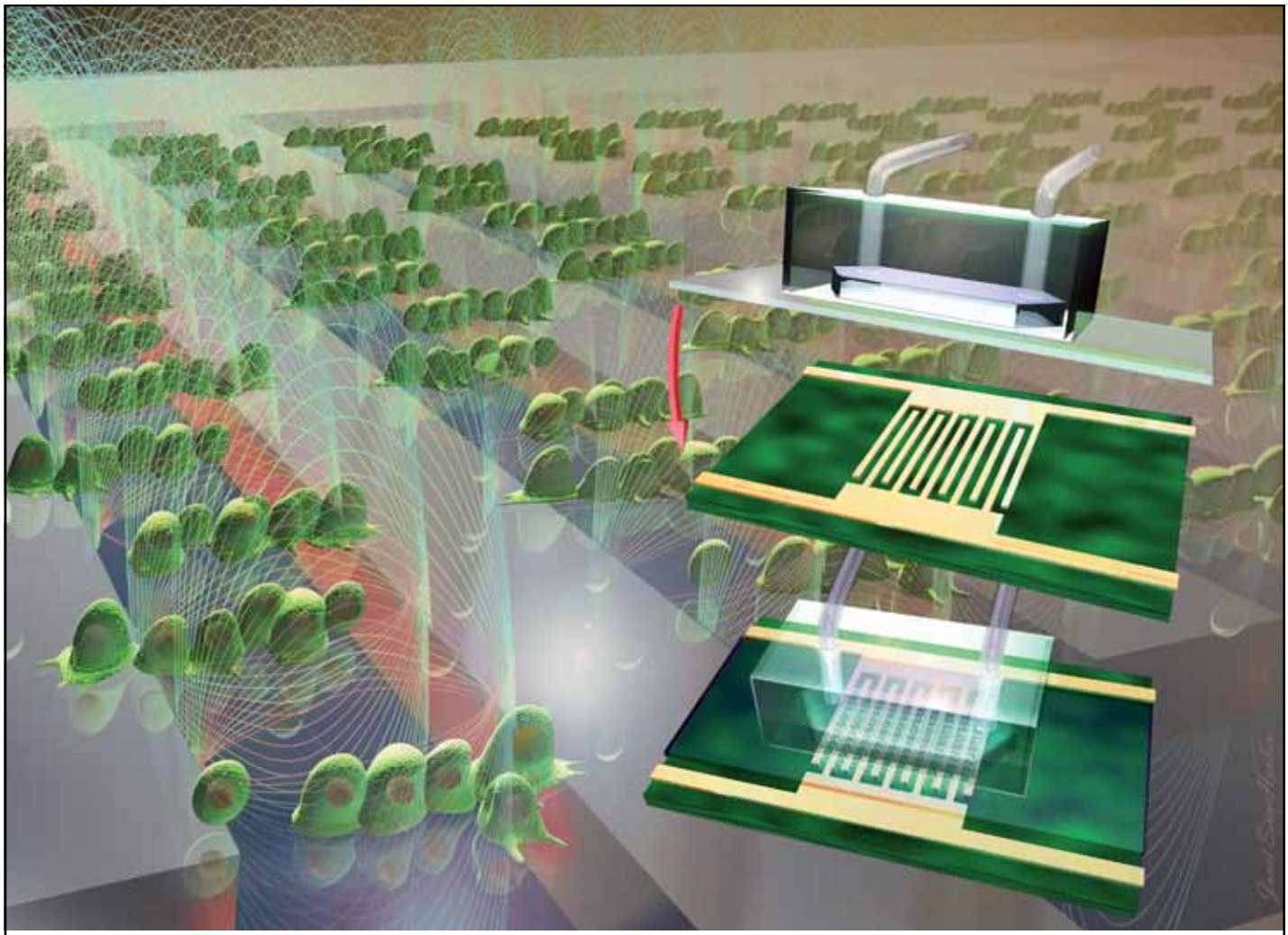
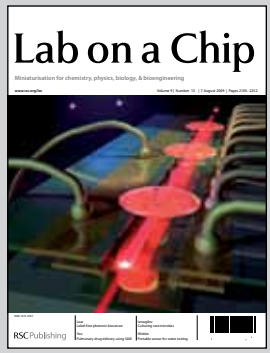


Featuring work from the group of Professor R. Bashir in the Department of Electrical and Computer Engineering & Bioengineering and the Micro and Nanotechnology Laboratory (MNTL), University of Illinois at Urbana-Champaign, Urbana, IL, USA.

Title: Dielectrophoresis-based cell manipulation using electrodes on a reusable printed circuit board.

A convenient and affordable approach to implement dielectrophoresis (DEP) particle manipulation without micro-fabrication on a chip is demonstrated. By using reusable electrodes on a printed circuit board and a PDMS microfluidic channel on a glass coverslip, mammalian cells and polystyrene beads are manipulated aligned with DEP.

As featured in:



See Bashir *et al.*, *Lab Chip*, 2009, **9**, 2224–2229.

RSC Publishing

www.rsc.org

Registered Charity Number 207890

Dielectrophoresis-based cell manipulation using electrodes on a reusable printed circuit board†

Kidong Park,‡[ab] Ho-Jun Suk,‡[bc] Demir Akin[d] and Rashid Bashir*[bce]

Received 3rd March 2009, Accepted 21st May 2009
First published as an Advance Article on the web 9th June 2009
DOI: 10.1039/b904328d

Particle manipulation based on dielectrophoresis (DEP) can be a versatile and useful tool in lab-on-chip systems for a wide range of cell patterning and tissue engineering applications. Even though there are extensive reports on the use of DEP for cell patterning applications, the development of approaches that make DEP even more affordable and common place is still desirable. In this study, we present the use of interdigitated electrodes on a printed circuit board (PCB) that can be reused to manipulate and position HeLa cells and polystyrene particles over 100 μm thick glass cover slips using DEP. An open-well or a closed microfluidic channel, both made of PDMS, was placed on the glass coverslip, which was then placed directly over the PCB. An AC voltage was applied to the electrodes on the PCB to induce DEP on the particles through the thin glass coverslip. The HeLa cells patterned with DEP were subsequently grown to confirm the lack of any adverse affects from the electric fields. This alternative and reusable platform for DEP particle manipulation can provide a convenient and rapid method for prototyping a DEP-based lab-on-chip system, cost-sensitive lab-on-chip applications, and a wide range of tissue engineering applications.

Introduction

Dielectrophoresis (DEP) was first defined by Pohl[1] as the movement of a neutral particle due to the interaction between its induced or permanent dipole and a non-uniform electric field. DEP can be very useful for inducing the translational motion of biologically important particles in fluid. For example, DEP can be used for filtering or concentrating target entities,[2] as well as aligning and patterning the target entities into predefined shapes.[3–5] Moreover, since DEP is affected by the AC frequency and the material properties of the target entities, DEP can be used to separate target particles from the mixture of similar particles.[6,7] As a result, DEP is becoming a versatile tool for lab-on-chip systems where particle manipulation in liquid environments might be required.

The implementation of DEP requires patterning of conductive electrodes for the application of the electric fields, and preferably a layer of insulator on the electrodes to prevent electro-thermal induced reactions at the electrode interface. In addition, depending on some applications, the electrodes need to be integrated within a microfluidic channel.[2] These requirements can lead to a microfabrication process that involves lithography, metal deposition, metal lift-off,[8] and dielectric deposition. Although there have been many studies for the rapid fabrication of microfluidic channels,[9–11] only a few approaches were successful in demonstrating the rapid prototyping of electrodes.[12–14] The microfabrication process necessary for the conventional DEP implementation increases the total fabrication cost and makes the lab-on-chip system with DEP less affordable for the cost-sensitive lab-on-chip and various biological applications.[15] Especially, the use of DEP with any standard cell culture dishes would be very attractive for realizing cell arrays and cell patterns for various biological applications. Here we present the use of a printed circuit board (PCB), widely used in electronic industries for mechanically holding and electrically connecting many components on a single board, for implementing DEP on thin glass coverslips, which can be used as cell culture substrates. Currently, PCB can be fabricated with 75 μm line/space resolution of electrodes, which is sufficient for many lab-on-chip applications.[16–18] PCB industries can also provide quick prototyping as well as cost-effective mass-production of the final devices. For these reasons, many researchers have investigated PCB technologies as an alternative platform for the lab-on-chip system.[19–22]

In this study, we demonstrate a simple approach for particle manipulation with negative and positive DEP using electrodes fabricated on a PCB that can be reused. Conventional methods for DEP-based particle manipulation, as shown in Fig. 1(a), not only require microfabrication process, but also the fabricated electrodes cannot be reused without thorough and complicated cleaning process. Fig. 1(b) shows our approach for a reusable platform for DEP particle manipulation. A microfluidic channel composed of a microscope coverslip and a patterned PDMS slab is placed on the PCB obtained from a commercial vendor. Then,

[a]*School of Electrical and Computer Engineering, Purdue University, West Lafayette, IN, 47907, USA*
[b]*Micro and Nanotechnology Laboratory, University of Illinois at Urbana-Champaign, Urbana, IL, 61801, USA. E-mail: rbashir@illinois.edu*
[c]*Department of Electrical and Computer Engineering, University of Illinois at Urbana-Champaign, Urbana, IL, USA*
[d]*Center for Cancer Nanotechnology Excellence, Stanford University School of Medicine, Stanford, CA, USA*
[e]*Department of Bioengineering, University of Illinois at Urbana-Champaign, Urbana, IL, USA*

† Electronic supplementary information (ESI) available: Movies of negative DEP and positive DEP. See DOI: 10.1039/b904328d

‡ Kidong Park and Ho-Jun Suk contributed equally to this work.

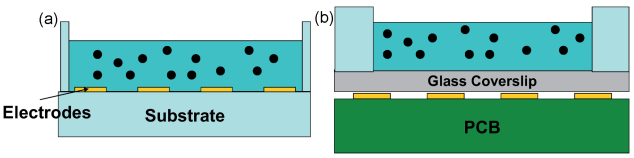


Fig. 1 (a) A schematic diagram of the conventional approach in implementing DEP. Electrodes and substrate should be disposed of after each experiment. (b) A schematic diagram of the proposed approach for DEP implementation. DEP electrodes are easily fabricated on a PCB and can be reused again and again since the electrodes are not in direct contact with the sample.

a non-uniform electric field generated by the PCB electrodes is applied to the fluid sample through the thin glass covership. In a previous work of Burt *et al.*,[23] a PCB was used to induce dielectrophoresis on particles to characterize their dielectric and surface charge properties. However, the PCB was in direct contact with the fluid samples, which leads to the possibility of the cross-contamination when the apparatus is reused. In our approach, a disposable thin glass coverslip isolates a PCB from the sample and makes the PCB reusable by avoiding any possible cross-contamination. Our approach can be ideal for cost sensitive lab-on-chip or cell-patterning applications, where particle manipulation with dielectrophoresis is desired and the cross-contamination should be minimized. We apply this approach to demonstrate the manipulation of polystyrene beads and HeLa cells with negative and positive dielectrophoresis, respectively.

Material and methods

Device fabrication and experimental setup

The electrode patterns were generated by a commercial IC layout software (IC station, Mentor graphics) and then converted into a gerber file, which is an industry standard file format for PCBs. To create a non-uniform electric field for DEP-based particle manipulation, an interdigitated electrode pattern shown in Fig. 2(a) was adopted. Various combinations of the width and the spacing of the interdigitated electrodes were analyzed using numerical analysis software (ANSYS, ANSYS, Inc.) as shown in Fig. 2(b). In this experiment, the interdigitated electrodes with 152 μm width/spacing and 229 μm width/spacing were chosen. The custom-made PCB (eCircuits Solutions Inc, USA) had a 1.6 mm thick FR-4 material as the insulating substrate and a 38 μm thick metal layer on each side patterned as the electrodes.

The DEP particle manipulation with the PCB electrodes was demonstrated with a microfluidic channel configuration and an open well configuration. For the microfluidic channel configuration, a 3 mm thick PDMS slab was casted on the master mold with 50 μm-high wedges for forming the microfluidic channel and then holes were punched for tubing connections. The PDMS slab was attached to a 100 μm-thick microscope glass coverslip (Ted Pella, Inc, USA) to form a complete microfluidic channel. For the open well configuration, a hole with 1.5 cm diameter was punched on a flat PDMS slab, which was then attached to the glass coverslip to form an open well. For proper electrical coupling between the PDMS/coverslip assembly and the electrodes, the coverslip and the electrodes should be in direct contact with each other. For this reason, a droplet of DI water was placed on top of the PCB electrodes. For both the microfluidic channel configuration and the open well configuration, the PDMS/coverslip assembly was then placed directly on the electrodes and was gently pushed down to bring the coverslip in a direct contact with the PCB electrodes. The surface tension of the DI water between the electrodes and the coverslip kept them in contact with each other during the experiment. After the experiment, the PDMS and glass coverslip assembly could be easily detached from the PCB and the PCB electrodes were readily reusable with the next microfluidic device.

Experimental setup and sample preparation

To apply AC voltages to the electrodes on the PCB, the top array of electrodes shown in Fig. 2(a) was connected to a set of function generator (33120A, Agilent) and RF power amplifier (2100L, EIN) that generated 76 V peak-to-peak at a frequency of 1 MHz. The bottom array of electrodes shown in Fig. 2(a) was connected to another set of function generator and RF power amplifier that generated the AC signal with the same peak-to-peak voltage and frequency as the signal applied to the top array, but 180 degrees out of phase. The AC signals were observed by an oscilloscope (TDS3012, Tektronix Inc.). Particle movements and patterns were monitored using optical microscopes.

HeLa cells were maintained and cultured in 25 cm^2 tissue culture flasks with 6 mL of Dulbecco's Modified Eagle Medium (DMEM, Sigma-Aldrich) supplemented with 10% fetal bovine serum (FBS, Sigma-Aldrich). The flasks were incubated at 37 °C with 5% CO_2 for no more than 3 days. After 3 days of incubation, cells reached confluence and were sub-cultured into new flasks. Before the DEP experiments, HeLa cells from sub-confluent flasks were trypsinized and centrifuged to form a pellet. The pellet was then rinsed three times in low conductivity medium (deionized water with 0.3% d-glucose, 8.5% sucrose) with a conductivity of 2 μS/cm. The low conductivity medium increases the efficiency of DEP while keeping the cells alive during the DEP manipulation.[24] The cells were finally suspended in the low conductivity medium by breaking the pellet. Similarly, polystyrene beads with a diameter of 3 μm were rinsed twice with DI water and centrifuged to form a pellet. Then, the beads were suspended in DI water at a target concentration.

Results and discussion

Electric field distribution from numerical analysis

The distribution of the electric field generated by the PCB electrodes was numerically analyzed as shown in Fig. 2(b). This numerical analysis assumed 'quasi static condition', which is valid from DC to about 300 MHz. By applying varying electrical potential with same magnitude but opposite polarities to the adjacent PCB electrodes, a non-uniform electric field was generated. From Fig. 2(b), it can be seen that non-zero electric potential develops above the glass coverslip and consequently creates the non-uniform electric field in the medium above the glass coverslip. The square of the electric field intensity at 10 μm above the glass coverslip has the maximum between the electrodes and the minimum directly above the electrodes. Since

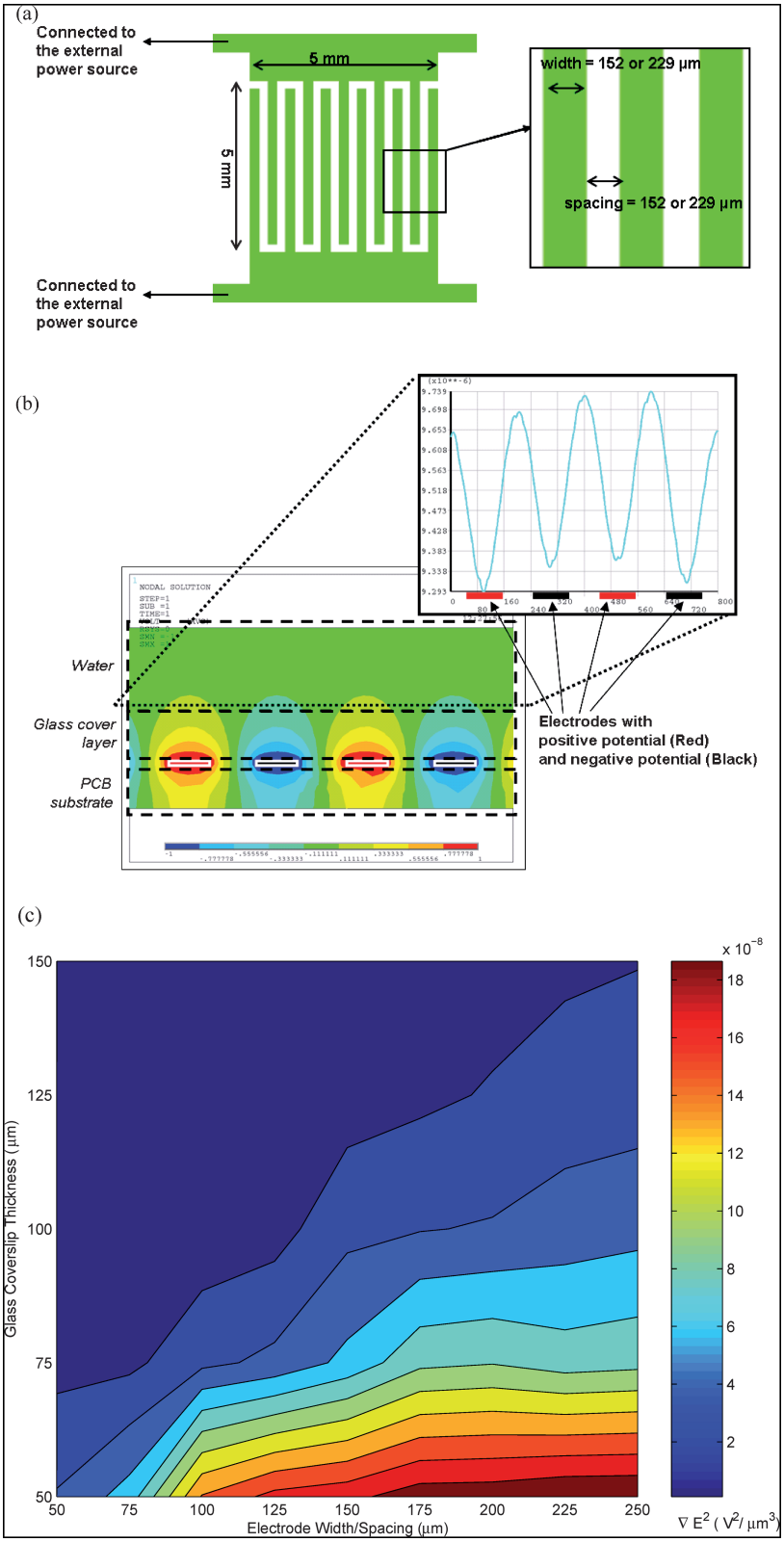


Fig. 2 (a) Layout of the PCB electrodes, (b) Numerical analysis of the electric potential generated by the PCB electrodes. Inset: Square of the electric field magnitude on the dotted line 10 μm above the glass cover layer. Black and red bar shows the position of the electrodes. The electric field intensity is weakest at the center of the electrode and strongest between the electrodes. (c) Numerical simulation of the maximum value of ∇E^2 with a range of glass coverslip thickness and electrode width/spacing. Each set of electrodes were excited with ±1 V.

HeLa cells experience positive DEP under non-uniform electric field, the cells that are near the glass coverslip will move to the high electric field regions between the adjacent electrodes. If the particles are less polarizable than the surrounding medium (*e.g.* polystyrene beads), and are near the glass coverslip, negative DEP will cause them to migrate and settle directly above the electrodes.

DEP force is closely related to the applied voltages and the geometric parameters, such as the thickness of the coverslip and the width/spacing of the electrodes. Fig. 2(c) shows the maximum $\nabla E^2$with a range of the coverslip thickness and the electrode width/spacing. As can be seen in the plot, the DEP force, which is proportional to ∇E^2, is decreasing with thicker coverslip and narrower electrodes. As a result, in order to improve the spatial resolution of patterning by making the electrode spacing smaller while keeping the magnitude of DEP force constant, the thickness of the coverslip should be decreased. In this work, commercially available 100 μm thick coverslip was used with 152 μm width/spacing and 229 μm width/spacing interdigitated electrodes.

Manipulation of polystyrene beads with negative DEP

Particle manipulation with negative DEP was demonstrated with a 3 μm polystyrene bead suspension. Fig. 3(a) shows the progression of the polystyrene bead manipulation with negative DEP. A bead suspension with a concentration of 7×10^7 particles/ml was introduced into a 50 μm high microfluidic channel placed on top of the electrodes and 76 V peak-to-peak, 1 MHz signal was applied to the electrodes. After about 60 seconds, most of the beads were aligned above the center of the electrode, where the electric field intensity was the weakest.

The DEP force applied on the polystyrene bead can be calculated from the velocity of the bead. Since the inertia of the beads is much smaller than the hydrodynamic drag force, the hydrodynamic drag force can be assumed to be in the opposite direction, but have the equal magnitude of the DEP force. The hydrodynamic drag force, F_{drag}, exerted on a spherical particle of radius R moving at velocity v in a medium of viscosity η is given by

$$F_{drag} = 6\pi\eta Rv \quad (1)$$

The beads moved at about 3.45 ± 0.88 μm/s in average and the viscosity of the DI water is 1 mPa s. As a result, the hydrodynamic drag force and the DEP force exerted on the polystyrene beads using the 76 V peak-to-peak, 1 MHz signals was about 0.0975 pN.

Fig. 3(b) shows the DEP manipulation of the polystyrene beads at a higher concentration, 10^9 particles/ml. Due to the high concentration of beads, most of the beads formed the pearl chains while moving to the center of the electrode. Also, the speed of the long pearl chain was higher than that of the individual beads, due to increased total DEP force and the same hydrodynamic drag force.

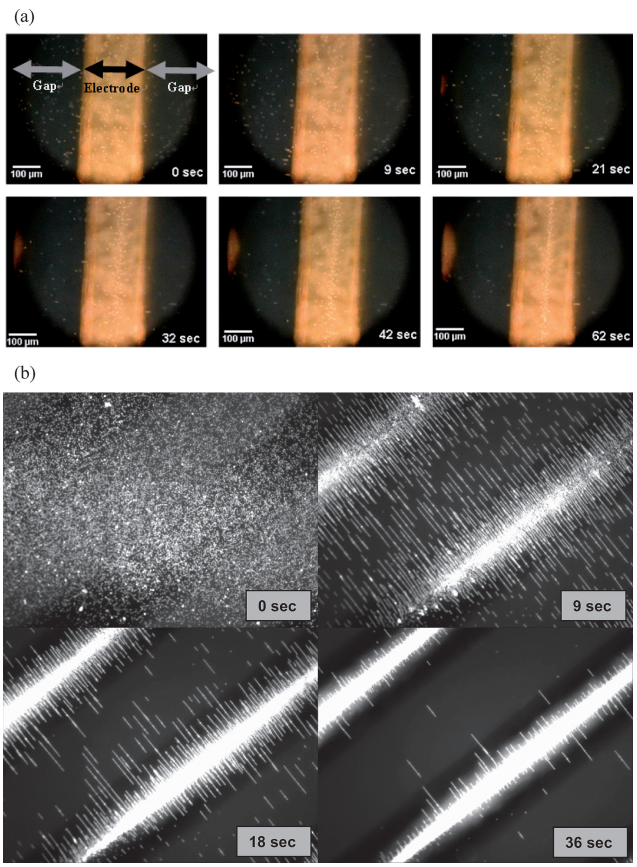


Fig. 3 3 μm polystyrene beads were loaded into 50 μm-high PDMS microfluidic channel, placed on the PCB. (a) With a concentration of 7×10^7 particles/ml, polystyrene beads were aligned in a single line in 60 seconds due to negative DEP. (b) With a higher concentration (10^9 particles/ml), more pearl-chains were formed.

Manipulation of HeLa cells with positive DEP

HeLa cells in the low conductivity media were patterned with positive DEP. Fig. 4(a) shows the aligned HeLa cells in the microfluidic channel. Since the Clausius-Mosotti factor[25] is a function of dielectric constants and conductivities of the medium and the particle, the cells should be thoroughly rinsed twice with the low conductivity media to keep the final conductivity of the media low enough for the positive DEP. The manipulated cells showed the formation of the pearl chains parallel to the electric fields and were located between the electrodes where the electric field intensity is the strongest. The DEP force exerted on the HeLa cells was calculated by the same approach used for the polystyrene beads. The radius of the

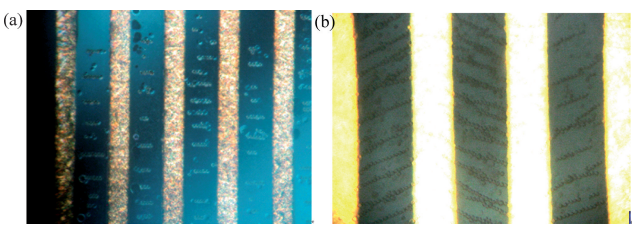


Fig. 4 HeLa cells were loaded into 50 μm-high PDMS microfluidic channel and 1.5 cm-diameter open well. (a) After applying AC voltages for 5 sec, cells in the microfluidic channel were aligned in horizontal lines between the electrodes. (b) Cells in the open well were aligned in slanted lines between the electrodes after applying AC voltages for 30 sec.

HeLa cells was assumed to be 10 μm and the low-conductivity medium viscosity of 1.274 mPa·s was used.[26] The cells in the microfluidic channel moved to the high electric field regions at about 17.35 ± 5.92 μm/s, leading to the DEP force of 4.17 pN, with 80 V peak-to-peak, 1 MHz signal. Fig. 4(b) shows the aligned HeLa cells in the open well, where the slanted pearl chains can be observed. The electric field generated by the interdigitated electrodes is strong, short-ranged and horizontal in Fig. 2(a). On the contrary, the electric field generated by the upper and lower part of the pattern for connecting the power source in Fig. 2(a), is relatively weak, long-ranged and vertical. Therefore, with the combination of these two forces, the total electric field is in a vertical direction where it's far from the PCB surface and becomes slanted and then horizontal as it gets close to the PCB. In the open well configuration, the cells precipitates slowly while experiencing DEP, and the pearl chains formed during the precipitation can be slanted due to the changing direction of the electric field.

The viability of the HeLa cells after the DEP manipulation was investigated by allowing the manipulated cells to grow in a growth medium inside a CO_2 incubator at 37 °C with 5% CO_2. Fig. 4(b) shows the HeLa cells patterned with positive DEP on a glass coverslip. The cells were located between the electrodes and the pearl chains were slanted. To enhance the attachment of the cells on the glass surface after turning off the DEP signals, the glass coverslip was coated with fibronectin before introducing the cell suspension. Fig. 5(a) shows the patterned cells in the open well after replacing the low conductivity medium with the growth medium. Fig. 5(b) shows that the cells are alive and proliferating on the fibronectin coated glass coverslip and can subsequently be used for various applications. The demonstrated technique can also be used for patterning and attachment of multiple cell types on the same substrate by repeating the patterning and growth steps.

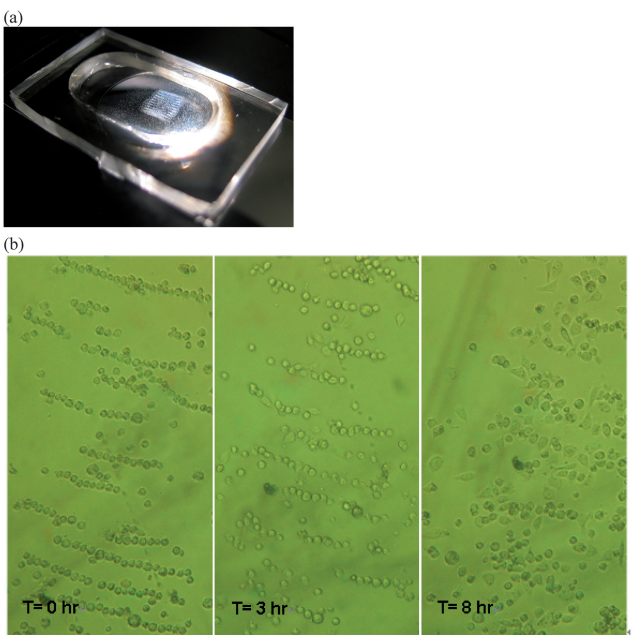


Fig. 5 (a) A PDMS open well after patterning HeLa cells with positive DEP. The size of the center rectangle patterned with cell is about 1.5 cm. (b) Status of the cells patterned on the fibronectin coated glass coverslip. Cells were attached to the surface and subsequently grew after 8 hours.

Conclusion

DEP-based particle manipulation can be a versatile and useful tool for microscale cell patterning and lab-on-chip applications. In this paper, we presented an alternative approach for DEP with reusable electrodes on a printed circuit board and a simple microfluidic channel to manipulate polystyrene beads and HeLa cells with negative and positive DEP, respectively. The use of PCB electrodes and coverslip enables convenient and quick prototyping of DEP platform, and provides a cost-effective alternative method for mass-production. The dimensional resolution of patterning is limited by the thickness of the glass coverslip. However, with a 100 μm glass coverslip 159 μm and 229 μm width/spacing electrodes can successfully manipulate 3 μm polystyrene beads and HeLa cells, respectively. This approach can provide a convenient and affordable method to prototype and develop a lab-on-chip system based on DEP particle manipulation

Acknowledgements

This work was supported by the National Science Foundation under Grant No. EEC-0425626 (NSF NSEC at OSU) which supported Kidong Park. Ho-Jun Suk was an undergraduate student at Cornell University and Summer Research Intern at Purdue University when the work was partially performed. Demir Akin was supported by NIH/NIBIB Grant R21/R33EB00778-01.

References

1 H. A. Pohl, *Journal of Applied Physics*, 1951, **22**, 869–871.
2 L. Yang, P. Banada, M. R. Chatni, K. L, M. Ladisch, A. Bhunia and R. Bashir, *Lab on a chip*, 2006, **6**, 896–905.
3 C.-T. Ho, R.-Z. Lin, W.-Y. Chang, H.-Y. Chang and C.-H. Liu, *Lab on a chip*, 2006, **6**, 724–734.
4 N. Mittal, A. Rosenthal and J. Voldman, *Lab on a chip*, 2007, **7**, 1146–1153.
5 M. Suzuki, T. Yasukawa, H. Shiku and T. Matsue, *Biosensors and bioelectronics*, 2008, **24**, 1043–1047.
6 G. H. Markx, M. S. Talary and R. Pethig, *Journal of Biotechnology*, 1994, **32**, 29–37.
7 I. Doh and Y. H. Cho, *Sensors and Actuators A*, 2005, **121**, 59–65.
8 M. Veiseh, Y. Zhang, K. Hinkley and M. Zhang, *Biomedical Microdevices*, 2001, **3**, 45–51.
9 G. V. Kaigala, S. Ho, R. Penterman and C. J. Backhouse, *Lab on a chip*, 2007, **7**, 384–387.
10 J. C. McDonald, M. L. Chabinyc, S. J. Metallo, J. R. Anderson, A. D. Stroock and G. M. Whitesides, *Analytical chemistry*, 2002, **74**, 1537–1545.
11 A. Tan, K. Rodgers, J. Murrihy, C. O'Mathuna and J. D. Glennon, *Lab on a chip*, 2001, **1**, 7–9.
12 R. W. Stark, M. S. Stalder and A. Stemmer, *Microelectronic Engineering*, 2003, **67-8**, 229–236.
13 D. Haefliger, B. P. Cahill and A. Stemmer, *Microelectronic Engineering*, 2003, **67-8**, 473–478.
14 M. Abdelgawad and A. R. Wheeler, *Advanced Materials*, 2007, **19**, 133.
15 X. Cheng, D. Irimia, M. Dixon, J. C. Ziperstein, U. Demirci, L. Zamir, R. G. Tompkins, M. Toner and W. R. Rodriguez, *Journal of acquired immune deficiency syndromes (1999)*, 2007, **45**, 257–261.

16 S. N. Bhatia, M. L. Yarmush and M. Toner, *J. Biomed. Mater. Res.*, 1997, **34**, 189–199.
17 C. Xu, Y. Wang, M. Cao and Z. Lu, *Electrophoresis*, 1999, **20**, 1829–1831.
18 N. N. Mishra, S. Retterer, T. J. Zieziulewicz, M. Isaacson, D. Szarowski, D. E. Mousseau, D. A. Lawrence and J. N. Turner, *Biosensors and Bioelectronics*, 2005, **21**, 696–704.
19 A. Wego, S. Richter and L. Pagel, *Journal of Micromechanics and Microengineering*, 2001, **11**, 528–531.
20 I. Moser, G. Jobst, P. Svasek, M. Varahram and G. Urban, *Sensors and Actuators B-Chemical*, 1997, **44**, 377–380.
21 N. T. Nguyen and X. Y. Huang, *Sensors and Actuators A-Physical*, 2001, **88**, 104–111.
22 J. Gong and C. Kim, in *Proc. of the International conference on Micro Electro Mechanical systems*, IEEE, Los Alamitos, CA, 2005.
23 J. P. H. Burt, T. A. K. Alameen and R. Pethig, *Journal of Physics E-Scientific Instruments*, 1989, **22**, 952–957.
24 X. Cheng, Y. S. Liu, D. Irimia, U. Demirci, L. Yang, L. Zamir, W. R. Rodriguez, M. Toner and R. Bashir, *Lab on a chip*, 2007, **7**, 746–755.
25 N. G. Green and H. Morgan, *Journal of Physics D: Applied Physics*, 1998, **31**, L25–L30.
26 D. R. Lide, ed., *CRC Handbook of Chemisty and Physics*, CRC Press, Cleveland, Ohio, 2007.