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High-throughput dielectrophoretic cell sorting assisted by cell sliding on scalable electrode tracks made of conducting-PDMS

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Highlights

- Ideally straight electrode-tracks made of silver-PDMS (AgPDMS) that assisted smooth sliding of cells under pDEP affinity were developed for continuous-flow DEP-activated cell sorting.
- The electrode-tracks were scalable by mirroring to form V-shaped tracks that enabled bidirectional cell sliding and involve elevated number of flow paths for further enhanced throughput.
- Greatly enhanced throughput with comparably high efficiencies for DEP-activated cell sorting were achieved as compared to the existing devices incorporating conducting-PDMS electrodes, with further simplified replica molding process.

Abstract

Dielectrophoresis (DEP) as a label-free cell separation approach in microdevices has been extensively investigated for a variety of applications. 3D microelectrodes made of conducting-PDMS inherit the merit of volumetric electrodes for generating influential DEP force throughout the entire channel depth and meanwhile, exploit low-cost fabrication process by soft lithography. However, the configuration of conducting-PDMS electrodes is limited to being embedded in sidewall of flow chamber, which leads to rather low flow rate and difficulties in extension of the flow rate. We previously reported a more effective configuration with 3D interdigitated electrodes made of silicon that assist cell sliding along solid tracks, yet such device requires expensive silicon dry etching and, moreover, the track appears to be patterned with non-straight and wavy outline, which not only hinders the flow rate but also allows cell sliding to occur only along its downstream side. Here we demonstrate low-cost silver-PDMS electrode-track featuring ideally straight outline that induces rather uniform drag to drive smooth cell sliding. Such design achieves live and dead cell separation at flow rate twice as that of silicon tracks with cell loading concentration 10 times higher. It also fully utilizes the track to enable cell sliding on both of the up- and down-stream sides. Notably, we also demonstrate that this track is expandable to be V-shape for more advanced bidirectional cell sliding, which is showcased here by tumor cells separation from lymphocytes at 1.2 ml/h. Such results greatly enhance the throughput as compared to the state-of-art conducting-PDMS based cell separator.

Keywords: Dielectrophoresis; Cell separation; High throughput; Conducting-PDMS; Straight track

1. Introduction

The cell sorting is imperative preparative step in fundamental biological research for isolating cell type from complex background with specific features in metabolism, apoptosis and molecule secretion, etc [1-3]. Besides, it also plays pivotal roles in clinical practices. For instance, enrichment of circulating tumor cells (CTCs) from peripheral blood enables liquid biopsy in cancer prognosis beyond the conventional tissue biopsy, which not only offers non-invasive way for patient monitoring but also increases applicability of therapeutics that rely on real-time feedback from routine CTCs measurements as cancer marker [2,4]. Compared with commercialized tools such as fluorescence-activated cell sorting (FACS) and magnetic-activated cell sorting (MACS), microfluidic platform offers solutions for cell sorting with cost-effective miniaturized device that exerts more sensitive control over cells based on diverse mechanism [5]. Among emerging microfluidic cell sorting technologies, dielectrophoresis (DEP), being capable of controlling the trajectory of cells in non-uniform electric field according to their dielectric polarity, has been extensively investigated due to the favorable

features such as label-free operation, flexible control of cell trajectory and easy scaling down for device miniaturization.

The methodology for integrating the microelectrodes with fluidic channel is critical for a DEP sorter. Smart integrations couple the non-uniform electric field with the flow field in a way that leads to cell sorting in advanced continuous-flow mode. Compared with DEP sorters working in discontinuous procedures [6,7], where the target cells are immobilized on the electrodes and subsequently collected after depletion of the entire sample batch, devices working in continuous-flow mode simultaneously deliver cells of different type toward separate outlets along diverging trajectories, which greatly expand sample volume capacity and simplify the operation.

In the last decade, researchers leveraged continuous-flow DEP activated cell sorting via various formats of microelectrodes configuration within the flow chamber. The most popular design was interdigitated thin film metal electrode array patterned on channel bottom with slanted electrode digits to have a certain cell type deflected from initial sheathed sample stream [8,9]. The digits also appeared symmetrically tilted at dual directions [10,11] to induce distinctive trajectories being divergent or convergent based on DEP polarity of the cells. Flow channel with such electrode configurations had electric field intensified only near the electrode surface, which led to limited height coverage and thus relatively low throughput generally no higher than 0.1 ml/h. Combination of such easily-fabricated thin film electrode with hybrid cells focusing effect such as gravitational-sedimentation or inertial focusing greatly enhanced throughput [12,13]. The former achieved 0.75 ml/h flow rate for separation of leukemic cell subpopulations, and the latter, though further increased the throughput to 6 ml/h, lacked the experimental verification with biological cells. Instead of patterning electrode only on bottom surface of the channel, researchers configured electrode that covered extended inner sidewall area around the channel to project non-uniform electric field being influential throughout the entire channel cross-section. Representative designs included top-bottom positioned electrode [14,15] and volumetric sidewall electrode made of electroplated metal [16]. Notably, Faraghat et al. presented separation of fibroblasts from red blood cells (RBCs) through 394 parallel flow paths with coaxial ring electrode around each channel that were fabricated by drilling through a 12-layer laminate with alternating conducting and insulating layers. Such high-degree parallelization enabled extremely high throughput of 30 ml/h yet at expense of compromised target cell recovery rate at ~50% [17]. Cheng et al. isolated tumor cells from blood cells using long-range top-bottom facing electrode patterned within a serpentine channel featuring V-shaped groove as bottom and achieved optimal flow rate at 1.2 ml/h [18]. These aforementioned electrodes typically required cumbersome and costly fabrication in clean room environment, such as silicon etching [18,19], wafer alignment [14,15], electroplating [12,16,18], or multi-layer metal patterning within laminate [17]. A low-cost DEP sorter that maintains effective

separation at high throughput is still desired. Existing devices fabricated through low-cost soft lithography process, such as liquid electrode formed by filling ionic buffer solutions into side channel within PDMS [20,21] and volumetric electrode made of conducting-PDMS composite [22-24] still fell short of sample flow rate being lower than 0.2 ml/h, due to very limited means of sidewall electrode configuration.

We previously demonstrated 3D interdigitated electrode fingers made of heavily-doped silicon that were fabricated through concise single-mask lithography process, and tailored with non-uniform sidewall profile through combination of anisotropic and isotropic dry etching of silicon [6,25]. We then improved the finger design to be slanted to enable continuous-flow cell separation assisted by cell railing along the finger at 0.5 ml/h sample flow rate [19]. The sequential silicon dry etching for shaping the sidewall profile on the silicon-on-insulator (SOI) substrate, led to high fabrication cost and required a wavy-outlined finger pattern for flow paths formation. As a result, cells being railed on such non-straight finger experienced periodically maximized drag force which hindered further enhancement of the flow rate and allowed cells railing only along the finger sidewall facing downstream. Here, as illustrated in Fig. 1a-b, we present 3D track electrodes made of silver-PDMS (AgPDMS) composite, as an outcome of low-cost replica molding process. The tilted track digits exhibit straight outline analogous to the 2D angled digits in prior work [8,9], yet such design enables effective control on cells over more extended region in the chamber and more rapid lateral cell migration. The perfectly straight edges of the tracks also induce rather uniform drag to enable smooth cell sliding on both sidewalls of such straight track electrode for elevated throughput. We also demonstrate that the electrode is scalable by simply mirroring the unidirectionally slanted track electrodes to V-shape for bidirectional cell sliding, which gives rise to further increased throughput without extending the cell sliding time.

The interdigitated track electrodes in Fig. 1a-b, either tilted with respect to the flow direction at 30° in unidirection or at $\pm 30^\circ$ in bidirection, have a two-layer structure for each digit. The upper layer featuring straight and uniform sidewall serves as the cell sliding track, bridging across the entire chamber and allowing parallel flow paths through the equally spaced posts supporting from the lower layer. We perform numerical modelling of the electric and flow fields tailored by such unique 3D track electrodes. We also investigate the effect of amplitude and frequency of the activation signal on the sliding effectiveness using the unidirectional sliding device, and show its applicability in continuous separation of live and dead Hela cells. Further, we perform the continuous separation of Hela cells from blood lymphocytes at 1.2 ml/h with bidirectional sliding device. Our device greatly enhances the sample flow rate as compared with the existing devices that exploiting low-cost conducting-PDMS electrode for continuous cell sorting and is also among the highest flow rates as applied

in the state-of-art DEP cell sorters. Meanwhile, it further simplifies the fabrication by one-step replica molding on a readily used SU8 master.

2. Materials and methods

2.1. Theory and chip design

Dielectrophoresis (DEP) refers to the force exerted on the induced dipole moment of an uncharged dielectric and/or conductive particle by an externally-imposed non-uniform electric field [26]. The time averaged DEP force acting on a cell with radius r is

$$\langle F_{DEP} \rangle = 2\pi\epsilon_m r^3 \text{Re}[K^*(\omega)] \nabla |E|^2 \quad (1)$$

and

$$K^*(\omega) = \frac{\epsilon_c^* - \epsilon_m^*}{\epsilon_c^* + 2\epsilon_m^*} \quad (2)$$

where ϵ_m is the permittivity of the suspension medium, E is the electric field and ∇ is the gradient operator. $\text{Re}[K^*(\omega)]$ is the real part of the Clausius-Mossotti (CM) factor $K^*(\omega)$. $\epsilon^* = \epsilon - j\sigma/\omega$ is the complex permittivity of the cell or the surrounding medium, as indicated by the subscript “c” or “m”, respectively. ϵ , σ , ω and j respectively denote the dielectric permittivity, electric conductivity, angular frequency and imaginary unit. The sign of $\text{Re}[K^*(\omega)]$ suggests the polarizability contrast between the cells and the suspension medium. Cells being more polarizable than the suspension medium have $\text{Re}[K^*(\omega)] > 0$, namely positive DEP (pDEP), and thus get attracted towards the electric field maximum region. Contrarily, cells experience negative DEP (nDEP) when $\text{Re}[K^*(\omega)] < 0$ and thus get repelled to the field minimum region.

Fig. 1a schematically illustrates the working concept of the device for cell sorting. The wider upper layer of the electrode digit overhanging the supporting posts gives rise to electric field in channel being more intensified in the upper layer than the lower layer and maximized near the track sidewall along its lower edge. For the unidirectional sliding device, cells under nDEP will remain in the initial sample stream being constrained by a sheath flow, and travel through the lower layer toward outlet I. In contrast, cells under pDEP will get trapped onto the upper track layer, displacing obliquely from the initial sample stream via sliding along the track, and subsequently enter outlet II. Similarly, sample mixture in the bidirectional sliding device is sandwiched between two sheath flows and delivered to the central outlet 2. Cells under pDEP in this device will get loaded onto the track and slide along the V-shaped digits to either side of the chamber, targeting outlet 1 or 3.

Cells carried by the fluids, without being trapped to the electrodes, experience hydrodynamic force mainly from the Stokes drag given by [27],

$$F_{drag} = 6\pi\eta(\mathbf{u}_m - \mathbf{u}_c)r \quad (3)$$

where η is the dynamic viscosity of the suspension medium. $\mathbf{u}_m$ and $\mathbf{u}_c$ are the flow velocities of the suspension medium and the cells, respectively. However, cells trapped on track and exhibiting sliding behaviour are subject to more complex hydrodynamic forces because they are carried by wall bounded flow with the presences of the ceiling wall and track sidewall nearby. Therefore, cells tend to exhibit cross stream migration due to the wall-induced lift force [28], and the presence of a wall also influences the drag force on a cell near the wall under Stokes condition [29]. The vertical position of a cell sliding along the track is affected by the DEP force and the hydrodynamic force acting on the cell along the vertical direction. A cell at equilibrium vertical position has balanced DEP force $\mathbf{F}_{DEP_z}$ and hydrodynamic force $\mathbf{F}_{hydr_z}$ along the vertical direction,

$$\mathbf{F}_{DEP_z} + \mathbf{F}_{hydr_z} = 0 \quad (4)$$

The sliding velocity then depends on the balance of the hydrodynamic force $\mathbf{F}_{hydr_t}$ and the friction $\mathbf{f}$ along the track

$$\mathbf{F}_{hydr_t} + \mathbf{f} = 0 \quad (5)$$

and

$$\mathbf{f} = \mu(\mathbf{F}_{DEP_n} + \mathbf{F}_{hydr_n}) \quad (6)$$

where $\mathbf{f}$ is proportional to the net normal force pointing inward the track sidewall as the sum of the decomposed hydrodynamic force $\mathbf{F}_{hydr_n}$ and pDEP trapping force $\mathbf{F}_{DEP_n}$ normal to the digits in the horizontal plane.

2.2. Fabrication

Fig. 1c illustrates the fabrication process from the cross-sectional view along AA'. The device fabrication begins with patterning of a two-layer SU8 master (MicroChem Corp, MA, US) on a silicon wafer using a multi-layer SU8 lithography process [30]. Briefly, the first and second SU8 layers, respectively denoting the lower supporting posts (50 μm thick) and upper tracks (20 μm thick) of the electrode digits, were sequentially spin-coated and exposed, followed by simultaneous development of these two layers (Step I to III). Note that the cavity patterns on the SU8 master respectively designing the bulk electrodes as well as the fluidic sidewalls of the inlet/outlet regimes are also formed within the same steps.

Next is the replica molding of the entire device structure homogeneously made of AgPDMS. The mold surface is pre-coated with a nonstick thin layer of 1H,1H,2H,2H-Perfluorooctyl-trichlorosilane (Sigma-Aldrich, MO, US) via evaporation in vacuum chamber to ease the removal of excess AgPDMS from the mold surface as well as the release of the molded structure. The AgPDMS composite is prepared by mixing the PDMS gel (Dow Corning, MI, US) and silver particles with diameter of 1~3 μm (Xingrongyuan, Beijing, China) at weight ratio of 15:85, followed by thorough mixing via grinding with a pestle in a mortar until that the

composite appears as paste-like substance. The AgPDMS composite is then substantially filled into the cavities by pressing the mold against a piece of copy paper, and meanwhile the mold is forced to slide on this paper with press applied to scrape off the extra AgPDMS on top of the mold. The filled AgPDMS is cured on a 70 °C hot plate for 3 hours (step IV). In the following step V, a blank PDMS layer is cured on top of the mold at 70 °C for 3 hours to form the device cap layer. The connective PDMS-AgPDMS structure is then peeled off from the mold, punched with inlet/outlet ports through the cap layer and bonded onto a glass slide using semi-cured PDMS as adhesive layer (step VI). The PDMS adhesive layer is spin coated on the glass slide with ~12 µm thickness and pre-cured at 70 °C for 5 minutes, followed by placement of the PDMS-AgPDMS structure onto the slide to form permanent bonding via complete curing of the PDMS adhesive layer for another 30 minutes at 70 °C. The outer sidewall of each bulk electrode is respectively sliced to expose the fresh AgPDMS, and cooper wires are subsequently interconnected to the AgPDMS sidewall with silver epoxy.

2.3. Cells and reagents

Hela cells were cultured in Dulbecco's modified Eagle medium (DMEM) containing 10% fetal bovine serum (FBS) and 1% penicillin/ streptomycin (P/S), and maintained in a humidified incubator with 37 °C and 5% CO₂ supply. Prior to the experiment, cells were washed with phosphate buffered saline (PBS) and then treated with trypsin to detach from culture dishes, followed by centrifuge to pellet the cells and re-suspending them in fresh culture medium. All reagents for cell culture were purchased from Life Technologies (CA, US). The cells were pre-stained with 2 µg/ml Calcein-AM (Aladdin, Shanghai, China) at 37 °C in the dark for 10 minutes, and then washed twice with DEP buffer. The DEP buffer was 300 mM D-mannitol (Yuanye, Shanghai, China) with its conductivity turned to 100 µS/cm using PBS. Dead Hela cells used in live and dead cell separation experiments were killed in culture medium by 15-minute water bath at 65 °C. The killed cells were then washed with DEP buffer, stained with 10 µg/ml propidium iodide (PI, Sigma-aldrich, MO, US) in the dark for 1 minute, followed by a second wash with DEP buffer. The dead cells, with concentration being fixed at 1×10^5 cells/ml, were finally mixed with live cells at different concentration ratios. The lymphocytes used in the experiment were obtained from whole blood using a density gradient method assisted by Ficoll-Paque PLUS (GE Healthcare Lifescience, MA, US) with detailed protocols in our previous paper [6]. The blood sample was donated by consented healthy volunteers and was drawn in China-Japan Friendship Hospital. Extracted lymphocytes were stained with Calcein-blue (AAT Bioquest, CA, US) for 10 minutes in the dark, washed in DEP buffer and mixed with Hela cells with concentrations respectively at $\sim 1.6 \times 10^7$ cells/ml and 1×10^6 cells/ml.

Cell viability after sliding along the electrode was assessed using a cell proliferation assay kit (CCK8, Dojindo, Kumamoto, Japan) according to the vendor protocol. Briefly, recovered cells from outlet II of the unidirectional device were seeded into 96-well plate (~1000 cells/well) and maintained in cell incubator up to 8 days with culture medium being refreshed daily. Wells for assay in a given day were replaced with 100 μ l culture medium containing 10 μ l CCK8 and kept in the incubator for 2 hours. Thereafter, the optical density (OD) at 450 nm was measured using a plate reader (Synergy HTX, BioTek Instruments, VT, US). As control, cells without passing through the device were also subject to the same assay as described above.

2.4. Experimental setup

The device was coated with FBS for 30 minutes to minimized cell adhesion and then flushed with DEP buffer. In experiment, the infusion flow rates for sample and sheath were independently controlled by different syringe pumps (LSP02-1B, Longer, Baoding, China). A sinewave voltage being gated by a 1 Hz pulse sequence with 90% duty cycle was generated from a function generator (DG1020, RIGOL, Beijing, China) and delivered to the chip through a power amplifier (AL-50HFA, Amp-Line Corp., NY, US). An oscilloscope (DS2302A, RIGOL, Beijing, China) was applied for monitoring the activation signal. The experiments were conducted under an inverted fluorescence microscope (Ti2, Nikon, Tokyo, Japan) equipped with a CMOS camera (ORCA-Flash4.0, Hamamatsu, Tokyo, Japan) for monitoring and micrograph sequence acquisition.

2.5. Numerical simulation

Numerical computations of the electric and flow field distributions were performed using Comsol Multiphysics (COMSOL Inc., MA, US), on a scaled-down 3D model of our device with three pairs of digits and the chamber width cut down by two-third. Besides, simulations on transformed versions of our device, including a 2D version with planar electrode digits patterned on chamber floor as well as a special case with the track layer going through the entire chamber depth, were also performed under the same boundaries settings for comparison [27,31].

3. Results and discussion

3.1. Fabricated device

Fig. 2 depicts the scanning electron microscopic (SEM) images respectively taken for unidirectional and bidirectional device with AgPDMS structures facing upward. Fig. 2a exhibits the 2.5 mm wide flow chamber between the bulk electrode pair of a unidirectional device. The 30° tilted interdigitated digits protruding from the bulk electrode bridge across the chamber with a gap between the end of each digit and counter bulk electrode. Each digit adopts a two-layer configuration: a 140 μ m wide overhang layer (lower position in the SEM) with

uniform sidewalls of 20 μm thick offers ideally straight track for cell sliding. Cylindrical posts (upper position in the SEM) of 50 μm thick and 100 μm diameter support the track from the device bottom, and the gaps between adjacent posts are maintained in line and thus divide the flow chamber to 19 parallel flow paths. Note that the bulk electrodes have narrow trenches that correspond to partitions on the SU8 mold. Such design aims to bypass the issue regarding AgPDMS filling of the shallow cavities (70 μm) with large area (3440 $\mu\text{m} \times 16500 \mu\text{m}$) for molding of the bulk electrodes. As a result, it warrants substantial filling of AgPDMS into the key regions electrically connecting the track electrode digits, as being sandwiched between the narrow trenches in the white dashed box. The bidirectional device in Fig. 2b has V-shaped track electrodes with extended digit length by mirroring the digits in the unidirectional design. Accordingly, the chamber width is doubled to 5 mm with the number of flow paths in parallel scaled up to 38.

The electrodes and the flow sidewalls are entirely made of AgPDMS. In contrast, existing devices incorporating conducting-PDMS electrode typically exploit different material, e.g. pure PDMS, to construct the fluidic sidewalls, and therefore require additional sacrificial lithography aligned to the SU8 master every time during device molding to pattern the fluidic boundary and the electrode in separate steps [22,23]. The fabrication here eliminates such cumbersome process by molding the entire structure in one step and, more importantly, becomes free of cleanroom operation once the SU8 master is fabricated.

3.2. Simulations

In order to investigate into the unique feature of the 3D track electrode design in our device and how it benefits the lateral cell migration via cell sliding in cell separation, we perform numerical modeling of the electric field and the flow field for our device and its 2D version [18,21,22,27,31,32]. The 2D version of our device (denoted as “2D device” later) has the same angled comb digits yet patterned on channel bottom as planar electrodes, and this type of electrode design has been widely investigated in previous studies [8,9,11]. For comparison, the model of the 2D device also has 30°-tilted electrode digits and 70 μm -deep flow chamber, and it induces cell deflection also by pDEP. Here we define two characteristic cross-sectional planes cut horizontally from different chamber depth. One is the plane-S at a depth where the electric field maxima emerge, and the other is the plane-M at mid-depth of the chamber ($z = 35 \mu\text{m}$). As schematically illustrated in Fig. 3a, Plane-S in our device (upper panel) refers to the lower edge of the upper track layer with $z = 50 \mu\text{m}$, whereas in the 2D device (lower panel), it refers to the chamber floor ($z = 0$) where the planar electrodes are deposited.

The 3D track electrodes, in contrast to the thin film planar electrodes on chamber floor, have the equipotential bulk bodies throughout the entire chamber height. Therefore, the 3D

track electrodes give rise to more intensified electric field strength and thus stronger DEP force in the channel area between adjacent digits. This has been verified by the electric field distributions in plane-S and plane-M from simulation for our device (Fig. 3b-c) as well as for the 2D device (Fig. S1a-b in the supplementary material). In plane-S, where the maxima of the electric field appear along the electrode edges in both device designs, our device (Fig. 3b) maintains higher electric field intensity than that of the 2D device (Fig. S1a) inside the channel between adjacent digits, as indicated by the color (red versus yellow) in such region. When moving to the plane-M at mid-depth for both devices, the planar electrodes (Fig. S1b) have the electric field diminish rapidly, whereas our device (Fig. 3c) exhibits noticeably stronger electric field intensity.

The more intensified electric field in our device also leads to stronger DEP force. Fig. 3d and e compare the $\nabla|E|^2$ magnitudes of the two designs at the plane-S and plane-M, respectively. $\nabla|E|^2$ is the gradient of the electric field intensity square and its magnitude is proportional to the DEP force strength, as indicated in equation (1). Fig. 3d reveals the line plots of $\nabla|E|^2$ across the channel between adjacent digits in plane-S, respectively along A-A' (Fig. 3b) in our device and a-a' (Fig. S1a) in the 2D device. Despite the comparably high $\nabla|E|^2$ peaked around the electrode edge (I & III) for the two designs, our device further maintains the $\nabla|E|^2$ an order of magnitude higher than that of the 2D device at the channel center line (II) away from the electrode edges. Fig. 3e depicts similar line plots in the plane-M of each device, which reveal a generally higher $\nabla|E|^2$ in our device, especially in the positions approaching the electrode edges (I & III).

More simulation results with discussions are given in the supplementary material. Briefly, we further compare our device respectively with its 2D version and a special case of it with the track layer going through the entire chamber height, for the electric field intensity and the $\nabla|E|^2$ magnitudes across a vertical cross-sectional plane (Fig. S2). The results reveal that the unique two-layer structure of the track electrodes project a beneficial-spatial DEP force field distributed not only in the horizontal planes but also along the vertical direction with more extended region along the depth under strong DEP force. Flow field simulations for our device and the 2D device are also given (Fig. S3a-b), and the results suggest that the coupled electric field and flow field in our device enable more rapid lateral cell migration via sliding than the 2D device, as discussed in detail in the supporting information.

3.3. Cell sliding

Successful cell sliding was the determining factor to deliver target cells to the outlet II during the cell sorting. A cell to slide underwent two sequential steps. The first was the loading step, where the cell was elevated onto the track by pDEP force, followed by the second step of

sliding, where the cell driven by drag force slid along the track with pDEP simultaneously being active to secure the cell on track. Failure in either step could lead to ineffective cell sliding. We characterized the collection efficiency, as indication of success rate of cell sliding, with respect to the varied DEP activation signal under increasing sample infusion flow rate, using the unidirectional device. Hela cells of 5×10^6 cells/ml were continuously infused into the device at 0.5, 1 and 1.2 ml/h, with sheath flow injected in parallel at fixed flow rate of 0.5 ml/h. The collection efficiency was calculated by taking the percentage of the escaped cells to outlet I as being recorded in image sequence, and subtracting it from one hundred percent.

Fig. 4a revealed the increasing trend of collection efficiency with the frequencies varying from 100 kHz to 400 kHz, which resulted in elevated $Re[K^*(\omega)]$ due to the higher polarizability contrast between the cytoplasm and the low-conductivity suspension medium when the capacitive cell membrane became short circuit with increased frequency. The $Re[K^*(\omega)]$ here increased from +0.72 to +0.92 as being calculated using the single-shell model [26] with the dielectric parameters of Hela cell given by the early publication [33]. The greater value of $Re[K^*(\omega)]$ at higher frequency resulted in stronger pDEP force that favored more effective cells loading as well as more secured stay of cells on track when sliding. On the other hand, the collection efficiencies were generally higher at lower sample flow rate with discrepancies being more pronounced at low frequency. For instance, the collection efficiencies at 0.5 and 1 ml/h, being rather close (i.e. 94.1% vs. 91.7%) at 400 kHz, respectively declined to 78.4% and 67.4% at 100 kHz. This indicated that the cells traveling with faster speed under higher flow rate, when subjected to insufficient pDEP force, encountered more difficulties to be loaded onto the track. Collection efficiency as function of applied voltage amplitude in Fig. 4b indicated significant improvement of cell sliding when increasing the voltage from 9 to 13 V_p . However, efficiency increment with further enhanced voltage of 17 V_p exhibited slowdown at 0.5 ml/h (i.e. from 94.1% to 96.1%) or even stagnation at 1- and 1.2-ml/h, remaining at round 92% and 88%, respectively. Saturation of the collection efficiencies suggested that the increased voltage, despite being sufficient to keep most of the loaded cells secured on track with amplitude no less than 13 V_p , failed to load more cells onto the track at the designated flow rate.

The cell sliding phenomenon was observed at both side of each individual track and interestingly exhibited different dynamic characteristics. We hence investigated further into the cell sliding characteristics on both up- and down-stream sides of the track. Fig. 5a-c displayed the representative cell sliding trajectories on both the upstream (green) and downstream (red) sides of the track respectively under flow rate of 0.5-, 1- and 1.2-ml/h. These trajectories were artificially overlaid from the image sequence displayed in Fig. S4-S6. The averaged sliding velocities at each flow rate were further revealed in Fig. 5g. Interestingly, cells sliding on the

downstream side exhibited velocity slower than those sliding on the upstream side, which possibly related to the higher vertical position being closer to the ceiling for cells slid along the downstream side as compared to the upstream side. According to equation (4), a sliding cell in vertical equilibrium position had balanced DEP force F_{DEP_z} and hydrodynamic force F_{hydr_z} along the vertical direction. As discussed in the theory part, cells sliding on track were subject to wall-bounded flow and thus experienced more complex hydrodynamic force incorporating wall-induced lift force as well as changed drag acting on a cell near-wall [28,29]. We performed direct numerical simulation suggested in prior publications [34,35] and extracted the hydrodynamic force (i.e., the net force of drag and lift) by integrating the sum of the shear stress and pressure across the surface of a spherical cell. Here we considered a spherical cell with 7.5 μm radius, placed individually along the track with its center at a depth the same as the plane-S (i.e., the lower edge of the track with $z = 50 \mu\text{m}$) and 7.5 μm from the track sidewall (either facing the up- or down-stream side). To assess the cell vertical position, we then depicted the hydrodynamic force acting on the cell in the vertical direction, i.e., the F_{hydr_z} , as it respectively slid along the upstream (U-U' in Fig. S3a) and downstream (D-D' in Fig. S3a) side. As revealed in Fig. S3d, the F_{hydr_z} as the net force of lift and drag in the vertical direction, emerged to be in opposite directions, i.e., downward in upstream side whereas upward in downstream side. Such feature coincided the vertical flow velocity profiles near the up- and down-stream sides of the track (Fig. S3c), which suggested that the fluid drag force contributed by the vertical velocity dominated the vertical hydrodynamic force. On the other hand, the DEP forces in the vertical direction (i.e., F_{DEP_z}) near the track sidewall all point toward the lower edge of the upper track layer where the electric field peaks, as indicated in Fig. S2b by the arrows. Therefore, as illustrated in Fig. 5d, sliding cell on the upstream side would have the cell center in a vertical position a bit lower than the lower edge of the track, where the DEP force pointing upward could balance the downward hydrodynamic force. On the contrary, sliding cell on the downstream side would have a vertical position higher than the lower edge of the track to balance the upward hydrodynamic force by the downward DEP force. Discrepancies in the vertical positions led to sliding velocity difference, where the cells closer to the ceiling wall on the downstream side exhibited lower sliding velocity because the fluid flow that drove them to slide diminished when approaching the ceiling wall. Such trend agreed with the sliding velocity difference exhibited in our experiment.

Fig. 5g also exhibited gradually vanishing discrepancies of the sliding velocities between the up- and down-stream sides, which could arise from the strengthened friction at the upstream side as the flow rate enhanced. As shown in Fig. 5e-f, the decomposed hydrodynamic force F_{hydr_n} normal to the track sidewalls had the same direction with the pDEP trapping force F_{DEP_n} on the upstream side but counter direction against it on the downstream side. Therefore, the

resultant net normal forces for F_{hydr_n} and F_{DEP_n} , respectively became strengthened (F_{N_up}) and weakened (F_{N_down}) with increased flow rate. According to equation (6), the strengthened net normal force on the upstream side then led to stronger friction, which slowed down the sliding and thus compensated the velocity discrepancies between the up- and down-stream sides.

To confirm the viability of cells after being subjected to DEP operation, we carried out proliferation assay on the collected cells from the device using the CCK8 method. Fig. 6a exhibits comparable proliferation curve for cells that are under strong electric field during sliding and those in the control group. Note that the viability assay was applied to cell sliding with 15 V_p activation voltage and 0.5 ml/h sample flow rate. The voltage was slightly above 13 V_p where the collection efficiency saturated, and the slowest flow rate ever applied here guaranteed longest exposure time of cells to the electric field. The micrographs in Fig. 6b-c reveal that the cells in experimental group have normal growth potential by referring to the control group.

3.4. Live and dead cell sorting in unidirectional device

Next, we applied the unidirectional device for continuous separation of live and dead Hela cells. DEP activation signal of 15 V_p at 400 kHz, as well as sample flow of 1 ml/h were applied according to parametric study in Fig. 4 to guarantee effective sliding of live cells and meanwhile maintain high throughput. The dead cells appearing to be with leaky membrane failed to maintain the conductivity contrast between cytoplasm and the DEP buffer, and thus had fairly weak DEP response. As a result, the dead cells being dominated by drag force remained in the injecting sample stream that target outlet I. Fig. 7a presents collection efficiencies of live cells and dead cells for outlet I and II, and the purities for live and dead cells respectively in their target outlets are presented in Fig. S7. Live and dead cells, being mixed with equal concentration at 1×10^5 cells/ml, yielded high collection efficiency respectively at 92.1% and 99.2% in their target outlet, which corresponded to high purities of 99.1% for the live cells in outlet II and 92.8% for the dead cells in outlet I. Elevated live cell concentration to 10 times of the fixed dead cell concentration maintained acceptably high live cell collection of 87.9%, which indicated excellent capacity for sliding cells offered by the double-sided and straight track. The superimposed fluorescence micrographs in Fig. 7b-c display continuous separation of live (red) and dead (green) cells being mixed at 10:1 concentration ratio (also see the movie in the supplementary material). Without DEP activation, the mixed cells flowed in parallel with the sheath flow and exited the device from outlet I. With DEP activation, live cells started to slide along both sides of the track electrodes (image highlighted with red border). These sliding cells, upon reaching the digit ends, got immobilized due to the local maxima of electric field exhibited there, and periodically released during the off-cycle of the gated activation voltage.

These cells then entered outlet II with most of them enriched near the right sidewall as a result of DEP focusing. Also note that the live cells, when sliding, dramatically decelerated in contrast to their flow velocities without DEP activation. In comparison, the dead cells exhibited similar trajectories and velocities before and after DEP activation, respectively.

Here the device achieved comparable separation efficiency (87.9% vs. 88.2% for sliding cells under pDEP; 97.8% vs. 91.1% for sheath-focused cells under weak pDEP) at doubled flow rate (1 ml/h vs. 0.5 ml/h) and cell loading concentration 10 times higher (1×10^6 cells/ml vs. 1×10^5 cells/ml) as compared to the silicon track introduced in our previous work [19]. The silicon track being tailored with non-straight wavy-outline periodically induced maximized and minimized drag along the track that respectively posed the cells in risk of escape or halt during sliding. Therefore, it required careful adjust of the flow rate. Besides, the silicon device allowed the cell sliding to occur only along the downstream side of the track. The straight track here eliminated such concern by inducing rather homogeneous drag acting on sliding cells along the track. Such improvement facilitated not only flow rate enhancement, but also full utilization of the track to allow cell sliding on both the up- and down-stream sides of the track, which offered greater capacity to accommodate more concentrated cells suspension.

The results also showed that further concentrated live cells of 1×10^7 cells/ml in the mixture resulted in live cell collection efficiency at 74.0%. Cells of high concentration formed elongated “pearl chain” under DEP polarization, of which the long “tail” extending to the channel between adjacent tracks could be easily disrupted by the flow to escape. On the other hand, removal of the dead cells remained effective, exhibiting slight decline to ~98% with increased live cell concentration. As a result, it ensured high live cell purity greater than 99% in outlet II for all three concentration ratios applied here (Fig. S7).

3.5. Continuous cell sorting via bidirectional cell sliding

The device here took full advantage of the track electrode design, where the number of parallel flow paths under the track could be easily scaled up by extending the length of the electrode digits without changing the separation distance between the adjacent digits. Here we doubled the electrode length by mirroring the unidirectional electrode. The resulted V-shape electrode generated enhanced throughput by enabling bidirectional cell sliding without prolonging the cell sliding time on track as compared to that in unidirectional device. We verified the capability of the bidirectional device for high-throughput separation of HeLa cells from lymphocytes with sample and each sheath flow injected respectively at 1.2 ml/h and 0.5 ml/h. As illustrated in Fig. 8a, the mixture of HeLa cells and the lymphocytes were hydrodynamically focused by the sheath buffers from both sides and delivered into the center

outlet 2 with DEP off. When DEP activation was applied at 17.5 V_p and 100 kHz, the Hela cells under pDEP were loaded onto the V-shaped track to slide bidirectionally to either outlet 1 or 3 at the chamber side, whereas the lymphocytes experienced fairly weak pDEP force were constrained in the initial sample stream targeting the center outlet 2. The fluorescence image taken on the midstream of the chamber in Fig. 8b, compared to that in Fig. 7c with the same exposure time, revealed that the cells here slid faster as indicated by the longer fluorescence tail. This was due to the smaller CM factor as well as double layer screening of the activation voltage at low frequency of 100 kHz, both of which led to weaker pDEP trapping force. The weaker pDEP force resulted in decreased net normal force and thus smaller friction that gave rise to higher terminal sliding velocity according to equation (6). We also noticed that some cells got rid of being tightly arrested on the track when sliding due to the weaker pDEP force. These cells exhibited slightly deviated trajectories from the track, yet they were still guided by the slanted track to successfully diverge to either side of the chamber.

Table 1 illustrated that the device collected 80.42% of Hela cells from the initial sample mixture from the target outlets 1 & 3 with 95.41% of lymphocytes being removed. The loss of Hela cells could be due to the compromised pDEP force at 100 kHz, whereas the lymphocyte contaminates could arise from the weak pDEP deflection on a small portion of the lymphocytes, as well as the dielectric interactions among these highly concentrated cells. The bidirectional sliding track, as compared to the silicon unidirectional track, further increased the flow rate by 2.4 times regarding the separation of tumor cells from blood lymphocytes [19].

The device here greatly improved the sample flow rate being applied for the cell sorting in other existing 3D electrodes made of conducting-PDMS. For instance, Lewpiriyawong et al. used AgPDMS electrode for separation of live and dead yeast with separation efficiency higher than 97% (92.1% and 99.2% respectively for live and dead Hela cells here using the unidirectional device), yet at low flow rate of 0.005 ml/h (1 ml/h here) [23]. Marchalot et al. exploited Carbon-PDMS electrode for isolation of tumor cells from RBCs and achieved tumor cells recovery at 78.7% (80.42% for tumor cells from blood lymphocytes here using the bidirectional device), but still with rather low flow rate of 0.08 ml/h (1.2 ml/h here) [24]. The results were also comparable with the representative DEP platform for CTCs isolation from peripheral blood mononuclear cells (PBMNs), where the tumor cells spiked to PBMNs had averaged 75% recovery efficiency with 1.2 ml/h sample infusion flow rate [36]. Compared with this early CTCs separator, where the sample infusion and target cells collection flow rate were equally high at 1.2 ml/h, the device here enriched the target cells into the flow stream with immediate contact to each of the chamber sidewalls, and it reduced the collection flow rate of the target cells to 0.46 ml/h with respect to 1.2 ml/h of the infusion flow rate (without optimization). Meanwhile, it also facilitated easier and low-cost fabrication by replica molding and thus eliminated the need for separate patterning procedures for the flow chamber and the

electrodes in the aforementioned DEP platforms. Therefore, the device was promising as a novel low-cost tool for CTCs isolation. Moreover, the flow rate of the device can be further elevated by easily extending the track length to incorporate more parallel flow streams, which on the other hand will benefit further enrichment of target cells by extending the infusion flow rate while keeping the collection flow rate of target cells unchanged at the chamber sides. In addition, the DEP-activated cell sliding can be further combined with affinity chromatography for increased affinity of the track to tumor cells. The track coated with specific antibody to the tumor cells will compensate the relatively weak pDEP force at low frequency and collected tumor cells at higher efficiency.

4. Conclusion

We have presented here 3D interdigitated microelectrode digits made of AgPDMS acting as cell sliding tracks that bridge over the entire flow chamber in oblique orientation. Such design advances the conducting-PDMS based microelectrodes, which are typically configured in channel sidewall in existing techniques, with greatly enhanced throughput at comparable separation efficiency in continuous DEP-activated cell sorting. Meanwhile, the device here inherits the low-cost soft lithography process for manufacturing of devices incorporating conducting-PDMS electrodes, and further simplifies such process by eliminating the need for additional sacrificial layer lithography on SU8 mold. Compared with similar design with angled electrode digits yet in 2D planar form, which is typically used to laterally deflect cells in DEP cell sorters, this 3D track design projects effective DEP force field influential over extended region within the flow chamber and enables more rapid lateral cell migration. Moreover, it exhibits great improvement of our early work introducing the silicon track with non-straight wavy outline. By demonstrating the perfectly straight track here, our design allows rather uniform drag force to drive cell sliding and thus facilitates further elevated flow rate as well as full utilizations of the track to enable cell sliding on both sidewalls facing up- and down-stream. As proof of concept, we demonstrate bidirectional cell sliding that targets increased throughput by easily mirroring the unidirectional track to incorporate more parallel flow streams. Such optimization achieves 2.4 times higher flow rate as compared to the silicon counterpart for separation of tumor cells from lymphocytes. The device also exhibits comparable performance to the existing CTCs platforms and thus holds potential for CTCs extraction in clinical practice.

Credit author statement

Concept, design and methods, X. Xing. Fabrication, experiments and data analysis, X. Nie, X. Xing and C. Han. Original draft and editing, X. Nie, X. Xing and Y. Luo. Revised draft, X. Xing, P. Shen and Y. Luo. Funding acquisition, X. Xing and D. Yu. Supervision, X. Xing and D. Yu.

Declaration of interests

None.

Appendix A. Supporting Information

Supplementary data associated with this article can be found in the online version at <http://dx.doi.org/XXXXXX/>.

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Figures

Fig. 1. (a) 3D schematic illustration of the device featuring interdigitated electrode tracks tilted at 30° with respect to the flow and the working concept for cell separation with cells under pDEP (red) sliding along the track toward outlet II and others under nDEP or weak DEP (green) being focused to outlet I by the sheath flow. The enlarged view of one electrode segment exhibits the two-layer electrode structure with track layer overhanging supporting posts and flow path penetrating through the gap between adjacent posts. (b) Device with V-shaped electrode track tilted symmetrically at $\pm 30^\circ$ as evolved from mirroring of the unidirectional device in (a) and thus featuring gained number of parallel flow path twice as that of the unidirectional device. This device allows bidirectional sliding of cells under pDEP to side outlet 1 or 3 whereas has those under nDEP or weak DEP being sheathed to the center outlet 2. (c) Fabrication process flow of the device illustrated by the cross-sectional view along AA' of the electrode segment in (a).

Fig. 2. SEM images of bottom-up positioned devices respectively with a portion of the flow chamber incorporating (a) unidirectional electrodes and (b) bidirectional electrodes, each with a zoom-in view of the electrode digits.

Fig. 3. (a) Schematic illustration of the probe-planes and lines for depicting the simulation results in vertical cross-sectional planes cut across adjacent digits respectively for the 3D track device (upper panel) and 2D (lower panel) device. (b-c) Electric field distributions across the horizontal (b) plane-S and (c) plane-M for the 3D track device. (d-e) $\nabla|E|^2$ in logarithmic scale along the line A-A' labeled in (b) in the 3D track device and the line a-a' labeled in Fig. S1a in the 2D device. (e) $\nabla|E|^2$ in logarithmic scale along the line B-B' labeled in (c) in the 3D track device and the line b-b' labeled in Fig. S1b in the 2D device.

Fig. 4. Collection efficiency of sliding HeLa cells in a unidirectional device as a function of (a) the frequency of an activation voltage applied at 13 V_p and (b) the amplitude of an activation voltage applied at 400 kHz, with the sample flow rates of 0.5, 1, and 1.2 ml/h. Sheath flows: 0.5 ml/h. DEP buffer: 100 μ S/cm. Data symbols and error bars denote mean $\pm$ s.d. (n = 3).

Fig. 5. (a-c) Superimposed fluorescent images showing representative cell sliding trajectories along the upstream sides (green) and downstream sides (red) of the electrode tracks under sample flow rates of (a) 0.5 ml/h, (b) 1 ml/h and (c) 1.2 ml/h. (d) Free-body diagrams illustrating the forces in the vertical direction acting on a cell sliding respectively on the up- and downstream sides of a track. The vertical cross-sectional plane is cut along the probe line I in (a). (e-f) Free-body diagrams illustrating the forces in horizontal plane acting on a cell sliding respectively along the (e) upstream side and (f) downstream side of the track. (g) Sliding velocities with respect to the sample flow rates for cells sliding along the up- and down-stream side of a track, respectively. Data symbols and error bars denote mean $\pm$ s.d. ($n = 7$). The image sequences for velocity calculation in (g) were recorded with image interval of 60 ms, and the images for the trajectories exhibition being depicted in (a)-(c) were down-sampled with 300 ms image interval. Each trajectory was generated by overlaying the images in a selected sequence, and four trajectories under the same flow rate were further fit into a fixed electrode area for concise exhibition. The original image sequences were shown in Fig. S4-S6 in the supplementary material. The labels U and D denote upstream and downstream, respectively. Activation signal: 15 V_p and 400 kHz. Sheath flows: 0.5 ml/h. DEP buffer: 100 μ S/cm.

Fig. 6. (a) Absorbance values measured at 450 nm in CCK8 assay for cells from the experimental (cells collected from outlet II with voltage applied at 15 Vp, 400 kHz and sample flow rates applied at 0.5 ml/h) and control group (cells without passing through the device). (b-c) Microscopic images of the cultured cells taken from the experimental and control groups at (b) Day 1 and (c) Day 3. Sheath flows: 0.5 ml/h. DEP buffer: 100 μ S/cm.

Fig. 7. (a) The collection efficiencies of live and dead cell populations at each outlet of unidirectional device with the concentration ratios (live cells: dead cells) in sample mixture at 1:1, 10:1, and 100:1 (from top to bottom). The dead cell concentration was fixed at 1×10^5 cells/ml. Data symbols and error bars denote mean $\pm$ s.d. ($n = 3$). (b-c) Superimposed fluorescent images respectively taken from up-, mid- and down-stream of the flow chamber with (b) DEP off and (c) DEP on. The concentration ratio for live (red) and dead (green) cells was 10:1. Sample flow rate: 1 ml/h, Sheath flow rate: 0.5 ml/h. Activation: 15 V_p and 400 kHz. DEP buffer: 100 μ S/cm.

Fig. 8. Superimposed fluorescent micrographs taken from up- to down-stream of a bidirectional device showing continuous-flow separation of HeLa cells (red) from lymphocytes (green) with (a) DEP off and (b) DEP on. Sample flow rate: 1.2 ml/h. Flow rate for each of the sheath: 0.5 ml/h. Activation: 17.5 V_p and 100 kHz. DEP buffer: 100 μ S/cm.

Table 1. The collection efficiencies of Hela cells and lymphocytes from the side outlets 1&3 and the center outlet 2 for the continuous cell separation using the bidirectional device as illustrated in Fig. 8. The averaged values and standard deviations were from three measurements.

	Outlet 1&3	Outlet 2
Hela	80.42% $\pm$ 3.37%	19.58% $\pm$ 3.37%
Lymphocytes	4.59% $\pm$ 4.79%	95.41% $\pm$ 4.79%