Developing a cantilever biosensor enhanced with electrokinetics to detect bacteria in real time

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Synopsis
Detecting pathogenic bacteria in real time could greatly benefit many applications including food and water analysis. Cantilever biosensors enhanced with electrokinetics have been shown to detect Escherichia coli in real time at a concentration of \(4.18 \times 10^6\) cells/ml and in batch measurements at a concentration of \(10^7\) cells/ml. Biosensors regeneration using high temperature heating has been demonstrated to be 82% effective. Further testing with improved piezoelectric cantilever biosensors should lead to lower detection limits.

Introduction
Detecting pathogenic bacteria in liquid is important in food and water analysis, clinical diagnostics, and environmental monitoring. In Canada, total coliform bacteria such as Escherichia coli are monitored weekly in water treatment plants and distribution systems [1]. Established methods for detecting bacteria rely on labelled reagents, immuno-beads, polymerase chain reaction or the enzyme-linked immunosorbent assay. These methods are time-consuming, laboratory dependent, and expensive [2]. Cantilever biosensors present themselves as a practical alternative for detecting bacteria because they can overcome these restrictions. Their operating principle (see Figure 1) is that the mass increase from the binding of an antigen of a bacterium to an antibody-immobilized cantilever causes a measurable resonance frequency shift. More development on cantilever biosensors is required in order to detect bacteria in real time. In particular, the binding rate of cells on the sensing surface needs to be improved. In this project, several cantilever biosensors enhanced with electrokinetics are being developed. To date, bacteria were detected using an hourglass design, a biosensor regeneration technique using a hot plate was presented, and piezoelectric cantilever biosensors were developed for future testing.

Methods and results
A biosensor featuring an hourglass design was developed with the SOIMUMPs micromachining process. An ac signal is applied across the anchors of the biosensor to actuate the structure and rapidly draw cells to the sensing surface. The hourglass features enable electrothermal actuation and electrokinetic cell collection. Escherichia coli were collected on the Poly-L-Lysine functionalized surface of the biosensor from a sample of \(10^7\) cells/ml within 15 min (see Figure 2). Resonant frequency shifts were measured in air using a vibrometer (see Figures 3 and 4). Escherichia coli were also collected from a sample of \(4.18 \times 10^6\) cells/ml and resonant frequency shifts were measured in real time (see Figure 5).

A technique for regenerating silicon biosensors containing bound biological material was presented. Such a technique can be especially valuable to biosensor development because it allows prototyping chips to be reused during testing. In this technique, a used silicon chip is heated on a hot plate at high temperature for a short period, and biological material is vaporized while the biosensor is left intact. Resonant frequency shifts showed that this technique removes 82% of bound biological material (see Figure 6).

Improved piezoelectric cantilever biosensor were developed with the PiezoMUMP micromachining process. Higher resonant frequencies, sample flow, stronger electrokinetic effects, and dynamic measurements using an impedance analyzer should lead to lower detection limits.

Conclusions
Cantilever biosensors enhanced with electrokinetics hold promise for detecting bacteria in real time. Images and resonant frequency shifts demonstrate cell collection from the sample and cell attachment to the sensing surface at higher concentrations within minutes. Real-time detection and biosensor regeneration have been demonstrated. Further testing with improved piezoelectric cantilever biosensors should lead to lower detection limits.

References

Fig. 1. Operating principle [3].
Fig. 2. Hourglass design with cells [4].
Fig. 3. Individual resonant frequency shifts [4].
Fig. 4. Average resonant frequency shifts [4].
Fig. 5. Real-time detection [4].
Fig. 6. Biosensor regeneration.