BioChip J. (2014) 8(2): 67-74
DOI 10.1007/s13206-014-8201-4

Original Article

Isolating and Concentrating Rare Cancerous Cells in Large Sample Volumes of Blood by Using Dielectrophoresis and Stepping Electric Fields

Guang-Hong Chen[1], Ching-Te Huang[1], Hsin-Hui Wu[1], Tatyana N. Zamay[2,3], Anna S. Zamay[2,3] & Chun-Ping Jen[1,*]

Received: 27 January 2014 / Accepted: 21 March 2014 / Published online: 20 June 2014

Abstract Detecting rare cells, such as circulating tumor cells (CTCs), circulating fetal cells, and stem cells, is vital during medical diagnostics and characterization. During carcinogenesis, cancer cells detach from the primary tumor into the blood stream, becoming CTCs. Typical rare cell samples are considered any sample that contains less than 1000 target cells per milliliter. The volumes of microfluidic devices typically range from several microliters to nanoliters; this is excessively small for experimenting using low-concentration samples. This study involved isolating cancerous cells in an open-top chamber with sub-milliliter volumes (0.1 mL) of blood samples by using a lysis buffer solution for red blood cells (RBCs), as well as concentrating cells employing the dielectrophoretic force generated using stepping electric fields, which were produced using a handheld electric module that comprised a voltage-frequency converter and an operational amplifier. To increase the sample volume, an open-top chamber was fabricated on and bonded to a glass substrate by using circular microelectrodes. The concentrations of cancer cells and RBCs were adjusted to 500 cells/mL and 4×10^5 cell/mL, respectively, for the experiments. To reduce the interference of blood cells during detection and isolate CTCs, the RBCs in the sample were lysed in a lysis buffer solution before the proposed chip was used to dielectrophoretically manipulate the rare cancerous cells. The findings indicated that the lysis buffer lysed the erythrocytes and the survivability levels of the cancerous cells (HeLa and MCF-7) remained high in the lysis buffer. The positive dielectrophoretic cancerous cells were guided based on the direction of the stepping electric field because of movement in the high-electric-field region; hence, the cancerous cells concentrated and collected at the central electrode.

Keywords: Dielectrophoresis (DEP), Circulating tumor cells (CTCs), Concentration, Isolation, Stepping electric field

Introduction

Oncological diseases are the second leading cause of death after cardiovascular diseases. During carcinogenesis, cancer cells detach from the primary tumor into the blood stream, becoming circulating tumor cells (CTCs) that can serve as early predictors of the metastatic process[1]. In addition to traditional cancer diagnostic methods, detecting the CTC levels in blood facilitates tumor growth prognoses and treatment monitoring. Despite the substantial diagnostic and prognostic potential of CTCs, such cells are rarely measured in clinical practice because of the challenges associated with CTC isolation and identification. Manipulating biological cells is essential in various biomedical applications, including the isolation and detection of rare CTCs, the concentration of cells from dilute suspensions, the separation of cells according

[1]Department of Mechanical Engineering and Advanced Institute of Manufacturing with High-tech Innovations, National Chung Cheng University, Chia Yi, Taiwan, R.O.C.
[2]Department of Biological Chemistry, Krasnoyarsk State Medical University, Krasnoyarsk, Russia
[3]Small innovative enterprise "MedBioTeh", Krasnoyarsk, Russia
*Correspondence and requests for materials should be addressed to C.-P. Jen (✉imecpj@ccu.edu.tw)

to specific properties, or the trapping or positioning of individual cells for characterization[2-4]. Numerous methods have been proposed for concentrating biological cells[2,5] such as immunoaffinity, filtration (isolation by the size of epithelial tumor cells)[6], fluorescence-activated cell sorting (FACS), magnetic-activated cell sorting[7], cell surface markers, and dielectrophoresis (DEP)[8]. The methods for enriching CTCs in clinical practice have been divided into size-based and immunomagnetic approaches, the advantages and disadvantages of which have been addressed in the literature[9]. Fu *et al.*[10] developed a microfabricated fluorescence-activated cell sorting (μFACS) device for recovering *Escherichia coli* HB101 cells, which were electrokinetically manipulated in a T-shaped fluidic microchannel comprising valves and pumps. Han *et al.*[11] used the continuous paramagnetic capture mode of a magnetophoretic microseparator to isolate breast cancer cells from peripheral blood by using a 0.2 T external magnetic flux. The micro-electrical impedance spectroscopy (μ-EIS) system was positioned downstream of the channel to measure the electrical impedances of various pathological stages (MCF-7, MDA-MB-231, and MDA-MB-435) of breast cancer cells as compared with a normal human breast cell line (MCF-10A). In an alternate study, biotinylated heat-shock protein 60 was applied as a receptor for viable *Listeria monocytogenes* in a microfluidic device[12] interdigitated electrodes were adopted to generate the dielectrophoretic force and enhance the capture rate.

DEP is achieved when a non-uniform electric field is generated by various electrode patterns. The direction of the DEP force is determined by the dielectric properties of the cells and medium, which are functions of frequency. DEP has been widely applied to manipulate DNA[13], bacteria[14], and cells[15,16] by using diverse microdevices. The microelectrode patterns used for DEP and their applications have been previously reviewed[17,18]. Furthermore, DEP is suitable for cell discrimination and isolation. Alazzam *et al.*[19] proposed an interdigitated comb-like electrode that allowed continuous CTCs separation by using DEP; the MDA231breast cancer cells appeared to generate a positive DEP response, whereas other blood cells exhibited negative DEP responses at a frequency of 25 kHz in a medium of 10 mS/m. In the current study, CTCs were separated from blood by using various DEP responses and precise counting. Becker *et al.*[20] used electro-rotation to measure the varying dielectric properties between the metastatic human breast cancer cell line MDA231 and RBCs, separating MDA231 from RBCs by combining dielectric differences and hydrodynamic force. The DEP capture voltage spectrum comprises dielectrophoretic and drag forces; it was previously employed to measure the dielectric properties of and separate HT-29 and human colon cancer cell lines from RBCs[21]. The dielectric properties of HT-29 cells are measured using the dielectrophoretic capture voltage spectrum at various media conductivities to obtain the operating conditions that allow separating HT-29 cells from RBCs. DEP field-flow fractionation (DEP-FFF) has been applied to isolate CTCs from clinical blood specimens by using simulated cell mixtures of cultured tumor cells and peripheral blood[22]. MDA-435, MDA-468, and MDA-231 cells were previously isolated from blood samples by exploiting the morphological characteristics of cells rather than using labeling procedures. Based on their varying dielectric properties, HeLa cells were previously isolated from normal peripheral blood cells on a silicon chip that comprised a 5×5 array of microlocations[23]. Moon *et al.*[24] proposed microfluidic cell separators comprising serially integrated multi-orifice flow fractionation (MOFF) and dielectrophoresis, facilitating the high-speed continuous separation of human breast cancer cells from a blood sample; the cells were first separated based on their size in the MOFF channel and subsequently based on their dielectrophoretic properties in the DEP channel.

Typical rare cells samples are considered any sample that contains less than 1000 target cells per milliliter[5]. The volumes of the microfluidic devices typically range from several microliters to nanoliters[25]; this is excessively small for experimenting using low-concentration samples. Therefore, various methods of increasing the device volume of are proposed herein. This study involved isolating cancerous cells in an open-top chamber at sub-milliliter sample volumes (0.1 mL) by using a lysis buffer solution for red blood cells (RBCs), as well as concentrating cells adopting the dielectrophoretic force generated by stepping electric fields, which were produced by a handheld electric module developed by the authors in a previous study, comprising a voltage-frequency converter and an operational amplifier[26]. Furthermore, to reduce the interference of blood cells during detection and isolate CTCs, the blood cells in the sample were lysed in a lysis buffer solution before using the proposed chip to assess the concentrations of rare cancerous cells.

Results and Discussion

The survival rates of cancerous cells in the RBCs lysis buffer were investigated before dielectrophoretic manipulation. Figure 1 shows the survival rates of the

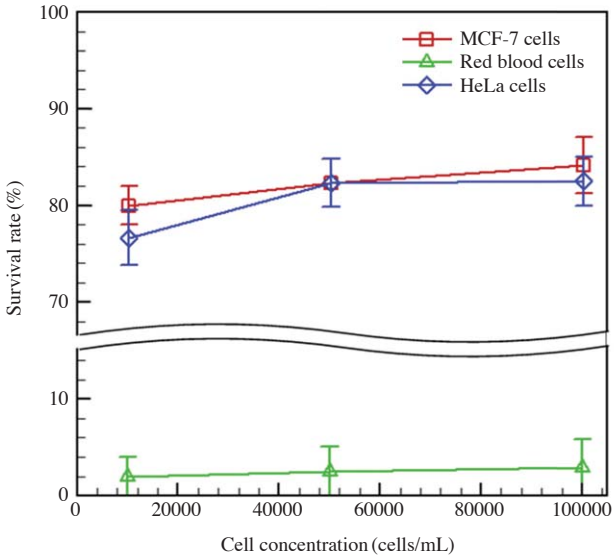

Figure 1. Survival rates of two cancerous cells (HeLa and MCF-7) and erythrocytes with different concentrations after suspended in the lysis buffer for one hour. Each data point represents the average value of the survival rate and the error bar depicts the standard error from the mean.

HeLa and MCF-7 cells and erythrocytes of various concentrations after 1 h of suspension in the lysis buffer. The RBCs survival rates were less than 10% for all concentrations ranging from 10^4 to 10^5 cell/mL; however, HeLa and MCF-7 cell survival rates were approximately 76%-80% and 80%-83%, respectively, indicating that the lysis buffer successfully lysed the erythrocytes, facilitating a high survivability rate after 1 h of lysing. To demonstrate the concentration of rare cancerous cells in subsequent experiments, the cancer cell and RBCs concentrations were adjusted to 500 cells/mL and 4×10^5 cells/mL, respectively.

The rare cancerous cell and RBCs sample mixtures were suspended in the lysis buffer solution that exhibited a conductivity of 10.66 μS/cm. A sample of the cells mixture (approximately 0.1 mL) was dropped into the open-top chamber. The proposed chip remained stationary for 30 min, forcing the cancerous HeLa cells and erythrocytes to precipitate on the substrate surface (Figure 2). After 30 min of suspension in the lysis buffer solution, fluorescent images were captured to demonstrate the viability of the calcein-AM-stained cells. Figure 2 (left side) shows optical microscopy images of the unstained erythrocytes being lysed; thus, cancerous cells were isolated from RBCs. A handheld electric module was then employed to concentrate the rare HeLa cells. In our previous work[27], HeLa cells can be concentrated successfully when a peak-to-peak voltage of 10 V was applied to the electrodes at a frequency of 1 MHz. Based on the previously described protoplast model, the viable HeLa cells suspended in the lysis buffer solution (ε_r=78; σ=10.66 μS/cm) exhibited strongly positive dielectrophoretic responses (i.e., the Clausius-Mossotti factor was 1.0 at a frequency of 1 MHz). Initially, HeLa cells distributed randomly on the substrate. When an electric field was applied the adjacent microelectrodes, a high-electric-field region was generated between the electrode pair. The applied electric field was subsequently switched to the adjacent electrode pair by using relays to generate a stepping electric field. The positive DEP cells were guided based on the direction of the stepping electric field because of movement in the high-electric-field region. The stepping electric field from the outmost electrode toward the central electrode was applied to concentrate the cancerous cells. The electric field was maintained for 20 s and then switched to the adjacent pair of electrodes. The HeLa cells were successfully concentrated, collecting at the central electrode (Figure 2, right side). The MCF-7 cells were examined following identical procedures. Because the MCF-7 cell diameter (approximately 17 μm) was larger than the HeLa cell diameter, less voltage was required to concentrate the MCF-7 cells, compared with the HeLa cells. The magnitude of the DEP force acting on MCF-7 cells is about five-fold that on HeLa cells under a given electric field distribution in the buffer medium. Several peak-to-peak voltages were examined to concentrate MCF-7 cells. After determining the optimal levels of the stepping electrical field, a peak-to-peak voltage of 8 V at a frequency of 1 MHz and 20 s of the time interval for relay switching were adopted to concentrate the MCF-7 cells; therefore, more than 80% of cells were collected to the central electrode. The MCF-7 cells were also successfully concentrated using the proposed chip (Figure 3). Repeated experiments yielded five or more identical results, indicating that the HeLa and MCF-7 cells were concentrated at the central electrode at approximate efficiencies of 76%-80% and 80%-83%, respectively. These results indicate that CTCs can be isolated and concentrated at the center electrode from a blood sample, making the proposed device suitable for the subsequent detection and characterization of CTCs. The enrichment of CTCs at the central electrode can improve the sensitivity of the subsequent detection, for example immunoassay.

Conclusions

Manipulating biological cells is vital in numerous biomedical applications, including isolating and detect-

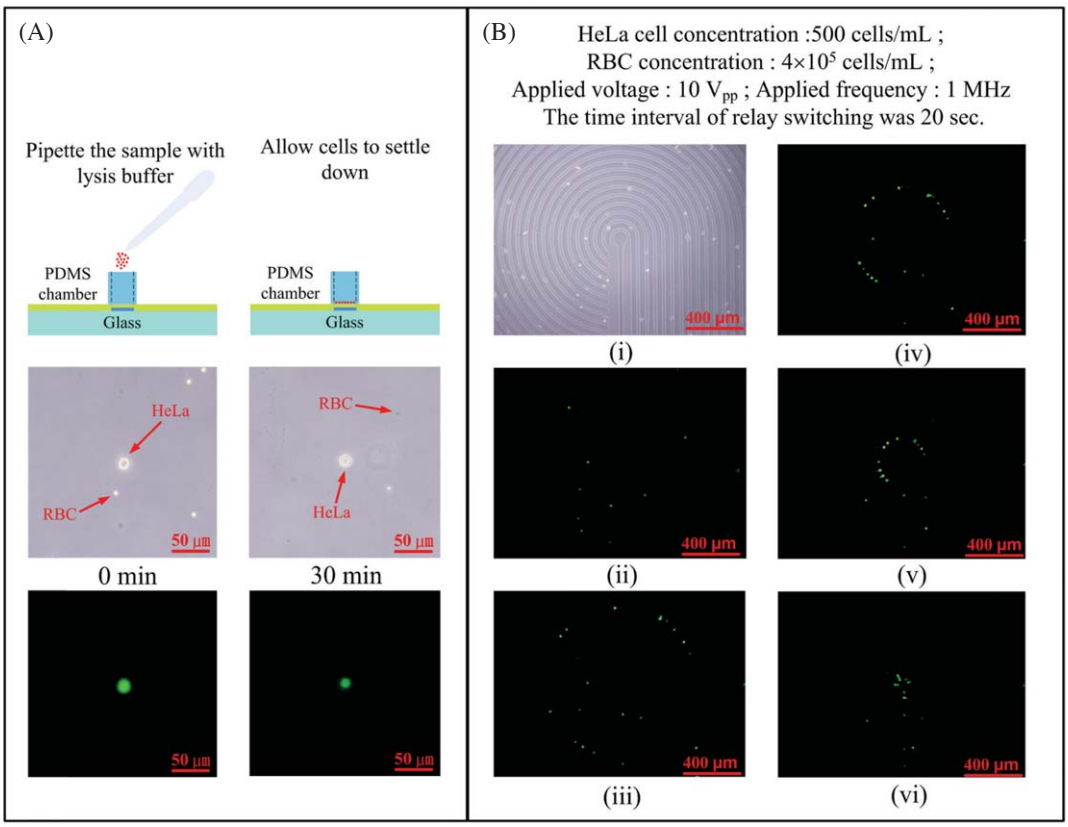

Figure 2. The experimental procedure and results of the isolation of rare HeLa cells from a blood sample. The concentrations of RBCs and HeLa cells in the microchamber were 4×10^5 cells/mL and 500 cells/mL, respectively. The voltage applied and frequency were 10 V_{pp} and 1 MHz, respectively. The time interval of relay switching was 20 seconds.

ing rare CTCs; detecting CTCs in blood facilitates tumor growth prognoses and treatment monitoring. Typical rare cells samples are considered any sample that contains less than 1000 target cells per milliliter. The volumes of microfluidic devices typically range from several microliters to nanoliters; this is excessively small for experimenting using low-concentration samples. To reduce the interference of RBCs during detection and isolate CTCs, the blood cells in the sample were lysed in a lysis buffer solution before the proposed chip was used to concentrate rare cancerous cells. Cancerous cells (HeLa and MCF-5) were isolated and concentrated in the open-top chamber at submilliliter sample volumes (0.1 mL) by adopting a lysis buffer solution for RBCs and using the dielectrophoretic force produced by stepping electric fields, which were generated by a handheld electric module that comprised a voltage-frequency converter and an operational amplifier. The results indicated that HeLa cells and MCF-7 cells concentrated at the central electrode at approximate efficiencies of 76%-80% and 80%-83%, respectively. The microdevice was proposed to isolate and concentrate cancerous cells, enhancing the sensitivity of CTC detection. Moreover, the proposed module is relatively simple and can be used in point-of-care applications or in the clinical diagnostics, biological assay, and biomedicine fields.

Theory and design

The DEP force (F_{DEP}) exerted on a spherical particle of radius R suspended in a fluid with permittivity ε_m is calculated as follows:

$$F_{DEP}=2\pi R^3 \varepsilon_m \mathrm{Re}(f_{CM})\nabla E_{rms}^2 \quad (1)$$

where $\mathrm{Re}(f_{CM})$ is the real part of the Clausius-Mossotti factor and E_{rms} is the root-mean-square of the external electric field in an AC field. The Clausius-Mossotti factor (f_{CM}) is a parameter of the effective polarizability of a particle; its value varies based on the complex dielectric properties of the particle and the surrounding medium, which are functions of the frequency of the applied field (f). The Clausius-Mossotti factor for a spherical particle is represented as follows:

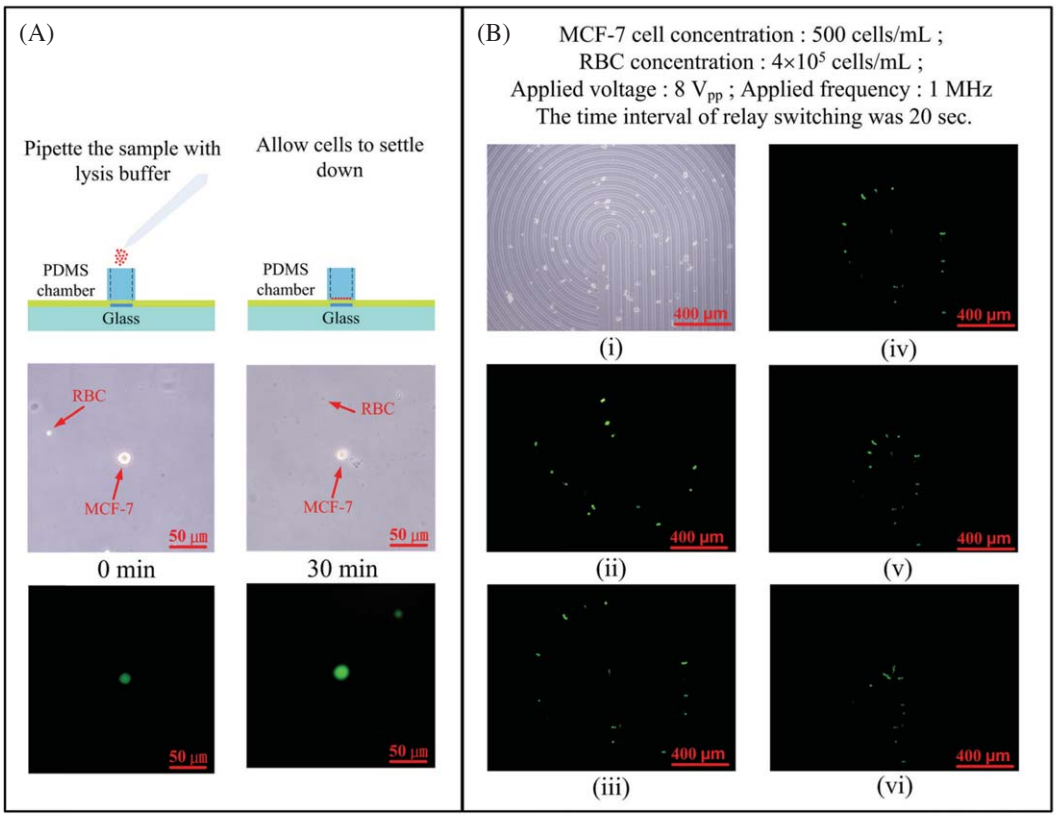

Figure 3. The experimental procedure and results of the isolation of rare MCF-7 cells from a blood sample. The concentrations of RBCs and HeLa cells in the microchamber were 4×10^5 cells/mL and 500 cells/mL, respectively. The voltage applied and frequency were 8 V_{pp} and 1 MHz, respectively. The time interval of relay switching was 20 seconds.

$$f_{CM}=\left[\frac{\varepsilon_p^*-\varepsilon_m^*}{\varepsilon_p^*+2\varepsilon_m^*}\right] \tag{2}$$

where ε_p^* and ε_m^* are the complex permittivities of the particle and the medium, respectively. The complex permittivity relates to the conductivity σ and angular frequency $\omega=2\pi f$ as follows:

$$\varepsilon^* \equiv \varepsilon - j\frac{\sigma}{\omega} \tag{3}$$

where j equals $\sqrt{-1}$. Therefore, the DEP force depends not only on the differing dielectric properties between the particles and the suspension solution, but also on the particle size. The DEP force can be either positive, pulling particles toward regions that exhibit high electric field gradients, or negative, repelling particles from the regions that exhibit high electric field gradients. The dielectric properties of viable mammalian cells can be determined using the protoplast model, which is used for spherical particles that comprise a cytoplasm and a lossless capacitive membrane[28,29]. The effective permittivity can be derived by neglecting the conductance of the membrane in the protoplast model; thus, the Clausius-Mossotti factor for viable cells can be rewritten as follows:

$$f_{CM}(\omega)=-\frac{\omega^2(\tau_m\tau_c^*-\tau_c\tau_m^*)+j\omega(\tau_m^*-\tau_m-\tau_c^*)-1}{\omega^2(2\tau_m\tau_c^*+\tau_c\tau_m^*)-j\omega(\tau_m^*+2\tau_m+\tau_c^*)-2} \tag{4}$$

where $\tau_c^*=c_{mem}R/\sigma_c$ and $\tau_c=\varepsilon_c/\sigma_c$ are time constants, and σ_c and ε_c are the electrical conductivity and permittivity of the cytoplasm, respectively. The parameters c_{mem} and R represent the effective capacitance of the membrane and the radius of the cell, respectively. Moreover, the constants τ_m and τ_m^* can be defined as $\tau_m=\varepsilon_m/\sigma_m$ and $\tau_m^*=c_{mem}R/\sigma_m$, respectively, where σ_m and ε_m are the electrical conductivity and permittivity, respectively, of the suspension medium.

The concept of concentrating cells by using circular electrodes and stepping electric fields was based on a previous investigation conducted by the authors[26]. When an electric field is applied to adjacent microelectrodes, a high-electric-field region is generated between the electrode pair. The applied electric field is

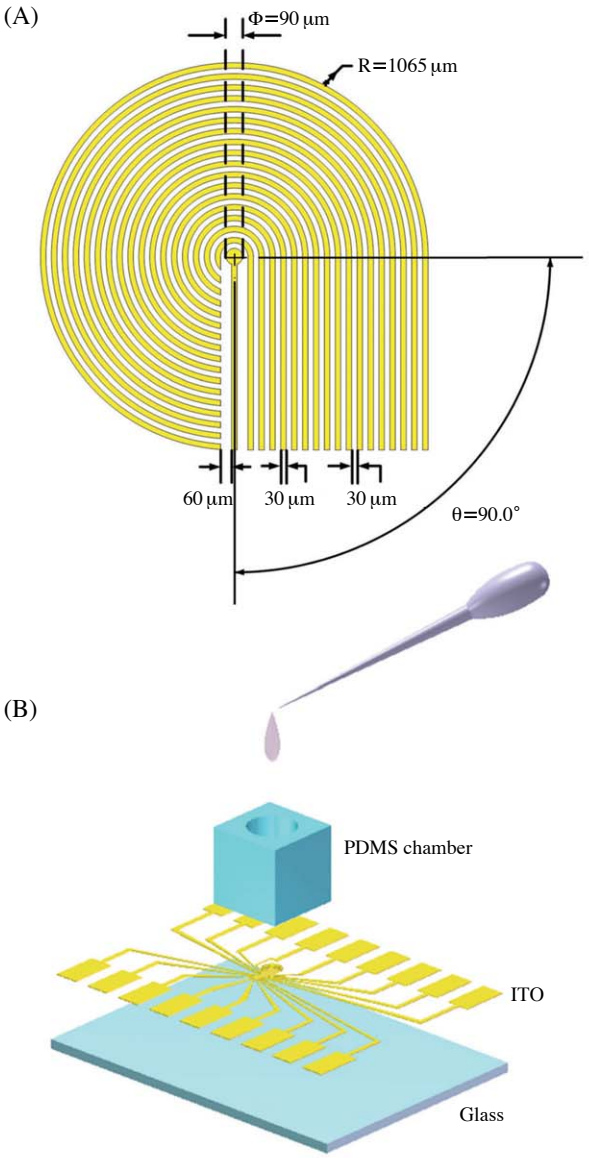

Figure 4. (A) Dimensions of the circular electrodes; and (B) The schematic diagram of the microchip for concentrating cells utilizing dielectrophoresis in stepping electric fields.

subsequently switched to the adjacent electrode pair by using relays, generating a stepping electric field. The positive DEP cells are guided based on the direction of the stepping electric field because of movement in the high-electric-field region; thus, the cancerous cells are concentrated as they move toward the center electrodes. Figure 4A shows a schematic diagram and the dimensions of the circular microelectrode. The width of the electrodes and the gap between them were both 30 μm and the radius of the circular microelectrode curvature was 1065 μm. A lollipop-shaped electrode (circular electrode diameter=90 μm) was employed as the central electrode to collect cells. To increase the sample volume, the open-top chamber was fabricated on and bonded to a glass substrate by using microelectrodes. Figure 4B provides a schematic of the proposed microchip, comprising circular microelectrodes.

Experimental section

Chip fabrication

A biocompatible material, polydimethylsiloxane (PDMS), was adopted to fabricate the open-top chamber in the proposed chip for concentrating rare cancerous cells. The microelectrodes were patterned by etching indium tin oxide (ITO) glass substrates by using HCl solution. The PDMS prepolymer mixture (Sylgard-184 Silicone Elastomer Kit, Dow Corning, Midland, MI, USA) was diluted with hexane, using a PDMS to hexane weight ratio of 1 : 5. The 3-μm-thick hexane-diluted PDMS prepolymer was spin-coated onto the electrodes to prevent electrolysis and cell adhesion. The undiluted PDMS prepolymer mixture was poured into a 10-mm-high culture dish. After the PDMS was cured and peeled off, an open-top chamber (4.5 mm diameter) was fabricated using a puncher. The open-top chamber was subsequently bonded to the ITO glass substrate after 50 s of oxygen plasma treatment in an O_2 plasma cleaner (Model PDC-32G, Harrick Plasma Corp., Ithaca, NY, USA). The chamber volume was approximately 160 μL. Figure 5A shows the microelectrode image captured using an optical microscope and the fabricated microchip.

Apparatus

In the current study, a low-cost and automated handheld electric module was developed, comprising a voltage-frequency converter and an operational amplifier. Two 12-V DC sources served as the power supply for the microfabricated module. An IC MAX 038 (MAXIM, USA) voltage-frequency converter and an AD817 amplifier (Analog Devices, USA) were employed to design an AC signal source that generated the electric fields required to facilitate dielectrophoretic manipulation in the chamber. An 8-bit microcontroller comprising 4 kB of flash memory (AT89C51, Atmel) was adopted to control the eight transistors (DG201CJ) that generated the stepping electric fields for 16 switching steps. The circuit module was fabricated on a printed circuit board. The microfabricated chip was mounted on the electric module, generating the stepping electric field that facilitated cellular concentration (Figure 5B). The cells were observed and recorded using an inverted fluorescence microscope (Model CKX41, Olympus, Tokyo, Japan), a mounted CCD camera (DP71, Olympus, Tokyo, Japan), and Olympus DP controller image software.

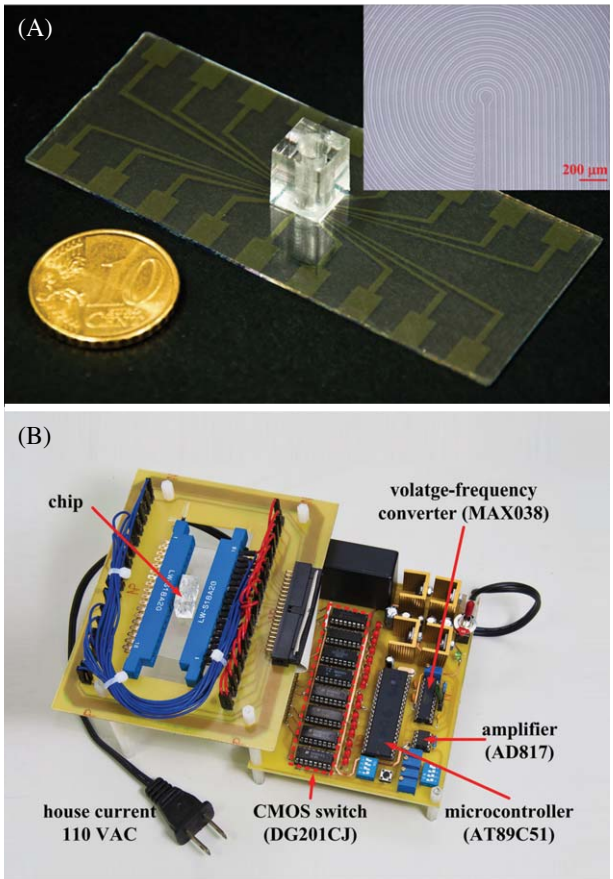

Figure 5. (A) The images of the microelectrodes taken by an optical microscope and the fabricated microchip; and (B) the electric module for providing the stepping electric field.

Cell sample preparation

Studies have shown that tumor cells typically exist in the bone marrow or peripheral blood of patients diagnosed with various carcinomas[30] such as cervical[31] or breast cancer[30]. Human cervical (HeLa cells) and breast (MCF-7) carcinoma cell lines were cultured to isolate rare cancerous cells by using the proposed microfabricated device. The cells were serially passaged as monolayer cultures in Dulbecco's modified eagle's medium (DMEM, Gibco, Grand Island, NY, USA), adding 3.7 g of $NaHCO_3$ per liter of medium, supplemented using 10% fetal bovine serum (Gibco, Grand Island, NY, USA) and 1% penicillin/streptomycin (Gibco, Grand Island, NY, USA). The cell culture dish (Falcon, Franklin Lakes, NJ, USA) was incubated in a humidified atmosphere containing 5% carbon dioxide at 37°C; the medium was replaced every 1 to 2 days. Cells grown to sub-confluence were washed with phosphate-buffered saline (Biochrome, pH 7.4) and harvested following a 5-min treatment of 0.25% trypsin and 0.02% ethylenediaminetetraacetic acid (Sigma, USA). The cells were stained using a standard fluorescence assay of calcein AM (Molecular Probes, Eugene, OR, USA) prior to the experiment. Calcein AM is a green fluorescent dye that penetrates the cell membrane, entering the cytosol and becoming fluorescent when it is hydrolyzed by the esterases located in cells. Peripheral blood was collected from volunteers and diluted in sucrose solution; cancerous cells were subsequently mixed into the blood samples. Erythrocytes were lysed in an RBCs lysis buffer (eBioscience, San Diego, CA, USA)[32] prior to conducting dielectrophoretic concentration experiments. The lysis buffer that exhibited a conductivity of 10.66 μS/cm was used in the subsequent experiments. The cells were sequentially stained with trypan blue dye, which is widely used in cell viability testing. Cell survival was evaluated by counting the number of total and dead cells using a hemocytometer. Multiple fields with at least 250 to 450 cells were counted for each run. The measured cell survival was based on at least three separate experiments. Most of the cells in a blood sample are RBCs and the concentration of white blood cells (WBCs) is about a thousandth of the total cell concentration in the blood[33]. The influence of WBCs is ignored in the present study. Moreover, lysates of RBCs might also affect the further detection of CTCs and the effect should be addressed in the future investigation. It should be noted that the size and shape of WBCs are similar to those of cancer cells; therefore, isolating cancer cells from the WBCs is an important issue in clinical applications.

Acknowledgements This work was supported by the National Science Council of the Republic of China (Taiwan) under grants NSC 102-2221-E-194-001 and by Directorate of State Scientific and Technical Programmes in Russia (grant number 14.512.11.0086).

References

1. Paterlini-Brechot, P. & Benali, N.L. Circulating tumor cells (CTC) detection: Clinical impact and future directions. *Cancer Lett*. **253**, 180-204 (2007).
2. Mostert, B., Sleijfer, S., Foekens, J.A. & Gratama. J.W. Circulating tumor cells (CTCs): Detection methods and their clinical relevance in breast cancer. *Cancer Treat. Rev*. **35**, 463-474 (2009).
3. Cheng, X. *et al*. Enhancing the performance of a point-of-care CD4+ T-cell counting microchip through monocyte depletion for HIV/AIDS diagnostics. *Lab Chip* **9**, 1357-1364 (2009).
4. Yu, M. *et al*. Circulating tumor cells: approaches to isolation and characterization. *J. Cell Biol*. **192**, 373-382 (2011).
5. Dharmasiri, U., Witek, M.A., Adams, A.A. & Soper,

S.A. Microsystems for the capture of low-abundance cells. *Annu. Rev. Anal. Chem.* **3**, 409-431 (2010).
6. Vona, G. *et al.* Isolation by Size of Epithelial Tumor Cells: A New Method for the Immunomorphological and Molecular Characterization of Circulating Tumor Cells. *Am. J. Pathol.* **156**, 57-63 (2000).
7. Jacob, K., Sollier, C. & Jabado, N. Circulating tumor cells: detection, molecular profiling and future prospects. *Expert Rev. Proteomic.* **4**, 741-756 (2007).
8. Hatanaka, H., Yasukawa, T. & Mizutani, F. Detection of Surface Antigens on Living Cells through Incorporation of Immunorecognition into the Distinct Positioning of Cells with Positive and Negative Dielectrophoresis. *Anal. Chem.* **83**, 7207-7212 (2011).
9. Alunni-Fabbroni, M. & Sandri, M.T. Circulating tumour cells in clinical practice: Methods of detection and possible characterization. *Methods* **50**, 289-297 (2010).
10. Fu, A.Y., Chou, H.P., Spence, C., Arnold, F.H. & Quake, S.R. An Integrated Microfabricated Cell Sorter. *Anal. Chem.* **74**, 2451-2457 (2002).
11. Han, K.H., Han, A. & Frazier, A.B. Microsystems for isolation and electrophysiological analysis of breast cancer cells from blood. *Biosens. Bioelectron.* **21**, 1907-1914 (2006).
12. Koo, O.K. *et al.* Targeted Capture of Pathogenic Bacteria Using a Mammalian Cell Receptor Coupled with Dielectrophoresis on a Biochip. *Anal. Chem.* **81**, 3094-3101 (2009).
13. Du, J.R., Juang, Y.J., Wu, J.T. & Wei, H.H. Long-range and superfast trapping of DNA molecules in an ac electrokinetic funnel. *Biomicrofluidics* **2**, 44103 (2008).
14. Koklu, M., Park, S., Pillai, S.D. & Beskok, A. Negative dielectrophoretic capture of bacterial spores in food matrices. *Biomicrofluidics* **4**, 034107 (2010).
15. Yang, F. *et al.* Dielectrophoretic separation of colorectal cancer cells. *Biomicrofluidics* **4**, 013204-013213 (2010).
16. Sancho, M., Martinez, G., Munoz, S., Sebastian, J.L. & Pethig, R. Interaction between cells in dielectrophoresis and electrorotation experiments. *Biomicrofluidics* **4**, 022802-022811 (2010).
17. Pethig, R. Preface to Special Topic: Dielectrophoresis. *Biomicrofluidics* **4**, 022701 (2010).
18. Pethig, R. Review Article-Dielectrophoresis: Status of the theory, technology, and applications. *Biomicrofluidics* **4**, 022811-022835 (2010).
19. Alazzam, A., Stiharu, I., Bhat, R. & Meguerditchian, A.N. Interdigitated comb-like electrodes for continuous separation of malignant cells from blood using dielectrophoresis. *Electrophoresis* **32**, 1327-1336 (2011).
20. Becker, F.F. *et al.* Separation of human breast-cancer cells from blood by differential dielectric affinity. *Proc. Natl. Acad. Sci. USA* **92**, 860-864 (1995).
21. Wu, L., Lanry Yung, L.Y. & Lim, K.M. Dielectrophoretic capture voltage spectrum for measurement of dielectric properties and separation of cancer cells. *Biomicrofluidics* **6**, 14113-1411310 (2012).
22. Gascoyne, P.R.C., Noshari, J., Anderson, T.J. & Becker, F.F. Isolation of rare cells from cell mixtures by dielectrophoresis. *Electrophoresis* **30**, 1388-1398 (2009).
23. Cheng, J., Sheldon, E.L., Wu, L., Heller, M.J. & O' Connell, J.P. Isolation of Cultured Cervical Carcinoma Cells Mixed with Peripheral Blood Cells on a Bioelectronic Chip. *Anal. Chem.* **70**, 2321-2326 (1998).
24. Moon, H.S. *et al.* Continuous separation of breast cancer cells from blood samples using multi-orifice flow fractionation (MOFF) and dielectrophoresis (DEP). *Lab Chip* **11**, 1118-1125 (2011).
25. Paguirigan, A.L. & Beebe, D.J. Microfluidics meet cell biology: bridging the gap by validation and application of microscale techniques for cell biological assays. *Bioessays* **30**, 811-821 (2008).
26. Jen, C.P. & Chang, H.H. A handheld preconcentrator for the rapid collection of cancerous cells using dielectrophoresis generated by circular microelectrodes in stepping electric fields. *Biomicrofluidics* **5**, 034101-034110 (2011).
27. Jen, C.P., Chang, H.H., Huang, C.T. & Chen, K.H. A microfabricated module for isolating cervical carcinoma cells from peripheral blood utilizing dielectrophoresis in stepping electric fields. *Microsystem. Technologies* **18**, 1887-1896 (2012).
28. Jones, T.B. Electromechanics of particles. Cambridge University Press, New York (1995).
29. Jen, C.P. & Chen, T.W. Selective trapping of live and dead mammalian cells using insulator-based dielectrophoresis within open-top microstructures. *Biomedical. Microdevices* **11**, 597-607 (2009).
30. Tong, X., Yang, L., Lang, J.C., Zborowski, M. & Chalmers, J.J. Application of immunomagnetic cell enrichment in combination with RT-PCR for the detection of rare circulating head and neck tumor cells in human peripheral blood. *Cytometry. B Clin. Cytom.* **72**, 310-323 (2007).
31. Mitsuhashi, A. *et al.* Detection of epidermal growth factor receptor mRNA in peripheral blood of cervical cancer patients. *Gynecol. Oncol.* **89**, 480-485 (2003).
32. Chen, W. *et al.* Nanoroughened Surfaces for Efficient Capture of Circulating Tumor Cells without Using Capture Antibodies. *ACS Nano.* **7**, 566-575 (2013).
33. Toner, M. & Irimia, D. Blood-on-a-Chip. *Annu. Rev. Biomed. Eng.* **7**, 77-103 (2005).