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Dielectrophoretic separation and manipulation of live and heat-treated cells of *Listeria* on microfabricated devices with interdigitated electrodes

Haibo Li[a], Rashid Bashir[a,b,*]

[a]*School of Electrical and Computer Engineering, Purdue University, West Lafayette, IN 47907-1285, USA*
[b]*Department of Biomedical Engineering, Purdue University, West Lafayette, IN 47907-1285, USA*

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Abstract

Dielectrophoresis, the movement of particles in non-uniform ac electric field, was used to separate live and heat-treated *Listeria innocua* cells with great efficiency on the micro-fabricated devices with interdigitated electrodes by utilizing the difference of dielectric properties between alive and dead cells. Both live and dead cells are found to be only able to collect either at the centers of the electrodes in negative dielectrophoresis or at the electrode edges in positive dielectrophoresis due to the dielectrophoretic force and electrohydrodynamic force. Cell viability was verified by a rapid method using epifluorescence staining. The dependency of the applied ac signal's frequency on the dielectrophoretic properties of *Listeria* cells is studied and discussed. This on-electrode manipulation and separation of cells can prove to be useful in micro-scale sample preparation and diagnostic applications in biochips.

Keywords: Cell separation; Dielectrophoresis; *Listeria*; Interdigitated microelectrode; Biochips

1. Introduction

Cell manipulation and separation has numerous biological and medical applications, e.g. identification and characterization of individual cells, purification of cell subpopulations from mixture suspension, etc. It is of great importance to separate and isolate pathogenic bacteria from non-pathogenic bacteria and to separate live bacteria from dead ones, for biomedical analysis or food safety detection. For example, *Listeria monocytogenes* is a gram-positive human pathogen and can be found not only in raw food but also on working surfaces in food-processing plants.

The effects of *listeriosis* include septicemia, meningitis, encephalitis, and intrauterine or cervical infections in pregnant women, causing 415 deaths in the US each year [1]. As few as 1000 or less bacteria can cause illness in persons with weak immune systems (elderly, children, cancer patients, etc.). Most of current detection methods of pathogenic bacteria involve enrichment by culturing food samples, followed by colony counts and/or immunoassays, the culturing can take 5–7 days and by the time the bacterium is identified the food products have already been shipped and probably sold [1].

The US Department of Agriculture is actively searching for fast methods of detecting *L. monocytogenes* in food-processing plants and our group is developing a new integrated micro-fabricated biochip to detect, manipulate and separate these bacteria cells from food sample much faster than the usual way. Thus, it is desirable to separate the dead bacteria from the live ones in order to detect effectively *L. monocytogenes* in food-processing since dead *L. monocytogenes* is not pathogenic.

The current methods commonly used for manipulation, concentration and separation of biological particles employ several kinds of physical forces from mechanical, hydrodynamic, ultrasonic, optical, and electro-magnetic origins [2]. Among these methods, dielectrophoresis on micro-fabricated electrodes has been proved especially suitable for its relative ease of micro-scale generation and structuring of an electric field on microchips. Furthermore, integrated dielectrophoresis biochips provide the advantages of speed, flexibility, controllability, and ease of application to automation [3].

Dielectrophoretic forces occur on cells when a non-uniform ac electrical field interacts with field-induced electrical

* Corresponding author. Tel.: +1-765-496-6229; fax: +1-765-494-6441.
E-mail address: bashir@ecn.purdue.edu (R. Bashir).

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polarization. The time-averaged dielectrophoretic force F for a dielectric sphere immersed in a medium in constant field phases in space (like the situation in our experiments) is represented as [2–4]:

$$F = 2\pi\varepsilon_0\varepsilon_m r^3 \mathrm{Re}[f_{CM}]\nabla|E_{rms}|^2 \quad (1)$$

where ε_0 is the vacuum dielectric constant, r the particle radius, E_{rms} the root mean square value of the electric field, and f_{CM} well known as the Clausius-Mossotti factor [3,4]:

$$f_{CM} = \frac{\varepsilon_p^* - \varepsilon_m^*}{\varepsilon_p^* + 2\varepsilon_m^*} \quad (2)$$

where ε_p^* and ε_m^* are the relative complex permittivities of the particle and the medium, respectively. When the dielectric constant of particle is larger than that of medium, i.e. $\mathrm{Re}[f_{CM}] > 0$, the dielectrophoresis (DEP) is called positive and the particle moves towards the locations with the greatest electric field gradient. Whereas, the dielectric constant of particle is smaller than that of medium, i.e. $\mathrm{Re}[f_{CM}] < 0$, the DEP is called negative and particle moves to the locations with smallest electric field gradient.

Biological cells consist of adjacent structures of materials that have very different electrical properties and exhibit large induced boundary polarizations that are highly dependent on the applied field frequency as well as their physiological states. For example, the cell membrane consists of a very thin lipid bi-layer containing many proteins and is highly insulating with a conductivity of around 10^{-7} S/m, while the cell interior contains many dissolved charged molecules, leading to a conductivity as high as 1 S/m. Upon death, the cell membrane becomes permeable and its conductivity can increase by a factor of 10^4 due to the cell contents exchanging freely material with the external medium through the small pores on the membrane. This large change in the dielectric properties on cell death indicates a large change in the dielectric polarizability [5]. Hence, a large difference in DEP responses (positive and negative, respectively) and a selective separation can be achieved between live and dead cells.

DEP is particularly useful in the manipulation and separation of microorganisms and has been employed successfully in isolation and detection of sparse cancer cells, concentration of cells from dilute suspensions, separation of cells according to specific dielectric properties, and trapping and positioning of individual cells for characterization [2], for example, for separations of viable and nonviable yeast cells [6,7], leukemia and breast-cancer cells from blood, and the concentration of $CD34^+$ cells from peripheral-stem-cell harvests [8]. Continuous separation can also be achieved by combining with a technique similar to field-flow-fractionation (FFF) [9]. Yet few reports have been seen about the applications of DEP in separation of live and dead cells of the same species of small bacteria as *Listeria* within micro-devices. Here, we demonstrate that dielectrophoretic separation of live and heat-treated *L. innocua* cells, a non-pathogenic strain of *L. monocytogenes*, was achieved with great efficiency on the microfabricated devices with interdigitated electrodes in water by considering the difference of dielectric constant between alive and dead cells. The dependency of the applied ac signal's frequency on the dielectrophoresis of different cells is studied and discussed.

2. Materials and methods

2.1. Cell preparation and fluorescent staining

For safety purpose, non-pathogenic *L. innocua* cells were chosen for the experiments since their dielectric properties are assumed to be similar to those of the pathogenic *L. monocytogenes*. The *Listeria* cells are rod-shaped with length of around 3 μm and diameter around 1 μm.

The cells were cultured in brain heart infusion (BHI) solution at 37 °C. They were harvested after 18 h with the concentration of $\sim 10^9$ cells/ml. Part of the cell suspension was heated at 80 °C for 20 min to kill the bacteria inside. Both live and dead cells were washed with Tris Glycine solution (3.6 mM Tris, 47 mM Glycine, pH 7.5 and conductivity ~3.3 μS/cm) for three times.

In order to observe the live and dead cells simultaneously under fluorescence microscope and verify the viability of the cells, they were stained (3 μl of the dye mixture is added to each ml of the bacterial suspension) at 37 °C for 15 min in dark with fluorescent dye of LIVE/DEAD® BacLight™ bacterial viability Kit L-7012 (Molecular Probes Inc.), which is composed of two nucleic acid-binding stains: SYTO 9 and propidium iodide. SYTO 9 penetrates all bacterial membranes and stains the cells green, while propidium iodide only penetrates cells with damaged membranes, and the combinations of the two stains produces red fluorescing cells [10]. This procedure can therefore provide a rapid method for ascertaining cell viability without selectively collecting the cells after the separation experiments in other methods.

The suspension was then diluted to $\sim 10^5$ cells/ml with DI water and mixed in the ratio of 50–50% right before the separation experiments. The conductivity of the final suspension was adjusted to 2 μS/cm as determined by a conductivity meter (Cole-Parmer Instruments).

2.2. Microelectrodes and cell separation

Interdigitated microelectrodes were manufactured on glass substrate by ABTECH Scientific Inc. using standard photolithography. The electrode material was gold, 1000 Å thick, magnetron sputtered onto a 100 Å thick seed layer of chromium. The width of the electrodes and the spacing between two adjacent electrodes are both 15 μm. Fig. 1 shows the schematic plot of the experimental devices and the top view of the interdigitated microelectrodes.

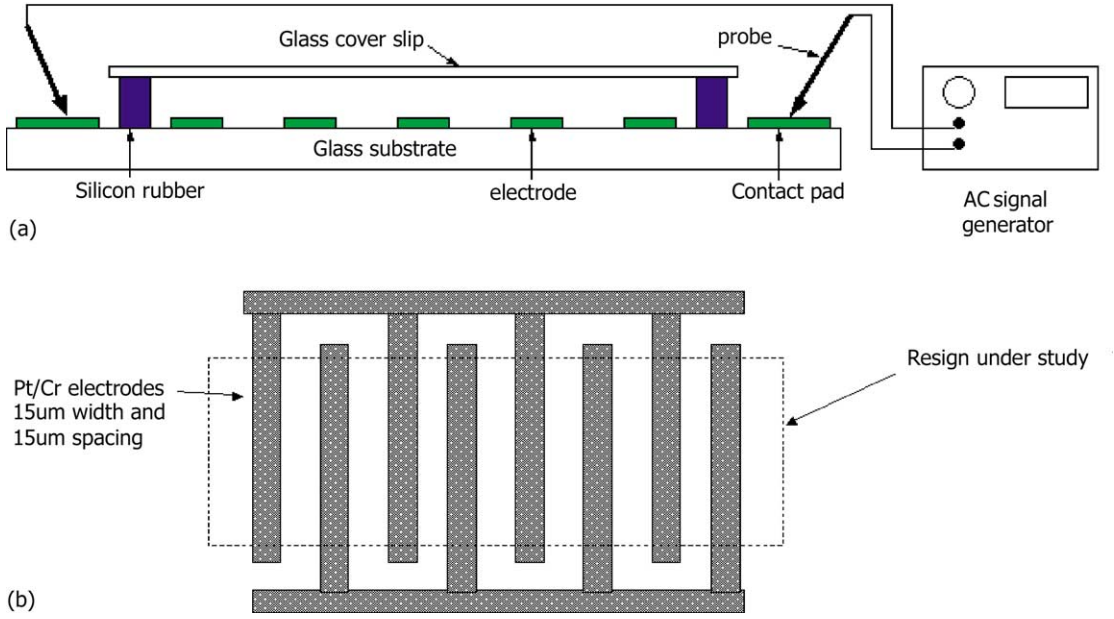

Fig. 1. A schematic plot of the experimental apparatus in (a) and the top view of the interdigitated microelectrodes in (b). The width of the electrodes and the spacing between two adjacent electrodes are both 15 μm. The silicone rubber wall is 0.3 mm thick. The electrodes are connected to a HP 33120A arbitrary waveform generator.

The electrodes are connected to a HP 33120A arbitrary waveform generator as the ac signal source by attaching two conducting wires to the contact pads. A rectangular electrode chamber was constructed by affixing with wax a silicone rubber (0.3 mm thick) to the glass substrate containing the interdigitated electrode array. About 20 μl of cell mixture suspension sample was pipetted into the chamber and a cover slip was gently pressed over the silicone rubber rectangular to form a tight seal. A sinusoidal signal with voltage of 1 V (peak-to-peak) and frequency ranging from 1 to 15 MHz was applied to the electrode array. The electrokinetic behaviors were then viewed on a TV monitor through a Kodak CCD digital camera on a Nikon fluorescence microscope. The separation efficiency was determined by counting the live and dead cells collecting at the electrode edges and centers, respectively.

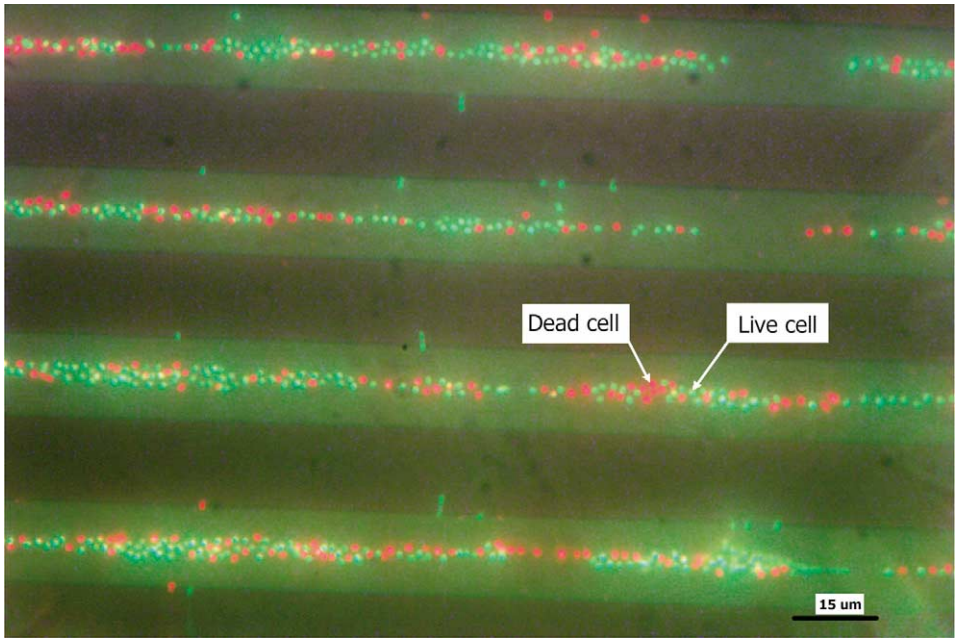

Fig. 2. Negative dielectrophoresis for both live and heat-treated *L. innocua* cells on interdigitated microelectrodes by applying a 1 V (peak-to-peak) and 10 kHz signal. Live cells are green and heat-treated ones are red. Cells collect on the top centers of the electrodes.

3. Results and discussion

At low frequencies ($\lesssim$30 kHz), both live and dead cells appear to exhibit negative dielectrophoresis. The result of applying a 1 V (peak-to-peak), 10 kHz voltage signal to the electrodes for a suspension containing both live (green) and dead (red) cells is shown in Fig. 2. Both cell types collect at the centers of the electrodes. As the frequency increases, the dielectrophoresis for live cells changes to positive and they are directed to the edges of the electrode strips and trapped there, while the dead cells still experience negative dielectrophoretic force and remain collected at the centers of the electrodes until around 100 kHz. In Fig. 3 the result of changing the frequency to 50 kHz is shown. A physical separation with the efficiency of about 90% between live and dead cells of *L. innocua* was achieved on the interdigitated microelectrodes by application of the 50 kHz signal. The efficiency was defined as the ratio of the number of the live cells collecting at the edges and that of the dead cells collecting at the centers to the total cell number in the counted area. At higher frequencies ($\gtrsim$100 kHz), both live and dead cells appear experiencing strong positive dielectrophoretic force. All cells are collected at the edges of the electrodes as shown in Fig. 4 in which a signal with 1 V and 1 MHz was applied. During the experiments, the dynamic movements of cells around the electrode were observed, and the results are the same for both positive and negative DEP whether or not we let the cells settle onto the bottom plane before the voltage is applied.

Eq. (1) indicates that the dielectrophoretic force is highly dependent on the gradient of the electric field, and hence, the geometry and configuration of the electrodes. The interdigitated electrode arrays have been well studied [11,12]. It is obvious that the electrode edges have the maximum electric field gradient and cells are expected to collect at the edges in positive dielectrophoresis. It seems that in negative dielectrophoresis, the cells are expected to collect at both the centers of the electrodes and the centers of the intervals between neighboring electrodes since there exists the minimum electric field gradient at these two places. But our experiments observed that cells only collect at the centers of the electrodes in negative dielectrophoresis. By further investigation about the electric field distribution above the electrode array using Green's theorem proposed by Wang et al. [12] and the electrohydrodynamic effect [13], the experimental observations can be explained very well. Fig. 5 plots the field parameter E^2_{rms} and $\nabla_y E^2_{\mathrm{rms}}$ at different heights above the electrode plane using Mathematica software. It can be seen from Fig. 5(a) that a minimum of electric field parameter E^2_{rms} only exists at points equidistant between two neighboring electrodes with height of less than ~5 μm. Above ~5 μm a maximum actually exists between two neighboring electrodes, while a minimum can always be seen at the points right above the center of any electrode. A minimum can be thought of as a "potential well" which holds cells in the negative dielectrophoresis. It can be seen from Fig. 5(a) that such potential well between two neighboring electrodes is much "shallower" in both space and energy than that on the top of the electrode. We can imagine that any cells with enough kinetic energy can easily move out of this potential well and go to the more "deeper" potential wells at the centers of the electrodes. Fig. 5(b) shows the gradient of the electric field parameter E^2_{rms} at different heights along the direction perpendicular to the electrode lines (labeled as "y"). Since $\nabla_y E^2_{\mathrm{rms}}$ is directly proportional to the dielectrophoretic force along y direction, it can be seen that the driving force directing cells to the interval between two electrodes is much smaller than the one pushing cells to the center of the electrode, and above 2 μm, the former can be negligible compared with the latter. Cells that happen to be above the electrodes or above the intervals but with height more than ~5 μm will be directed to the electrode centers due to the dielectrophoretic force. For those cells happening to be above the intervals and with height less than ~5 μm, the dielectrophoretic force tends to push them to the centers of the intervals. However, in this case the electrohydrodynamic effect may dominate since the dielectrophoretic force is small in this region. Electrohydrodynamic effect is caused by the high field around the electrodes. Under the influence of the high electric field, heat is generated in the medium, resulting in local temperature gradients, which in turn give rise to gradients in the conductivity and permittivity of the medium. These gradients can induce fluid movement and this fluid movement can cause particle movements through the viscous drag force [13]. Green and Morgan have shown the fluid flow patterns observed on the microelectrodes at frequencies below 500 kHz: the fluid moves in from the intervals to the electrode edges and runs parallel to the substrate; at the center of the electrode the converging flows move upwards, perpendicular to the substrate; cells experience a viscous drag force and are pushed into the center where they settle at the stagnation point [13]. This is clearly demonstrated by our results. As seen in Fig. 2, the rod-shaped *Listeria* cells look like small round dots at the centers of the electrodes from the top view in negative dielectrophoresis, indicating that the cells align themselves upwards with the converging flow, and no cells were found in the regions in between the electrodes. This on-electrode manipulation and separation of cells by using a combination of dielectrophoretic and electrohydrodynamic forces may prove useful in diagnostic applications.

It is believed that at low frequency (here, $\lesssim$30 kHz for live cells and $\lesssim$100 kHz for dead cells), the applied electric field is mainly dropped across the outmost membrane of a cell, and it behaves as a poorly conducting sphere with a high permittivity, and hence, negative dielectrophoresis is expected in this frequency range [2]. With increased frequency, the applied field gradually penetrates into the cell, which then behaves as a more conducting sphere having the permittivity of the cell interior, and positive dielectrophoresis is expected in this frequency range (<15 MHz). It has

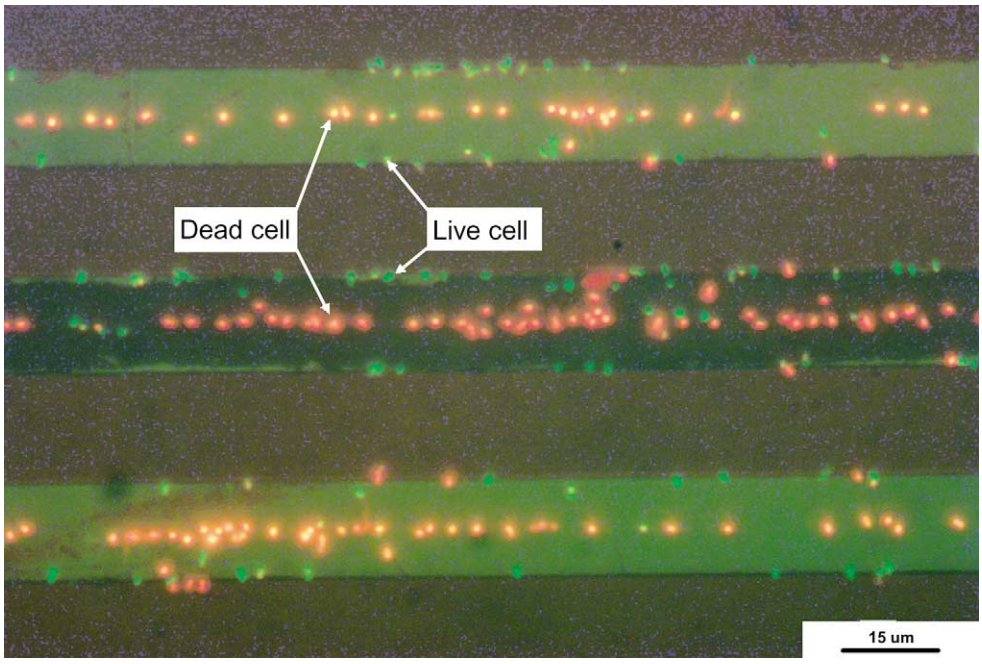

Fig. 3. Dielectrophoretic separation of live and heat-treated *Listeria* cells using interdigitated microelectrodes and a 1 V (peak-to-peak) 50 kHz signal. Most live cells (green) collect on the edges of the electrodes, whilst the heat-treated cells (red) collect on the top centers of the electrodes.

been demonstrated that cells at different physiological states have different dielectric properties, which can be identified through their dielectrophoretic behaviors. Huang et al. [6] used the smear-out sphere approach and showed that the cytoplasmic membrane conductivity of yeast cells increased on heat treatment from 2.5×10^{-7} to 1.6×10^{-4} S/m, while that of the cell interior decreased from 0.2 to 7×10^{-3} S/m. However, no data about the electric properties of live and heat-treated *Listeria* cells is available so far, and simply using the parameters in [6] and the medium conductivity of 2 μS/cm gives a result inconsistent with our observations. Based on our experimental results, a qualitative plot of the

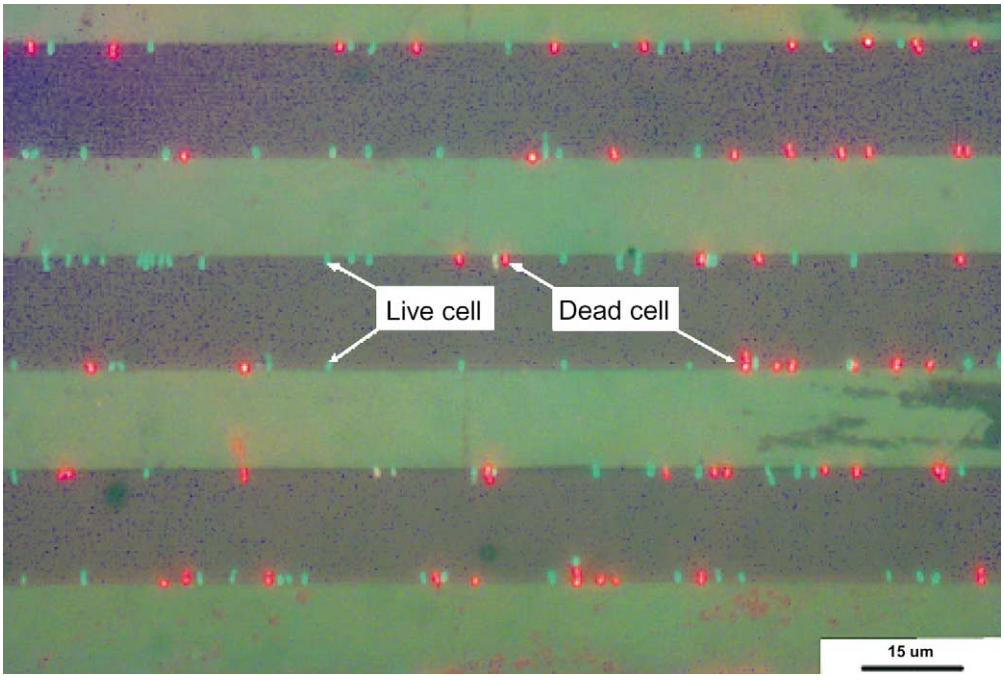

Fig. 4. Positive dielectrophoresis for both live and heat-treated *L. innocua* cells on interdigitated microelectrodes by applying a 1 V (peak-to-peak) and 1MHz signal. Live cells are green and heat-treated ones are red. All cells collect on the edges of the electrodes.

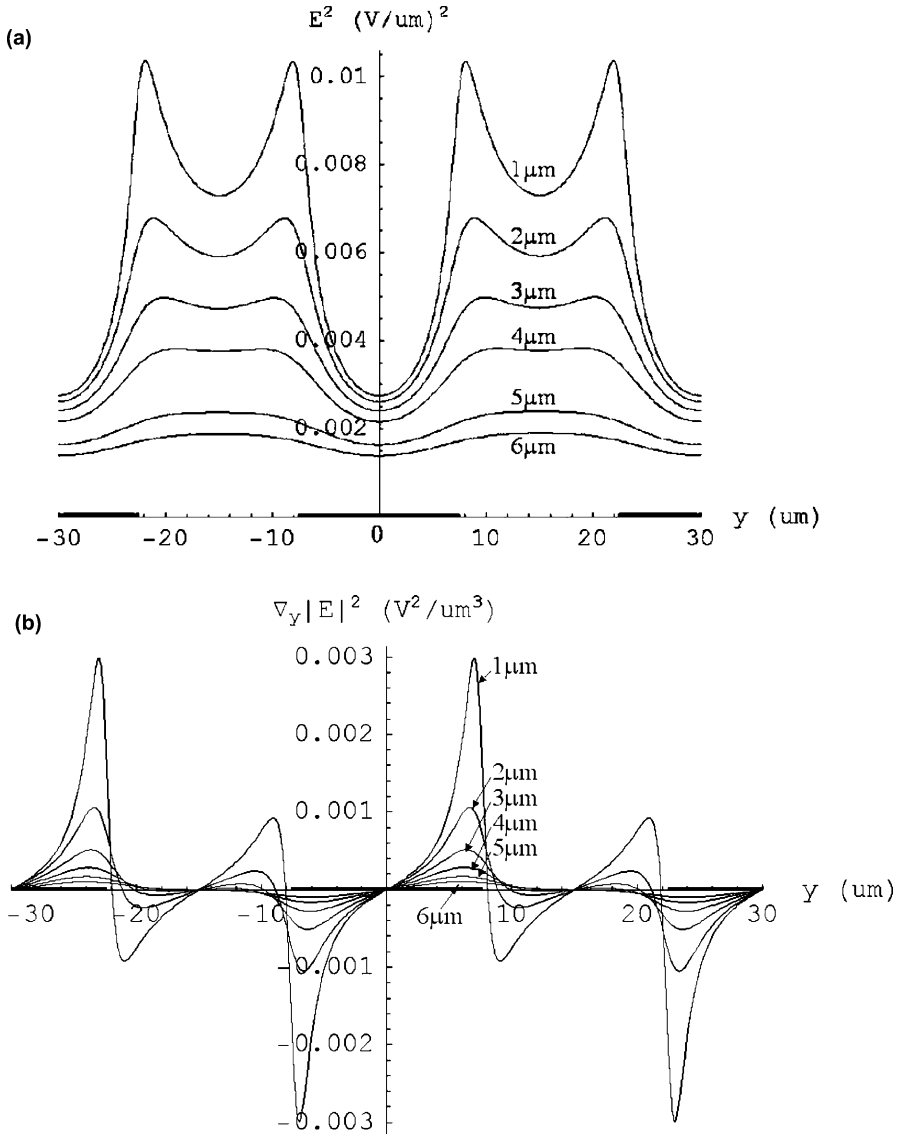

Fig. 5. Electric field parameter E^2_{rms} and $\nabla_y E^2_{rms}$ at different heights above the electrode plane using Mathematica in (a) and (b), respectively. Results are for a parallel array with electrode width 15 μm and interval 15 μm, and an applied voltage of 1 V at different heights labeled on the curves. Electrodes are represented by the bold lines on the *y*-axis.

parameter Re[f_{CM}] versus the applied frequency is shown in Fig. 6, indicating the range of the signal frequency where separation of live and heat-treated cells was achievable in our experiments. This plot looks rather similar to the DEP responses for the same cell types of MDA-231 and MDA-468 calculated from the single-shell dielectric model by Gascoyne et al. [14]. It is possible that the membrane capacitance is the dominant factor in determining the cell's dielectrophoretic response in the frequency range and suspension conductivity we used. The heat-treatment may make differences in the thickness, composition and folding of the membrane, and hence, in the membrane capacitance between the live and dead cells, giving rise to the difference in their dielectrophoretic behaviors observed here. The electrorotation (ROT) study will be used for the dielectric characterization of *Listeria* cells in our future work.

Though, most live cells collect at the edges of the electrodes when their dielectrophoresis changes from negative to positive, a few of them still remain at the centers. Similar thing happens to the a few dead cells that move to the electrode edges at frequency lower than ∼100 kHz. This indicates that the dielectric properties may still have some variability, even within the same type of cells, and the behavior of moribund cells may be different from that of both live and dead cells. By carefully selecting the appropriate suspension medium, the separation conditions (e.g. frequency of the applied field) can be chosen so that the difference in the Re(f_{CM}) values between live and dead cells are maximized, and separations with 100% efficiency can be achieved. Another future area of our work includes the use of cell separation using dielectrophoresis in combination with a flow in micro-channels.

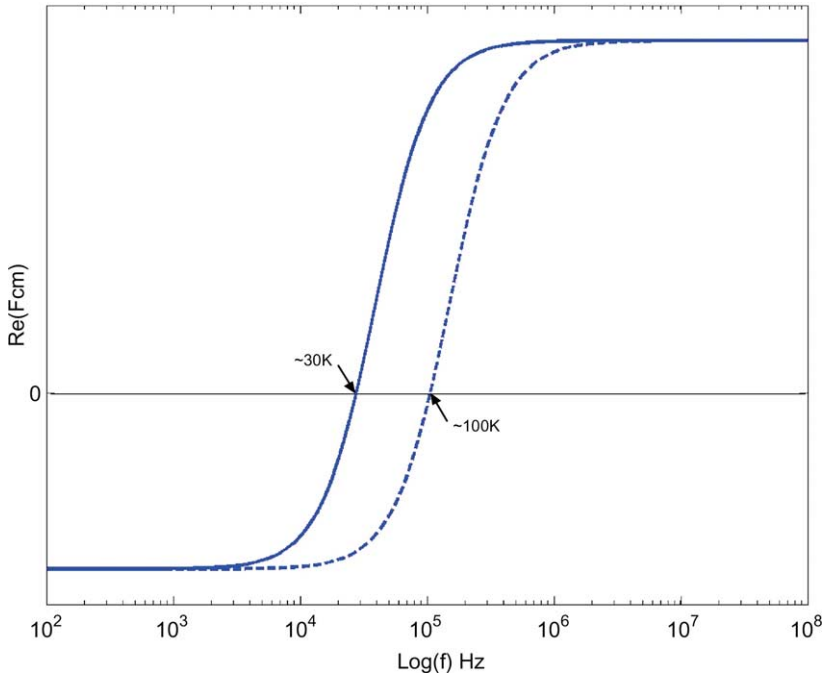

Fig. 6. A qualitative plot of the parameter Re[f_{CM}] vs. the applied frequency, solid line for live cells and dashed line for dead ones, indicating the range of the electric frequency where separation of live and heat-treated cells is achievable.

4. Conclusion

The dielectrophoretic separation of live and heat-treated *L. innocua* cells was achieved in water with the efficiency of about 90% by applying a 1 V and 50 kHz signal. It was observed that the dielectrophoretic behaviors of live and dead cells differ in the frequency range from ∼30 to ∼100 kHz in the selected medium (water). Both live and dead cells can only collect either on the top centers of the electrodes in negative dielectrophoresis or at the electrode edges in positive dielectrophoresis as results of a combination of dielectrophoretic and electrohydrodynamic effects. The viability of the cells was characterized by a rapid epifluorescence staining method using the LIVE/DEAD Bacterial Viability Kit (BacLight) and the live and dead cells can be monitored simultaneously in the experiments. Separation and manipulation of these microorganisms on biochips using DEP can be very useful in sample preparation and preprocessing and in diagnostic applications.

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References

[1] Foodborne Pathogenic Microorganisms and Natural Toxins Handbook, Center for Food Safety & Applied Nutrition, USDA.
[2] X. Wang, Y. Huang, P.R.C. Gascoyne, F.F. Becker, IEEE Trans. Ind. Appl. 33 (3) (1997) 660–669.
[3] Y. Huang, K.L. Ewalt, M. Tirado, R. Haigis, A. Forster, D. Ackley, M.J. Heller, J.P. O'Connell, M. Krihak, Anal. Chem. 73 (2001) 1549–1559.
[4] H.A. Pohl, J. Appl. Phys. 22 (1951) 869–871.
[5] R. Pethig, G.H. Markx, TIBTECH 15 (1997) 426–432.
[6] Y. Huang, R. Hölzel, R. Pethig, X. Wang, Phys. Med. Biol. 37 (7) (1992) 1499–1517.
[7] G.H. Markx, M.S. Talary, R. Pethig, J. Biotechnol. 32 (1994) 29–37.
[8] M. Stephens, M.S. Talary, R. Pethig, A.K. Burnett, K.I. Mills, Bone Marrow Transpl. 18 (1996) 777–782.
[9] G.H. Markx, R. Pethig, Biotechnol. Bioeng. 45 (1995) 337–343.
[10] L. Boulos, M. Prévost, B. Barbeau, J. Coallier, R. Desjardins, J. Microbiol. Methods 37 (1999) 77–86.
[11] P.V. Gerwen, W. Laureyn, W. Laureys, G. Huyberechts, M.O.D. Beeck, K. Baert, J. Suls, W. Sansen, P. Jacobs, L. Hermans, R. Mertens, Sens. Actuators B 49 (1998) 73–80.
[12] X. Wang, X. Wang, F.F. Becker, P.R.C. Gascoyne, J. Phys. D: Appl. Phys. 29 (1996) 1649–1660.
[13] N.G. Green, H. Morgan, J. Phys. D: Appl. Phys. 31 (1998) L25–L30.
[14] P.R.C. Gascoyne, X. Wang, Y. Huang, F.F. Becker, IEEE Trans. Ind. Appl. 33 (3) (1997) 670–678.