbead and bacteriophage, 3) MC-1 with microbead, bacteriophage and targeted bacteria, 4) other bacteria or micro-organisms. The microelectronic circuit should identify the difference of these four situations and report the number of the targeted bacteria.

The circuit mode of situation 3 is given in Figure 6. Beside the impedance measurement function, this circuit also can address each pair of electrodes, read the counting number of bacteria. Furthermore, by changing the threshold of the impedance, this sensor can be modulated to detect different kinds of bacteria or micro-organisms. This simulation result shown in Figure 7 is based on the 0.18 μm CMOS technology. The parameters of the circuit are designed for adjustment to a wide range of impedances, from few kilo-ohms to mega-ohms.

Figure 7: The simulation result of the impedance measurement.

Conclusion:

Combining the specificity of bacteriophage, the large surface area of microbeads, the sensitivity of the microelectrode and the mobility of the MTB, this biosensor provides a new approach to detect specified bacteria rapidly and precisely. Preliminary design and experiments prove that the MTB can attach to microbeads and push them under the control of the micro-electromagnetic field. Experimental data also show that MC-1 bacteria can survive in the human blood and most of the media used to cultivate the pathogenic bacteria. Not only for biosensor, MTB can also be a micro-carrier for many applications for example, drug delivery, cell separation, chemical analysis and bio-marker. However, there are several challenges that need to be solved. For example, the reliable attachment of MTB to microbeads, the precise measurement of single bacteria’s impedance and the fabrication process of high density micro-electrodes.

Acknowledge:

This work was initially fully supported by a grant from the Canadian Institute for Robotics and Intelligent Systems (IRIS). It is presently supported in part by a Canada Research Chair (CRC) in Development, Fabrication, and Validation of Micro/Nanosystems, the
Canada Foundation for Innovation (CFI), the National Sciences and Engineering Council of Canada (NSERC), and the Government of Québec. The authors wish to thank Prof. Dennis A. Bazylinski and Dr. Timothy J. Williams from Iowa State University, for providing the MC-1 bacteria, Dr. Dirk. Schüler from the Max Planck Institute for marine microbiology in Germany for providing the Magnetospirillum gryphiswaldense bacteria.

Reference: