Long-Range Electrokinetic Bioparticle Trap

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Detection of microorganisms such as bacteria and viruses is important in a variety of fields such as bioscience research, medical diagnosis, screening analysis in food processing and for environmental testing. Bacterial contamination of food, water and the environment is on the rise around the globe. The Centers for Disease Control and Prevention (CDC) estimates 76 million food-borne illnesses each year in the US, with 325,000 hospitalizations and 5,000 deaths [1]. Contaminated dinking water, beaches and improperly disinfected swimming pools [2] have also been implicated as a source of infection. Current practices of prevention rely on real-time monitoring of various types of pathogenic bacteria. However, at present real-time bacteria detection has a typical sensitivity on the range of 10^6-10^7 CFU/ml [3], while pathogenic bacteria are generally present at very dilute concentrations (< 100 CFU/ml) and still be an infectious dose.

Miniature medical and environmental diagnostic kits that are portable and fast have yet to be developed. One reason is that biosensors function effectively as detectors only when the bacteria or viruses are proximal and only when sufficient particles are present to stimulate signal generation. For practical applications, biosensors must offer a sensitivity that can detect fewer than 1,000 microorganisms per milliliter of suspension. Conventional microbiological detection methods rely upon enrichment techniques where bacteria are incubated on nutrient media or viruses are incubated on cell cultures, which are well established and reliable. However, the time required for cultures to produce unambiguous indications of growth may range from days to several weeks, rendering these methods cumbersome and occasionally impractical for point of care diagnosis or emergencies. Realizing that detection sensitivity could be improved by expediting bioparticle diffusion to the sensors, so instead of elaborating on sensing mechanisms as in many prior efforts, this work seeks to improve the detection sensitivity by incorporating electroosmotic (EO) particle collection with sensing.

Several bacteria-trapping strategies that are compatible with microtechnology have been reported in the literature. One is to create a non-uniform AC electric field and attract bacteria to regions of high field near electrodes, also known as dielectrophoresis (DEP). It has been well documented that DEP can be used for the manipulation and characterization of particles, and the separation of mixtures, such as cells, bacteria, and latex spheres [4, 5, 6, 7]. Suehiro *et al.* [8] have demonstrated a combination of DEP with electrical rupture of cell structure to increase detection sensitivity. However, DEP is size sensitive and its magnitude

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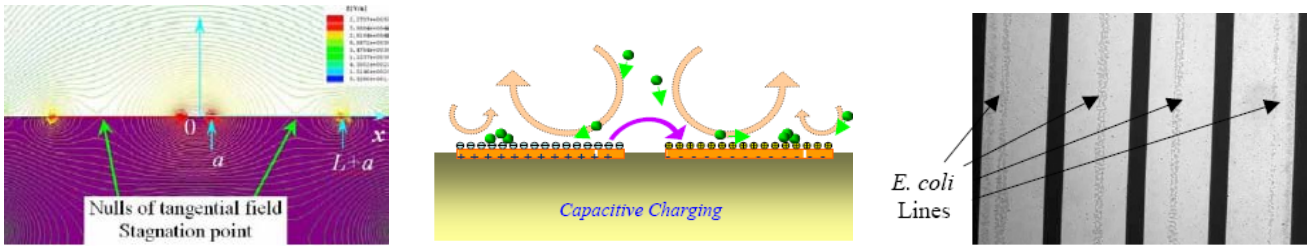
scales with the square of electric field strength. Hence, DEP force is weak for microorganisms and also short-ranged, decreasing rapidly away from electrodes. Currently, DEP bacteria traps cannot concentrate enough bacteria for sensing from a macroscopic volume of sample. Another suggested strategy is to use nano-magnetic particles with functionalized antibodies that dock with the bacteria in the bulk [9]. However, the need to synthesize the nano-magnetic particles, to immobilize anti-bodies on them and to manipulate them in miniature diagnostic kits render this approach highly complex, expensive and difficult to handle.

We have developed a new strategy of using long-range AC electro-osmotic (EO) flow to carry bacteria to a certain location and to trap the bacteria at that location. Electroosmotic motion has no dependence on particle size and scales much more favorably with the distance from the electrode (1/r versus $1/r^3$ for DEP). Therefore, EO force is particularly advantageous for collecting micron/submicron particles from the electrolyte. We use AC EO to direct particles to certain locations, and in doing so, greatly enrich the local particle concentration at the electrode surface to a detectable level.

There are prior observations of particle aggregation on electrode surface [10, 11] and concentration of bacteria on electrodes [12, 13] by EO flows, where the particle deposition was attributed to dielectrophoresis and surface forces. We have investigated the electric field distribution around electrodes and are able to offer a new perspective on particle concentration. Applying an AC potential over a pair of microelectrodes, our work [14, 15] suggests that particles prefer to deposit at certain locations due to local gradients of electric fields at electrode surface, as shown in Fig. 1. The stagnation lines are located at positions where there is a change in the tangential field direction, which is $1/\sqrt{2}$ of electrode-width away from its inner edge for isolated, wide electrodes, as shown in Fig. 1 (a). Because flow directions depend on the tangential fields, four counter-rotating vortices were formed at the electrode surface, and the stagnation takes place at locations where tangential fields become zero, as shown in Fig. 1 (b). This technique has been extrapolated to attract bioparticles from the bulk of a suspension to electrode surface, thus reducing the diffusion time of bioparticles to the detectors. In Fig. 1 (c), about 60 *E. coli* were concentrated onto a 10 μm x 10 μm area from a 10^6 CFU/ml suspension in 30 seconds, which is faster and more effective than commonly-adopted dielectrophoresis and electrostatics. Measuring the cell impedance with signals appropriate for particle assembly, we observed an increased differentiation of



(a) (b) (c)

Fig. 1 (a) Electric fields around a planar electrode pair. The tangential component changes sign at $1/\sqrt{2}$ of electrode-width. (Axes: relative dimensions.) (b) Four counter-rotating vortices are formed above the electrodes due to changes in tangential electric fields, which facilitates particles aggregation on electrodes. (c) Assembled *E. Coli* lines on electrodes.

impedances between bacteria suspensions and control solutions. As shown in Fig. 2, the electrode impedances were measured with an Agilent 4294A impedance analyzer from 40 Hz to 5 MHz at an open oscillation level of 5 mVrms and 1.0 Vrms. *E. coli* were resuspended in tap water of 2 mS/M at $5x10^3$ CFU/ml. For the measurements at 5 mV, little difference between *E. coli* suspensions and control tap water can be detected. As a comparison, the measurements of the same samples at 1 Vrms exhibit impedance difference by a factor of two, which indicates a sensitivity better than 10^4 bacteria/ml (Fig. 2).

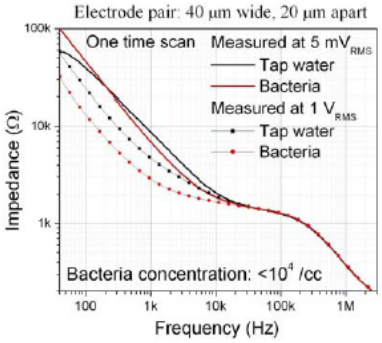


Fig. 2 Impedance of *E. Coli* in tap water with comparison to control sample.

Because most bioparticles [16] are negatively charged, asymmetric-polarization AC EO is devised with a synergy of AC and DC electrokinetics for more efficient particle collection. Energized by biased AC signals $V_{appl} = V_O(1+\cos\omega t)$, electrodes in a pair undergo differential polarizations, i.e. the positively-biased electrode electrochemically produces co-ions and the negatively-biased electrode capacitively attracts counter-ions. Consequently a unidirectional flow is induced at the electrode surface, generating a large vortex to convect bioparticles, as conceptually shown in Fig. 3.

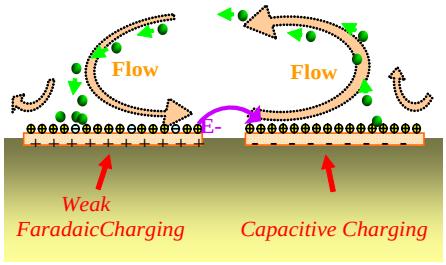
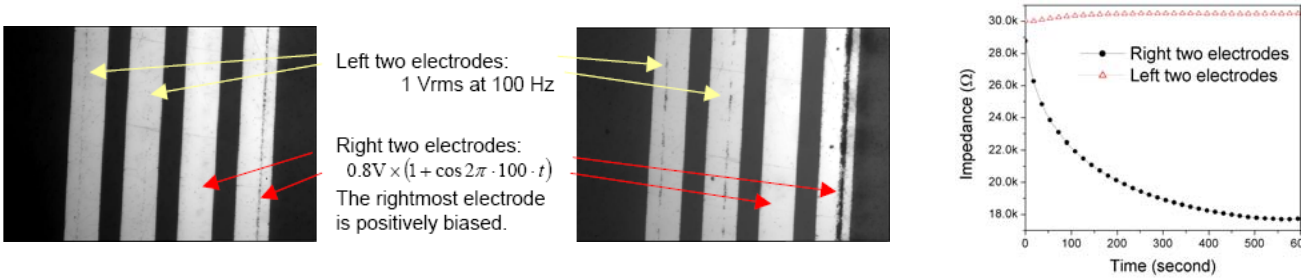


Fig. 3 Schematic of particle trapping by asymmetric-polarization AC EO. Two differential electrode polarizations produce a large vortex over the electrode pair. Particles are preferentially convected to positively biased electrode.

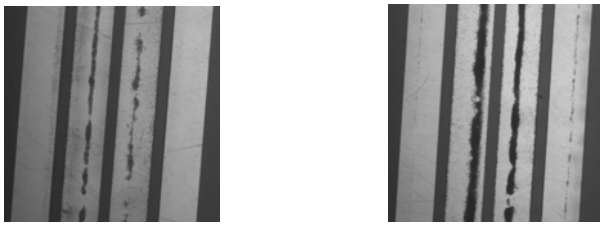
Another benefit of asymmetric-polarization AC EO is that electrophoretic/electrostatic force is exerted simultaneously with AC EO to move bioparticles towards positively-biased electrodes. Therefore, A-P AC EO exhibits robust and continuous attraction of particles to electrode surfaces, as shown in Fig. 4. At $5x10^4$ *E. coli*/ml and energizing signals as in Fig. 4, the impedances of the two electrode pairs versus time (Fig. 4c) shows consistent impedance changes with continuous particle collection by A-P scheme. Combining two A-P particle-collectors and using the center electrodes for EIS as shown in Fig. 5, the detection limit can be

further improved. With an appropriate choice of signal frequencies, magnitudes, biases and sequencing when energizing electrodes, we strive to achieve particle detection limits on the order of 10^2/mL.



(a) 30 sec. after the field is on. (b) 5 minutes after the field is on. (c) Impedance vs time.

Fig. 4 Comparison of particle concentration by electrodes excited by unbiased and biased AC signals.



(a) 1minute after "on." (b) 5 minutes after "on."

Fig. 5 Combination of A-P AC EO particle collection with detection.

This trap can be used in conjunction with a biosensor located at the specific location on the electrode where the bacteria are trapped. Alternatively, in an analogy to vortices formed by two pairs of electrodes, we can generate two artificial vortices by pumping two flows towards each other. Bioparticles are to be brought to site where the two flows meet and be trapped to DEP electrodes located there. This design can generate flow convection over as a large volume of fluid as necessary. This scheme is promising for bioparticle detection in highly diluted biofluids. We are currently developing a prototype for AC EO bioparticle trap. High sensitivity is expected with further improvements.

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