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Review

Emerging on-chip electrokinetic based technologies for purification of circulating cancer biomarkers towards liquid biopsy: A review

Early detection of cancer can significantly reduce mortality and save lives. However, the current cancer diagnosis is highly dependent on costly, complex, and invasive procedures. Thus, a great deal of effort has been devoted to exploring new technologies based on liquid biopsy. Since liquid biopsy relies on detection of circulating biomarkers from biofluids, it is critical to isolate highly purified cancer-related biomarkers, including circulating tumor cells (CTCs), cell-free nucleic acids (cell-free DNA and cell-free RNA), small extracellular vesicles (exosomes), and proteins. The current clinical purification techniques are facing a number of drawbacks including low purity, long processing time, high cost, and difficulties in standardization. Here, we review a promising solution, on-chip electrokinetic-based methods, that have the advantage of small sample volume requirement, minimal damage to the biomarkers, rapid, and label-free criteria. We have also discussed the existing challenges of current on-chip electrokinetic technologies and suggested potential solutions that may be worthy of future studies.

Keywords:
Biomarker / Cancer / Electrokinetic / Liquid-biopsy / On-chip
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1 Introduction

Cancer is a cluster of diseases involving alterations in the status and expression of multiple genes that confer a survival advantage and undiminished proliferative potential to somatic or germinal cells [1]. Cancer burden continues to be one of the leading causes of morbidity and mortality across the world, exerting tremendous physical, emotional, and financial strain on individuals, families, communities, and health care system. Each year, more than 11 million people are diagnosed with cancer, the majority of whom live in low- and middle-income countries [2]. To this day, the only certain approach to defeat cancer is early diagnosis; however, unfortunately the traditional cancer diagnosis is highly dependent on the invasive sampling of tumor tissues or imaging techniques [2], and thus, causes high levels of patient discomfort [3]. Consequently, plenty of efforts have been devoted to exploring new technologies based on liquid biopsy. Compared to the traditional tissue biopsy, the liquid biopsy relies on the detection of cancer biomarkers circulating in biofluids, including circulating tumor cells (CTCs), cell-free nucleic acids (cell-free DNA and cell-free RNA), small extracellular vesicles (exosomes), and proteins. Liquid biopsy is a less invasive method and can potentially serve as better representatives of the primary and metastatic sites [4]. However, different challenges within each biomarkers' physiological characteristics limit their rigorous and reproducible purification and detection. For instance, CTCs have an extremely low concentration in the bloodstream, which poses challenges to their isolation with high purity and recovery rate from small sample volumes. Cell-free nucleic acids (*cf*DNA and *cf*RNA), on the contrary, are relatively abundant in the blood, but their high fragmentation and low stability nature make their isolation challenging. The major challenge with exosome purification is to differentiate them from other nanovesicles with similar size and density (e.g., lipoprotein) circulating in biofluids. As to cancer-related proteins, different cancer types have different specific protein markers, and there is no

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Abbreviations: **cDEP**, contactless dielectrophoresis; ***cf*NAs**, cell-free nucleic acids; **CTCs**, circulating tumor cells; **DEP-FFF**, dielectrophoretic field flow fractionation; **DU**, differential ultracentrifugation; **eDEP**, electrodeless dielectrophoresis; **EDL**, electrical double layer; **EFD**, electro-fluid-dynamics; **EHD**, electro-hydrodynamics; **EO**, electroosmosis; **EOF**, electroosmotic flow; **EP**, electrophoresis; **iDEP**, insulator-based dielectrophoresis; **MOFF**, multi-orifice flow fractionation; **ODEP**, optically induced dielectrophoresis; **PCB**, printed circuit board; **ROT**, electrorotation; **SEC**, size exclusion chromatography; **TWD**, traveling wave dielectrophoresis; **UC**, ultracentrifugation

Color online: See article online to view Figs. 1–5 in color.

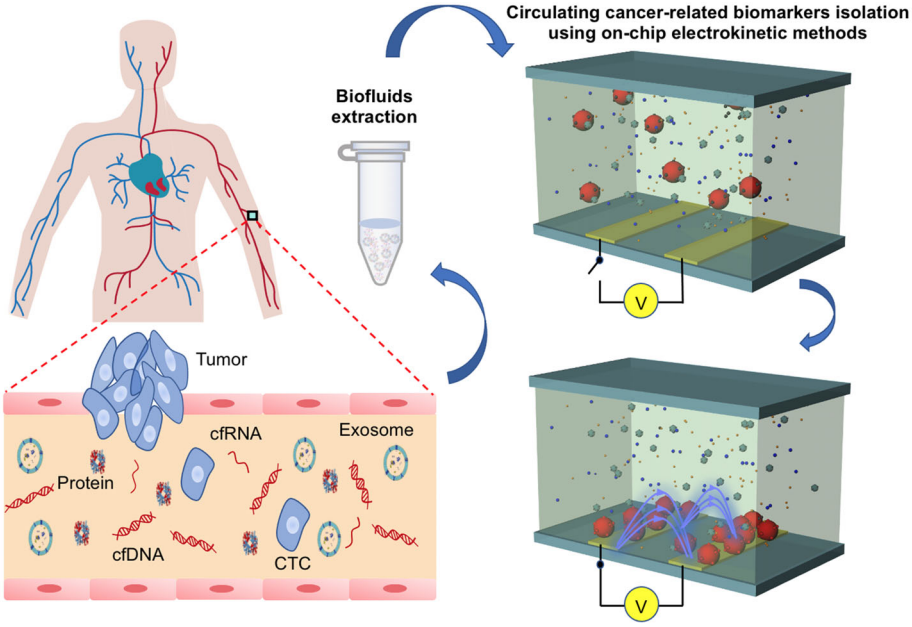

Figure 1. The schematic illustration of cancer-related biomarker isolation and purification using on-chip electrokinetic based methods. The whole process includes biofluids extraction from patients, and biomarkers isolation with the on-chip electrokinetic methods.

generalized protein marker that can be applied to diagnose all cancer types. As a result, routine isolation of circulating cancer biomarkers is challenging in clinical settings and faces a number of drawbacks, including long procedure time, high cost, and difficulties in standardization [5]. In the past decades, numerous new approaches have been developed for isolating cancer-related biomarkers from biofluids, such as differential ultracentrifugation [6,7], affinity-based [8–10] and precipitation-based kits [11,12], size-based filtration [6,13], immune-affinity capture [14,15], microfluidic-based assays [16–18], acoustic-based methods [19,20], and on-chip electrokinetic-based techniques [21–23]. Among these technologies, on-chip electrokinetic-based method has gained significant attention because of its small sample volume requirement, rapid, and label-free criteria (Fig. 1).

Electrokinetics, also known as electro-fluid-dynamics (EFD) or electro-hydrodynamics (EHD), is the study of the relative motion between two phases as mediated by electric fields and a charged interfacial layer [24]. The electrokinetic phenomena include electrophoresis (EP), electroosmosis (EO), streaming potential, sedimentation potential, dielectrophoresis (DEP), traveling wave dielectrophoresis (TWD), and electrorotation (ROT) [25,26]. Among those electrokinetic forces, EP, DEP, and EO are the most popular methods for biomolecular separation. EP involves the separation of charged analytes based on the difference between their electrophoretic mobilities, leading to different migration velocities [27]. In the 1960s, the pioneer of capillary electrophoresis (CE), Hjerten, used CE to separate a range of analytes from small molecules in a tube with the inner diameter of a few millimeters [28,29]. CE became popular in the early 1980s, when Jorgenson and Lukacs demonstrated that it is possible to conduct high-performance analytical electrophoretic separations in capillaries with inner diameters of less than 100 μm [30,31]. Since that time, thousands of papers and books have been published in this area, along with extensive development of necessary equipment and instrumentation [27,32–35]. CE was proved to be a powerful separation technique for various samples due to the major advantage of much faster isolation compared to the traditional thick slab gel electrophoresis [36,37]. DEP was first discovered by Pohl in 1951 [38], which refers to the movement of particles induced by polarization effects in a non-uniform electric field. The non-uniformity of the electric field can be either generated by applying an alternating current (AC) across an array of electrodes [39,40] or by placing obstacles such as micro-pillars and rectangular hurdles in microfluidic channels (insulator-based approach) [41,42]. Due to its rapid and truly label-free criteria, DEP has been widely used for sorting, isolating, and manipulating biomolecules and particles based on their size and dielectric properties [43–45]. Also, when the electrolyte solution is filled in microfluidic channels with charged surfaces (e.g., glass and polydimethylsiloxane (PDMS) are negatively charged), an electrical double layer (EDL) will be formed at the surface/liquid interface. If an electric field is applied across the channel, EDL will interact with the electric field, resulting in EDL displacement. Because of the liquid viscosity, EDL will drag the bulk of the liquid and the suspending particles along the direction opposite to the electric field to form an electroosmosis flow (EOF). With DC voltage applied, the presence of the EOF could eliminate the need for an external syringe pump to drive fluid and drag particles along from the inlet to the outlet.

In recent years, due to the rapid development of microfabrication techniques, on-chip electrokinetics have attracted widespread of attentions, and on-chip electrokinetic-based devices have become an alternative for separation and isolation of the bio-analytes when compared to the conventional

electrokinetic methods such as capillary electrophoresis [46]. For example, DNA fragments could be separated by the on-chip electrophoresis device in a matter of minutes compared to tens of minutes for CE and hours for slab gels [47]. In addition, on-chip electrokinetics can facilitate the realization of an integrated and automated micro total analysis system (μTAS) [48], in which sample preparation, separation, and detection are performed on a single miniaturized platform. Many excellent articles have been published to review and summarize the on-chip techniques for isolating cells, nucleic acids, exosomes, and proteins [5,16,49–63]. For instance, the focuses on microfluidic technologies for isolation and detection of cancer-related biomarkers were comprehensively reviewed in a few articles [5,16,49,52,54]; recent DEP-based technologies and their biological applications were reviewed in [57]; the challenges for next-generation point-of-care molecular diagnostics using the cancer-related biomarkers in blood have been highlighted in [58]; the electrokinetic strategies for exosome isolation were summarized in [59]; the electrokinetically-driven microfluidics for CTC manipulation were reviewed in ref. [61]; and the recent innovation in protein separation on microchips by electrophoretic methods was highlighted in [60]. Our review here is distinct from these reviews as we solely focus on the on-chip electrokinetic-based isolation and purification techniques for all circulating cancer biomarkers including CTCs, cell-free nucleic acids (*cf*NAs), exosomes and proteins. In addition, the existing challenges and the potential solutions in the future research directions have been discussed. We especially emphasized the importance of utilizing a low electric field and directly working with highly conductive biofluids (0.5–1.2 S/m) such as undiluted blood in DEP and other electrokinetic-based technologies for practical applications.

2 Circulating tumor cells

Circulating tumor cells (CTCs) can be transported to potential metastatic sites after they are shed into the vasculature from the primary tumor [64]. Studies have shown that CTCs can be used as a marker to predict disease progression and survival in metastatic cancer patients [65–68]. High CTCs numbers are correlated with disease aggression, increased metastasis, and decreased time to relapse [64,69]. However, the major challenge for utilizing CTCs as a biomarker for early-stage cancer diagnosis is that they have an extremely low concentration in the bloodstream (1–10 CTCs per mL of whole blood in patients with metastasis) and are co-existed with a large number of white blood cells (WBC), erythrocytes and numerous other biological entities which make their isolation a daunting task [16,70].

The current techniques for isolating CTCs from whole blood include density gradient centrifugation [71,72], positive immunoaffinity selection-based methods [73,74], and negative selection methods [75–77]. Density gradient centrifugation separates CTCs from other cellular components of blood based on their differential densities [71,72]. It is a common macroscale technique for clinical and research applications. However, this method is time-consuming and has a low recovery rate [13]. Positive immunoaffinity selection-based method could capture CTCs immunomagnetically by means of ferrofluidic nanoparticles conjugated to a monoclonal antibody against the Epithelial Cell Adhesion Molecule (EpCAM) [73,74]. Although plenty of clinical studies substantiate the prognostic value of the immunoaffinity capture method, especially Cellsearch™ which is the only FDA approved automated system to capture and assess CTCs, the immunoaffinity only selects EpCAM positive (epithelial) CTCs, which may underestimate CTC number and potentially miss a critical subpopulation of cells [64,69,78]. More importantly, the majority of CTCs are not viable after capturing with this method, which hinders the cell culture and molecular diagnosis in the subsequent analysis [15]. In addition, the immunoaffinity assay is expensive, labor-intensive, and subject to large variability in the recovery rate (9–90%) mainly due to the variability in expression of surface markers [13,72]. Therefore, negative selection methods are developed to avoid some of the major disadvantages of the positive selection method, such as losing non-EpCAM CTCs and a relatively poor recovery rate [79]. In this approach, red blood cells are lysed followed by a CD45 (a common antigen of leukocytes) immuno-depletion process to remove leukocytes. Therefore, all CTCs, either epithelial marker positive or negative, can be fully recovered, which makes the number of isolated CTCs larger than those by the positive method [75]. However, the background cells in negatively isolated samples are often mixed with numerous white blood cells and red blood cells, and hence, prohibit the downstream molecular analysis [79]. Therefore, more efficient and reliable methods are required for CTCs isolation.

2.1 On-chip electrokinetic based methods for isolation of CTCs

During the last few decades, numerous electrokinetic technologies have been developed to overcome the limitations of the traditional CTC isolation methods. The earliest study of dielectrophoresis (DEP) based CTC separation could track back to 1995, in which an interdigitated gold microelectrode array was developed by Becker et al. for separating cancer cells from blood by balancing the hydrodynamic and DEP forces acting on the cells [80]. In their study, breast cancer cells MDA231, T lymphocytes, and erythrocytes were suspended in a sucrose solution with the conductivity of 0.01 S/m. The results showed that breast cancer cell could be separated based on their unique dielectric properties as the AC field was applied, and the recovery rate was higher than 95%. In a later study by Chiu et al., an optically induced dielectrophoresis (ODEP)-based cell manipulation device has been developed for CTCs purification after the negative selection-based CTCs isolation process [81]. The ODEP device is comprised of four layers, including a polydimethylsiloxane (PDMS) layer, an indium-tin-oxide (ITO) glass substrate, an adhesive tape and, an ITO coated photoconductive material (Fig. 2A). To

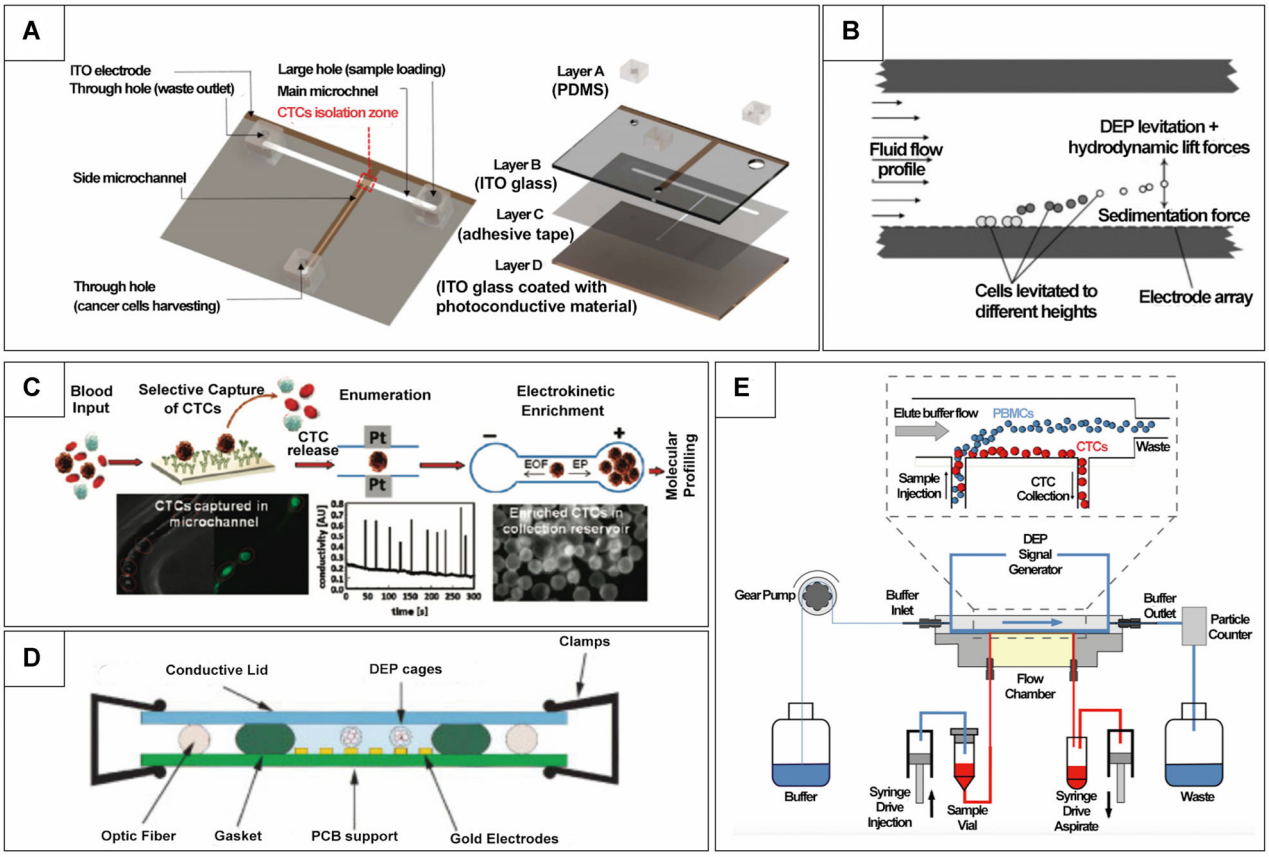

Figure 2. (A) Schematic illustration of the ODEP microfluidic system developed by Chiu et al. (B) The DEP-FFF approach developed by Gascoyne's group for characterization and capture of cancer cells. (C) The schematic illustration of the electrokinetic-based CTCs enrichment device developed by Dharmasiri et al. (D) Schematic illustration of the PCB device developed by Altomare et al. for levitation and movement of human tumor cells. (E) Schematic diagram of the ApoStream device; inset shows cell flow and separation in the flow chamber. (A) Reprinted with the permission from [81], copyright (2016) Springer Nature. (B) Reprinted with the permission from [88], copyright (2009) John Wiley and Sons. (C) Reprinted with the permission from [93], copyright (2011) American Chemical Society. (D) Reprinted with the permission from [94], copyright (2003) John Wiley and Sons. (E) Reprinted with the permission from [95], copyright (2012) AIP Publishing.

electrically polarize the cells, an AC field was exerted between the top and bottom substrates (layer B and D in Fig. 2A) to generate uniform electric fields. As the photoconductive layer (layer D in Fig. 2A) was illuminated by a light bar with 150 μm bandwidth, the projected light produced a locally non-uniform electric field at the light-illuminated regions, which could induce the DEP force on the polarized cells and manipulate them locally. To test the performance of the device, a cell suspension sample was prepared by spiking PC-3 cells (500 cells) (as a CTCs model) into a whole blood sample (8mL), followed by a negative selection-based CTCs isolation process. The obtained cell pellets were stained with immunofluorescent dyes and re-suspended in 30 μL of sucrose solution (conductivity: 0.34 μS/m). The treated cell was subsequently loaded into the microfluidic system for further CTCs isolation and purification. After the ODEP-based CTCs isolation process, the cell purity of harvested cancer cells was examined microscopically. The results revealed that the purity was as high as 100%, and the cell recovery rate was 41.5%. In a following work by the same group, the design was improved with four rectangular photo-active zones (termed "light bar arrays") to simultaneously create four different flow velocity conditions in the microfluidic system [21]. In each zone, ODEP was used to isolate cells with specific size under the corresponding flow velocity condition. The performance of CTC isolation was reported as 94.9% purity, and the recovery rate was improved to be 54%.

In another study, Cen et al. developed an integrated system that combined three complementary techniques of dielectrophoresis (DEP), traveling wave dielectrophoresis (TWD), and electrorotation (ROT) on a single chip for the manipulation and characterization of human malignant cells [82,83]. The chip employs planar microelectrode arrays fabricated on a silicon substrate to facilitate the generation of non-uniform electric fields required for the controlled manipulation, measurement, and characterization of cells

from lymphoma and myeloma cell lines (Daudi and NCI-H929). In their experiments, Daudi and NCI-H929 cells were suspended in a dextrose/PBS medium (conductivity: 5.7–26.2 μS/m) and subsequently loaded into a microfluidic channel containing four sets of electrode arrays. The results showed that the membrane capacitance of the Daudi and NCI-H929 was lower when compared to the B-lymphocytes cell from healthy individuals. The authors of the paper postulated that the difference between the cell membrane capacitance of healthy and malignant cells came from the composition difference of their cell membrane (e.g., cell surface proteins) and suggested that the low membrane capacitance can be one of the striking characteristics of malignant cells. In addition, they demonstrated that the viable and nonviable lymphoma/myeloma cell lines could be separated by TWD at frequencies above 1 MHz.

Another DEP isolation strategy is called contactless DEP (cDEP) in which electrodes were isolated from the main microfluidic channel by a thin membrane to eliminate the direct sample-electrode contact [84–87]. For instance, Davalos's group has developed a cDEP device to isolate cancer cells from a diluted whole blood sample with a conductivity of 10–11.5 μS/m [84]. In their study, both analytical modeling and experimental observation demonstrated that the target cancer cells, THP-1, could be continuously isolated from diluted whole blood with a strong positive DEP in response to a frequency of 50–90 kHz. Meanwhile, the red blood cells experienced a negligible negative DEP response due to their relatively smaller complex permittivity and size. In a later study by this group, they further investigated the application of cDEP for isolating cancer cells from diluted plasma samples with the conductivity of around 12 μS/m [87]. Devices with a throughput of 0.2 mL/h (equivalent to sorting 3×10^6 cells per minute) were used to entrap breast cancer cells while allowing blood cells through the channel. They demonstrated that the cDEP technique can isolate cancer cells in concentrations as low as 1 cancer cell per 10^6 blood cells (equivalent to 1000 cancer cells in 1 mL of blood), and the recovery rate was 96%.

A continuous DEP field flow fractionation (DEP-FFF) approach was proposed by Gascoyne's group to characterize and capture cancer cells from peripheral blood mononuclear cells (PBMCs) in a rapid processing manner (Fig. 2B) [88–91]. In this approach, DEP, sedimentation (resultant force of gravity and buoyant force), and hydrodynamic lift forces act to control the position of cells in a hydrodynamic flow profile. Cells having different properties, and therefore, transit the chamber at different speeds and become fractionated. The direction of the DEP force, toward or away from the microelectrode plane during DEP-FFF, is determined by whether the field frequency is greater or less than the cell crossover frequency (the frequency where the DEP force makes the transition from a negative to a positive force). Since the crossover frequency for tumor cells, granulocytes, and lymphocytes were ∼30, ∼90, and ∼140 kHz in the dextrose/sucrose suspending medium with the conductivity of 0.03 S/m, an operating frequency of 60 kHz was applied to result in a positive DEP for tumor cells, and a negative DEP for the other cells. As a result, tumor cells were pulled toward the microelectrodes embedded on the bottom of the chamber, and PBMCs were repelled in the running buffer. Tumor cells then experienced steric retardation to slowly transit through the chamber while PBMCs would be carried away by the running buffer. Once the elution of PBMCs was completed, the DEP frequency was switch to 15 kHz to experience a negative DEP, and thus tumor cells were levitated into the flow stream and eluted from the chamber in just a few seconds.

Similarly, Moon et al. realized continuous separation of breast cancer cells (MCF-7) from blood samples using a passive hydrodynamic method combined with DEP, named multi-orifice flow fractionation (MOFF) [92]. Hydrodynamic separation took advantage of the massive and high-throughput filtration of red blood cells (RBC) and white blood cells (WBC), and DEP separation played a role in precise post-processing to enhance the efficiency of the separation. In this device, cells were moved laterally by hydrodynamic inertial forces driven by a multi-orifice structure, and the extent of lateral movement varies according to the size of the cells. As a result, cells with different sizes could be focused at different locations of the channel. After MOFF separation, the cells entered the DEP module for further purification. In their experiments, RBC, WBC, and MCF-7 were suspended in a working buffer with conductivity of 57 μS/m. With a 10 V_{pp}, 900 kHz applied electric field, MCF-7 cells were pulled upward along the electrodes, while RBC and WBC passed straight through the channel. Throughout the device, the separation efficiency of MCF-7 cells was 75.18%, and the separation efficiencies for RBC and WBC were 99.24% and 94.23%, respectively. The serial combination of MOFF and DEP sorting techniques enabled high-speed continuous flow-through separation.

In another study, an electrokinetic-based device was used for CTCs enrichment after selective capture with the immunoaffinity method (Fig. 2C) [93]. The microfluidic device was designed for high-throughput processing of whole blood with high recoveries of CTCs. The device comprises of CTC selective capture unit, the enumeration unit, and the electrokinetic manipulation unit. Anti-EpCAM antibodies immobilized on the surface of the device to capture CTCs by their specific EpCAM protein markers. The captured CTCs were then enzymatically released from the surface and hydrodynamically transported through a pair of platinum electrodes for conductivity-based enumeration. Afterward, CTCs were enriched in the collection reservoir under the combined effect of electrophoresis and pressure-driven flow, providing an enrichment factor of 500. The microfluidic device was able to process 1 mL of whole blood in less than 40 minutes at an optimized flow rate of 2 mm/s. Moreover, the enriched sample was free from leukocytes and erythrocytes, and the device had the ability to recover CTCs with the efficiency of 96% ± 4% from whole blood.

Altomare et al. developed a printed circuit board (PCB) device to manipulate tumor cells based on a software-controlled DEP platform (Fig. 2D) [94]. Depending on the dielectric properties of eukaryotic cells, different cells could

be "entrapped" in various DEP cages under the suitable electric potentials applied to the electrodes. Because the electric potential applied to the electrodes was controlled by the software, the movement of the cells could be manipulated by altering the code. The experimental data concluded that the PCB device allowed the manipulation of different tumor cells suspended in 280 mM mannitol, including chronic myelogenous leukemia K562, B-lymphoid Raji, T-lymphoid Jurkat, erythroleukemic HEL, and melanoma Colo38 cell lines.

Moreover, the commercialized ApoStream device has been developed to capture CTCs from blood using a DEP-based microfluidic flow chamber (Fig. 2E) [95]. In their experiments, PBMCs were spiked with prestained cancer cells in a buffer solution with a conductivity of 0.03 S/m. With an AC applied, cancer cells were affected by positive DEP forces and pulled towards the electrodes, while PBMCs were levitated by negative DEP into the hydrodynamic flow. This separation was accomplished by applying the voltage at a frequency 45–85 kHz, which was in between the DEP crossover frequency of cancer cells (30–40 kHz) and PBMCs (90–140 kHz). The system also enhanced the throughput by operating in continuous mode for efficient isolation and enrichment of CTCs from blood. The recovery rate was reported to be higher than 70%, and the viability of cells was more than 97.1%.

3 Cell-free nucleic acids

Cell-free nucleic acids (*cf*NAs), including cell-free DNA (*cf*DNA) and cell-free RNA (*cf*RNA), have been identified as circulating cancer biomarkers in the past few decades [96–98]. The presence of *cf*NAs in human blood was first reported by Mandel and Metais in 1948 [99]. After that, *cf*NAs have been studied in a wide range of physiological and pathological conditions, including pregnancy, trauma, inflammatory disorders, and malignancy [100]. Recently, *cf*NAs have been found to be present at higher concentration in the blood of patients with advanced-stage (stage II-IV) cancer rather than in healthy individuals [101,102], or patients with early-stage cancer (stage I) [103,104]. As a result, *cf*NAs have been widely studied as a prognostic biomarker in breast, lung, and colon cancers [105–111].

The conventional methods for *cf*NAs isolation from plasma, serum, and urine mostly rely on commercially available kits [8,9,112,113]. The majority of the available kits are designed based on the fact that special silicon matrix materials adsorb *cf*NAs in a certain high-salt buffer condition. Other commonly used methods include organic-based reagents (phenol/chloroform) and the ion-exchange binding based approaches [113–115]. However, the major drawbacks of these methods are high cost, long and tedious procedures [116]. Thus, more efficient and reliable methods need to emerge for purification of *cf*NAs from biofluids to be utilized as biomarkers for early-stage cancer diagnosis in the clinical settings.

3.1 On-chip electrokinetic based methods for isolation of *cf*NAs

To address the limitations of the conventional methods for the purification of *cf*NAs, electrokinetic technologies have recently been developed by several groups. One of the most popular techniques is the AC DEP separation method, in which the non-uniformity of the electric field can be generated by applying an alternating current across an array of electrodes [39,40]. This technology has been advanced for *cf*NAs isolation, and Heller's group made significant contributions to this field [22,116–126]. They have developed an AC electrokinetic device for purification of *cf*DNA that could directly work under high conductivity conditions using undiluted or minimally diluted blood, plasma, or serum (conductivity range of 0.7–1.2 S/m). For instance, they used an AC electrokinetic device (Fig. 3A) to rapidly purify *cf*DNA from 25 μL of unprocessed blood and plasma from chronic lymphocytic leukemia (CLL) patients within a total processing time of 10 minutes [116,118]. In their report, *cf*DNAs could be trapped at the DEP high-field region by applying 11 volts peak-to-peak (V_{pp}) at 10 kHz AC field for 3 min. After washing with 1×TE buffer, other blood components from the microarray were removed, and the fluorescently stained *cf*DNA was analyzed on-chip by fluorescence microscopy. In addition, the same group used the AC electrokinetic device to trap and isolate circulating cell-free DNA from CLL plasma samples to verify the existence of specific cancer mutations in the SF3B1, NOTCH1, and TP53 genes [117]. The *cf*DNA that recovered from the microarray was sufficient to allow for PCR amplification and sequencing for five of the 12 CLL patient samples, and the sequencing results matched those obtained from DNA extracted from CLL cells.

Insulator-based DEP (iDEP) is another electrokinetic technology that was used for isolating NAs and other cancer-related biomarkers. This technology can avoid issues prevalent in AC-based DEP methods including electrode fouling and undesirable electrode reactions [127–130]. Also, the electrodeless design reduces the complexity of the device and omits the costly metal deposition procedure in their fabrication. Chou et al. developed an iDEP device consisted of a constriction array etched on quartz substrate which could be used for concentrating the single-stranded and double-stranded DNA fragments from 0.5×TB-EDTA (TBE) buffer [127]. The results showed that there was a strong DEP response of the DNA molecules in the audio frequency range. The DEP force was depended on the length of the DNA fragments, and the longer fragments showed significantly larger DEP force due to their larger polarizability effect. Since there is a great dispersion of the DEP force with length, the potential application for this device could be selective trapping of the specific size of DNA fragments. Similarly, Li et al. developed a microchip with microchannels and nano-slits in between for DNA isolation (Fig. 3B) [131]. The results demonstrated that DNA molecules could be concentrated at the nano-slit in both 0.5× TBE buffer and 500 mM NaCl solutions. Moreover, the applied voltage was only 5V (10 V/cm), which was significantly

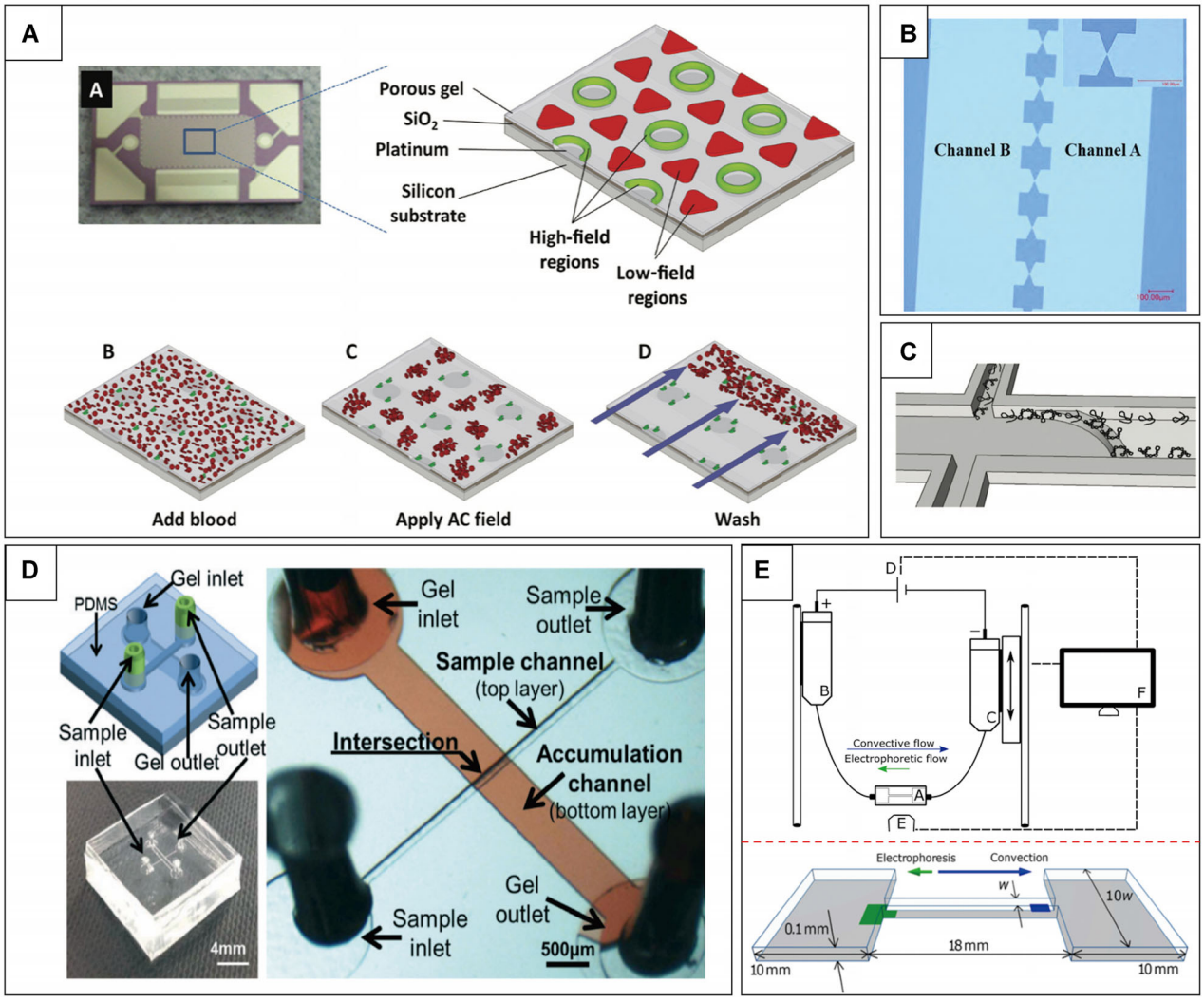

Figure 3. (A) AC electrokinetic microarray device operation developed by Heller's group for segregating *cf*DNA from blood. (B) Schematic illustration of nanofluidic device for DNA trapping developed by Li et al., which consists of two microchannels and nano-slits in between. (C) Schematic of the continuous-flow separation of DNA based on the conformation developed by Täuber et al. (D) Schematic illustration of the microfluidic device for rapid extraction and quantification of *cf*DNA developed by Yang et al. (E) Diagram of the experimental apparatus (top) and microfluidic device (bottom) for DNA trapping with the hybrid of convective flow and electrophoretic flow developed by Montes et al. (A) Reprinted with the permission from [116], copyright (2014) P.B. HOEBER. (B) Reprinted with the permission from [131], copyright (2015) AIP Publishing. (C) Reprinted with the permission from [135], copyright (2017) The Royal Society of Chemistry. (D) Reprinted with the permission from [138], copyright (2015) The Royal Society of Chemistry. (E) Reprinted with the permission from [140], copyright (2018) John Wiley and Sons.

lower than the other similar design. The low voltage could also suppress the Joule heating effect and make the device more stable.

Ros's group has also made significant contributions to DNA manipulation using the iDEP strategy [132]. They demonstrated the proof of concept for DNA separation based on DEP trapping in the high-field regions. In their study, a structured microfluidic device that consisted of an array of insulating rectangular posts has been fabricated. DNA molecules were injected into the channel filled with 10 mM phosphate solutions. After injection, the driving voltage was switched to a sinusoidal AC voltage to create a DEP potential landscape, superimposed by a static DC voltage component to induce directed electrophoretic motion of DNAs. The results showed that the device was able to perform efficient and fast DNA separation according to their lengths for two different DNA conformations: linear DNA and super-coiled covalently closed circular plasmid DNA (cccDNA). In addition, this device can separate DNA fragments with a size difference of 7 kbp (7 and 14 kbp), which was comparable to other microfluidic separation methods, such as magnetic bead arrays or entropic traps [133,134].

Furthermore, Täuber et al. fabricated a lab-on-chip device with an arc-shape insulating ridge in the channel for DNA

separation (Fig. 3C) [135]. In this device, DNA molecules were injected into the channel filled with 1 mM phosphate buffer. After injection, DC voltage was applied to induce the electrophoretic migration of the DNA towards the ridge; meanwhile, an AC voltage was applied over the interval to affect the DNA motion at the ridge by varying the frequency from 50 to 700 Hz. As a result, DNA fragments with different dielectric properties exhibited distinct DEP migration profiles that lead to separation. The results showed that the device had the capability to separate DNA fragments based on their size and topological variants (linear and super-coiled DNA conformation). In addition, they realized the separation of DNAs with a size difference of 16.7% (6.0 kbp and 5.0 kbp DNA molecules), which addressed the current limit of size resolution in the DEP-based DNA separation techniques.

Similar to the arc-shape insulating ridge design, the curved microchannel was another iDEP based strategy for DNA separation, in which the electric field achieves the maximum and minimum strengths near the inner and outer walls of the channel respectively due to the variation in path length of the electric field [136,137]. Parikesit et al. demonstrated that iDEP induced at the sharp corner of a U-turn-shaped channel could cause size-dependent trajectories of DNA macromolecules [137]. In their experiments, fluorescently stained DNAs were diluted with Milli-Q purified water, and the motion of the DNA molecules was observed under the microscope. The numerical simulation of the electrokinetic force distribution inside the channels was in qualitative agreement with their experimental observation. Compared to other iDEP methods, the device continuous operation allowed for high throughput analysis of DNA with lower applied electric field (10 V/cm) to avoid denaturation of the DNA.

Besides DEP, electrophoresis on a chip has also been used for DNA separation. For example, Yang et al. demonstrated a low-cost microfluidic device capable of rapid quantification of *cf*DNA in a small droplet (<10 μL) of plasma and whole blood in 5 minutes (Fig. 3D) [138]. In this study, *cf*DNA samples were spiked into the whole blood or blood plasma from both healthy donors and severe septic patients. After the sample loading, *cf*DNA was successfully extracted and concentrated by electrophoresis with a 9V DC applied across the channel filled with 1% agarose gel. Moreover, Marshall et al. developed an isotachophoresis (ITP) assay for pathogenic nucleic acids extraction from the whole blood spiked with Plasmodium falciparum [139]. The device is able to automatically mix, lyse, and extract nucleic acid into a downstream reservoir. The system's integrated heaters were used to lyse and mix the samples simultaneously. After lysing, the nucleic acids were extracted into a secondary buffer (50 mM Tris titrated with 50 mM HEPES and 1 μL of protein K) via ITP. The results showed that the device realized up to 90% lysis efficiency and achieved a clinically relevant limit of detection of 500 parasites per microliter.

Another strategy for *cf*DNA isolation is the integration of electrokinetics with other mechanisms such as pressure-driven flow and polyelectrolyte migration. For instance, Montes et al. reported a microfluidic device for DNA isolation using a mechanism that combines polyelectrolyte migration with electrophoretic recirculation (Fig. 3E) [140,141]. In their experiments, fluorescently stained λDNA were diluted in a buffer solution containing 0.25× Tris-EDTA (TE) and loaded into a microfluidic device. With suitable combinations of geometry, pressure, and voltage, long DNA molecules (>10 kbp) could be trapped within a small volume close to the inlet. Experimental observations confirmed that the rapid accumulation of DNA at the inlet is caused by an outward migration of polyelectrolyte towards the capillary boundaries, followed by electrophoresis of DNA within the stagnant fluid layer next to the walls.

4 Exosomes

Exosomes are nanoscale membrane vesicles (30–150 nm) secreted by all cells and can be found in biofluids including blood [142,143], saliva [144], urine [145], breast milk [146], human semen [147], as well in cerebral fluids [148]. Recent studies have shown that exosomes play important roles in cell-cell communication by carrying a wide variety of functional proteins, mRNAs, and miRNAs [149,150]. Exosomes could either fuse with the target cell to transfer the proteins and RNAs to the recipient cell through endocytosis or bind the target cell through receptor-ligand interaction [151]. Intercellular communication mediated by exosomes not only plays a role in the regulation of normal physiological processes but also in pathological processes of many diseases, including cancer [152–156]. Because of exosomes' presence in most biofluids and the resemblance of their contents to the parental cells, exosomes have great potential as the biomarkers for early diagnosis for various diseases [157,158]. In particular, cancer-derived exosomes likely serve as a circulating biomarker for early-stage cancer diagnosis since they carry the contents reflecting the genetic or signaling alterations in their parental cells [159–161].

Differential ultracentrifugation (DU) is currently considered the gold standard for exosome isolation [6], which relies on multiple centrifugation steps to sequentially remove impurities based on their size and density [162]. However, this technique is highly time-consuming (> 6 h), requires significant capital investment and large sample volumes, and causes damage, fusion, and/or aggregation of vesicles [162–167]. In addition, the DU method requires a well-optimized protocol. Unfortunately, there is no standardization of these protocols. Laboratories are using different pre-processing protocols to remove cell debris, as well as different protocols for ultracentrifugation itself. Although many attempts have been made to keep the spin time and centrifugal force consistent, the pelleting efficiencies vary since different laboratories often use different rotors and different sample dilution steps [162,168]. Another technique for the purification of exosomes is the density-gradient ultracentrifugation, in which the sample is spun in a tube that contains a density gradient of viscous material and thus, the objects are separated based on their buoyant density [169]. Although this technique can

achieve higher purity and recovery rate than DU, it cannot separate exosomes from viruses or microvesicles due to their similar buoyant density [170].

Besides the ultracentrifugation, ultrafiltration and size exclusion chromatography (SEC) have been used for exosomes purification. The fundamentals of ultrafiltration are same as the conventional membrane filtration in which the suspended particles can be isolated depending on their size or molecular weight. The ultrafiltration technique is faster than ultracentrifugation and does not need special equipment; however, it often requires applied shear stress, which may result in the deformation and denaturation of vesicles and potentially skew the results of downstream analysis [171]. SEC separates molecules or particles based on their size by filtration through a gel. The gel consists of spherical beads containing pores of specific size distribution. Small molecules/particles can diffuse into the pores and have more volume to traverse and elute later in the process. On the contrary, large macromolecules/particles, including exosomes, do not enter the pores and therefore elute sooner. In this manner, exosomes could be separated from other vesicles and contaminants of different sizes. [171,172] However, the results usually become considerably diluted, and efficient separation of all lipoproteins remains a challenge [165,173,174]. Moreover, this technique requires dedicated equipment and expensive chromatographic sorbents [6,11].

Current state-of-the-art exosome precipitation kits provide an easy-to-use method to isolate exosomes from biological fluids. Several commercially available precipitation kits have been developed, which include ExoQuick™, Exospin™, Total Exosome Isolation Reagent from Invitrogen™, ExoPrep, PureExo Exosome Isolation kit, and miRCURY™ Exosome Isolation Kit [5]. Typically, these commercial products use special reagents (e.g., polymeric additives) to decrease the solubility of exosomes and lead to precipitation. The procedure usually contains mixing of the sample and polymer solution, incubation, and sedimentation of exosomes by low-speed centrifugation. The advantages of precipitation kits include the possibility of working in the physiological pH range and their independence on the ionic concentration. In addition, exosome precipitation kits are easy to use and do not require any specialized equipment [171], which allows easy integration into clinical usage by exploiting existing technologies [6]. However, this technique has several disadvantages including, the need for multiple incubation steps ranging from several hours to overnight [160,175], the co-precipitation of other non-exosome contaminants (such as proteins and polymeric materials) [176], and the fact that residual matrix can affect exosomes' biological function [177].

The immunoaffinity-based method is another isolation technique, which relies on the antibodies that bind with the surface antigens of exosomes. The immunoaffinity isolation method can effectively isolate specific exosome populations. In addition, compared to the ultracentrifugation method, the immunoaffinity capture needs lower sample volumes (usually less than 100 μL) [6,164]. However, it suffers from the high reagents cost and the dependency on the antibodies' selectivity, specificity, and the affinity/binding constant that may exclude the important exosomes population [6,14,178].

4.1 On-chip electrokinetic based methods for isolation of exosomes

In recent years, on-chip electrokinetic-based methods have gained a lot of attention for exosome isolation due to their rapid and label-free criteria. For example, the alternating current electrokinetic (ACE) microarray chip could rapidly isolate and recover exosomes from highly conductive undiluted human plasma (conductivity: 0.7–1.2 S/m) in less than 30 min (Fig. 4A) [178]. Exosomes were isolated and concentrated in the DEP high-field regions around the edges of the microelectrodes after 10 minutes of applying an alternating current (AC) at 10 V_{pp} and 15 kHz. With the AC field still on, the exosomes remained concentrated in the DEP high-field regions, while a 1×TE buffer wash was used to remove the bulk plasma and any other materials collected in the DEP low-field regions of the ACE chip. In addition, the same platform has been used for the isolation and characterization of exosomes derived from plasma specimens from patients with brain and pancreatic tumors [179,180]. The on-chip immunofluorescent analysis showed distinguishable results between exosomes derived from blood sample of patients and healthy individuals. They also found that the AC electrokinetic chip assay readily discriminated those colon cancer samples with highly aggressive metastatic phenotypes from those with less aggressive phenotypes. Thus, this method has the potential utility in differentiating early-stage and advanced-stage cancers. With further validation, the integrated AC electrokinetic biomarker assay has the potential to be translated to clinical use as a rapid, sensitive, noninvasive blood test for different cancers.

Utilizing a similar principle, Chen et al. developed another AC DEP exosome isolation chip with interdigitated electrodes [181]. In their study, exosomes harvested from A549 lung cancer cell line were spiked into the exosome-deleted plasma and diluted four times with 8.5% sucrose solution. The results showed that exosomes could be isolated in 30 min with higher recovery efficiency (>83%) compared to the conventional ultracentrifugation method. To facilitate the subsequent analysis, this device integrated exosome isolation with *in situ* lysis module, in which a square wave electrical field (1 V, 10 Hz, 2 min) was used for electroporation of exosomes. They found that exosomes isolated from the plasma of lung cancer patients contained higher levels of miRNA (miR-21, miR-191, and miR-192) and exosomal protein markers (CD81, HSP70, and EGFR) compared to those from healthy subjects. Overall, this study provided an efficient and practical approach to the isolation and detection of exosomes, which could be used for the early diagnosis of lung cancer.

Besides AC DEP strategy, iDEP is another label-free technique that has been used to isolate exosomes from biofluids. Our group has recently demonstrated the capability of a glass nanopipette to isolate exosomes from biofluids with the

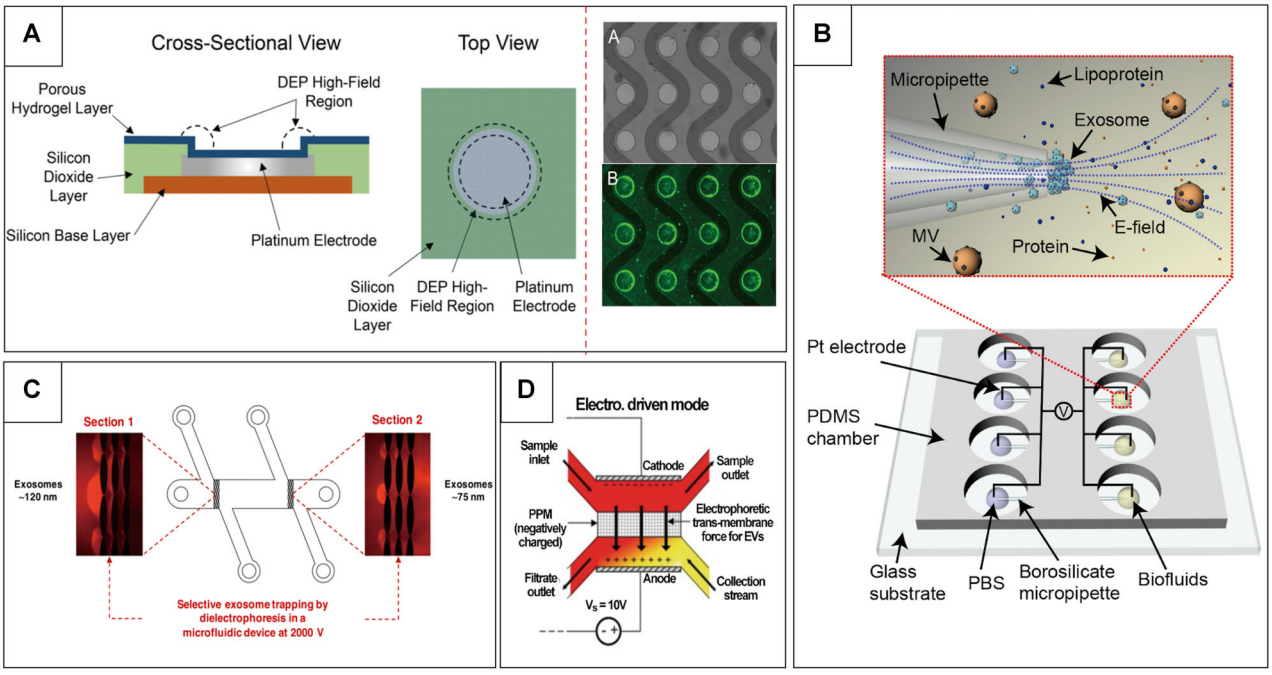

Figure 4. (A) Schematic of the microelectrode array chip used for rapid exosome isolation developed by Ibsen et al. (B) Schematic of the electrokinetic nanopipette device for rapid exosomes isolation developed by Esfandiari's team. (C) Schematic illustration of the DC-driven insulator-based DEP microfluidic device for capture and separation of exosomes developed by Ayala-Mar et al. (D) Schematic of the electrophoresis-driven filtration for exosome isolation developed by Davies et al. (A) Reprinted with the permission from [178], copyright (2017) American Chemical Society. (B) Reprinted with the permission from [23], copyright (2019) The Royal Society of Chemistry. (C) Reprinted with the permission from [183], copyright (2019) American Chemical Society. (D) Reprinted with the permission from [143], copyright (2012) The Royal Society of Chemistry.

force balance of three electrokinetic forces, including DEP, electrophoresis (EP), and electro-osmosis (EO) [182]. With a low DC voltage (10 V/cm) applied across the pipette, a non-uniform electric field was induced across the conical pore, and thus, the DEP force was applied on the suspended exosomes. Also, because the exosomes are negatively charged, the EP force toward the anode was induced. Another electrokinetic force affecting the exosomes in the solution is an electro-osmosis flow (EOF) toward the cathode introduced by the negatively charged glass pipette. By adjusting the pore size and electric field strength, the three electrokinetic forces were balanced at the pipette's tip and created a trapping zone. This device was further improved by using an array of parallel nanopipettes to enhance the trapping efficiency (Fig. 4B), and we have demonstrated that the nanopipettes platform was capable of rapid purification of exosomes directly from 200 μL of highly conductive undiluted plasma, serum, saliva, and cell culture media within 20 min [23]. The yield of purified exosomes was around two orders of magnitude higher compared to the traditional differential ultracentrifugation method. This simple to fabricate device omits the need for specialized equipment and extra reagents while maintaining the high yield and good purity of exosomes extracted from biofluids.

Another iDEP device was developed by Ayala-Mar et al. (Fig. 4C) [183,184]. In their work, a DC-driven iDEP microfluidic device was developed to isolate exosomes within 20 s. This device contained a channel with two different arrays of oval-shaped electrically insulating post sections, in which the gap distance for the first and second section were 15 μm and 10 μm, respectively. Each section was tailored to generate different non-uniform electric field distribution, and thus, different DEP forces were applied on exosomes suspended in bi-distilled water. By applying an electric potential difference of 2000 Volts across the device main channel (1 cm in length), exosomes with a mean diameter of 113.23 ± 10.34 nm were dielectrophoretically captured in the first section, while exosomes with a mean diameter of 72.86 ± 8.71 nm were captured in the second section. Afterward, the trapped exosomes were released and collected by the electroosmotic flow through the side channels.

Moreover, Davies et al. developed a different approach to isolate exosomes by sieving exosomes from whole blood through a porous membrane embedded in a microfluidic device (Fig. 4D) [143]. Using the membrane as a size-exclusion filter, exosomes were separated from cells and large debris by injecting whole blood under pressure through the channel. To enhance isolation purity, DC electrophoresis was employed as an alternative driving force to propel particles across the filter and increase the separation efficiency of exosomes from proteins. In this configuration, 25× Tris-acetate-EDTA (TAE) buffer was added to the blood sample in a volume ra-

Table 1. Previously reported cancer-associated protein markers in biofluids and their applications

Biomarker	Cancer type	Reference
α-Fetoprotein (AFP)	Testicular, germ cell tumors, HCC	[195,199,200]
Bladder tumor-associated antigen (BTA)	Bladder	[195] [199]
Cancer antigen 125 (CA125)	Ovarian, fallopian tube	[195,199–201]
Cancer antigen 15-3 (CA15-3)	Breast	[195,199,200,202]
Cancer antigen 19-9 (CA19-9)	Colon, pancreatic	[195,199,200,202]
Cancer antigen 27–29 (CA27-29)	Breast	[185,195,199,202]
Cancer antigen 242 (CA242)	Colon	[202]
Carcinoembryonic antigen (CEA)	Colon	[203]
Fibrin degradation protein (FDP)	Bladder	[195,199,204]
Heat shock protein 27 (HSP27)	Gastric	[205]
Heat shock protein 70 (HSP70)	Prostate, bladder	[206,207]
Heat shock protein 90 (HSP90)	Gastric	[205]
Human chorionic gonadotropin-β (hCGβ)	Testicular	[195,199] [202]
Human epidermal growth factor receptor 2 (HER-2)	Breast	[195,199] [202,208–211]
Epidermal growth factor receptor (EGFR)	Colon	[212,213]
KIT	GIST	[214]
Thyroglobulin (Tg)	Thyroid	[96] [195] [211]
Cytokeratins	Breast	[215]
Lactate dehydrogenase (LDH)	Testicular cancer	[216,217]

tio of 1:25, while the collection stream consisted of 1× TAE. DC electrophoresis propelled exosomes through the fabricated membrane while sample and collection streams were injected at equal flow rates of 2 μL/min. The results demonstrated that the electrophoretic-driven filtration could selectively eliminate some soluble proteins and isolate exosomes with higher purity compared to the pressure-driven filtration. In addition, they isolated exosomes from the whole blood of melanoma-grown mice and performed RT-PCR to verify their contents of RNA. The results showed that Melan A mRNA derived from melanoma tumor cells were found enriched in filtered samples, confirming the recovery of exosomes via their cargo.

5 Cancer-associated proteins

Proteins and other macromolecules released by cancer cells in the extracellular fluids can also serve as biomarkers for diagnosis. Protein-based biomarkers, including peptides, globular proteins, fibrous proteins, and membrane proteins, can be obtained from various biofluids and have been used for screening cancer for decades [2,185,186]. For example, prostate-specific antigen (PSA), a 33 kDa serine protease, is the most widely studied biomarker for early detection of primary prostate cancer [2]. The other most commonly used cancer-associated protein markers are cancer antigens 1 (CA125, CA15-3, CA19-9), carcinoembryonic antigen (CEA), alpha-fetoprotein (AFP), and heat shock proteins (HSP27, HSP70, HSP90) and more, which are listed in Table 1 [185,187–193]. Among those protein biomarkers, AFP, human chorionic gonadotropin-β (hCGβ), and lactate dehydrogenase (LDH) have been formally used in the TNM staging system, which is promulgated by the American Joint Committee on Cancer (AJCC) [194]. Other proteins, although not formally used for staging, are important for prognosis and selection of therapy. For instance, the human epidermal growth factor receptor 2 (HER-2) and cytokeratins can be used to refine the prognosis of breast cancer. HER-2, Epidermal growth factor receptor (EGFR), and KIT are used clinically to predict if breast cancer, colon cancer, or Gastrointestinal Stromal Tumors (GIST) will respond to trastuzumab, cetuximab, or imatinib, respectively [195].

Although protein biomarkers offer tremendous potential for early-stage cancer detection due to their great diversity and close involvement in physiology [196], the major limitation of using them in clinical application is the lack of specificity, meaning that the elevated level of one specific protein marker could be associated with multiple types of cancer. For example, a high level of AFP in the serum is associated with the germ cell tumors, hepatocellular carcinoma (HCC), gastric, pancreatic, colonic, and bronchogenic carcinomas [96,197]. Thus, to overcome this issue, some studies proposed to use cocktails of antibodies or panels of protein-based biomarkers to increase the accuracy and specificity of diagnosis [198].

The conventional methods used for the isolation of cancer-related protein markers are mostly based on different chromatographic approaches, including ion-exchange, size-exclusion, hydrophobic, reverse-phase, and affinity chromatography [218–221]. Since proteins do not have a generalized purification method, the type of chromatography to be used depends entirely on the physical and chemical properties of the target proteins. Thus, they could be isolated by using either a single chromatography method or combined chromatographic approaches sequentially [222]. Although proteins with high purity could be obtained by using

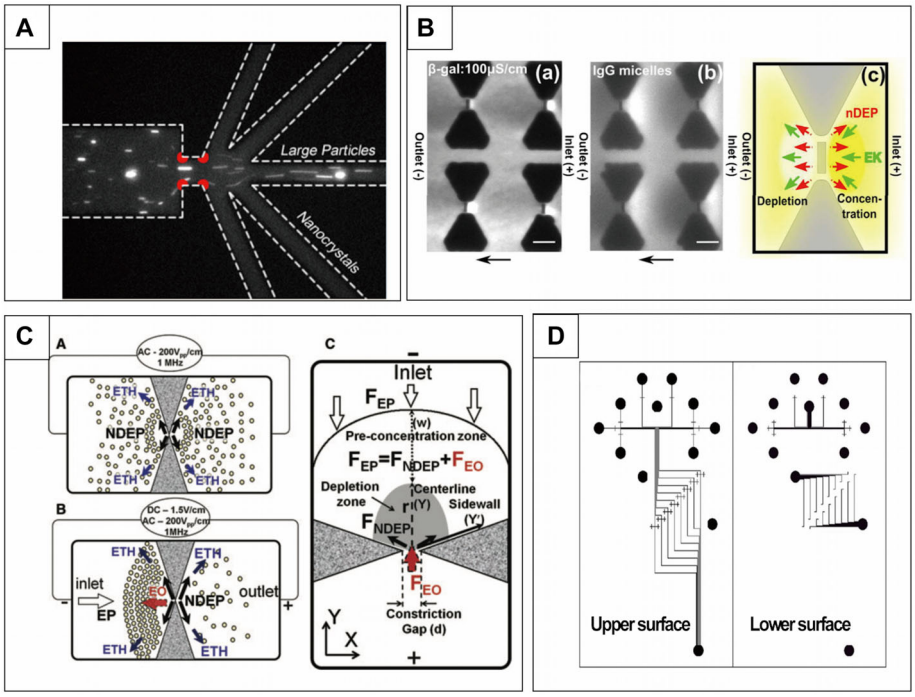

Figure 5. (A) The illustration of the microfluidic device for sorting membrane protein nanocrystals developed by Abdallah et al. (B) Fluorescence microscopic images and the working principle illustration for the iDEP concentration of proteins at the inlet side of insulating nanoposts. (C) Schematic of protein preconcentration through an electrokinetic force balance on a nanoconstriction device reported by Liao et al. (D) Layout of the multilayer polymer microchip capillary array electrophoresis devices developed by Yu et al. (A) Reprinted with the permission from [128], copyright (2013) American Chemical Society. (B) Reprinted with the permission from [129], copyright (2015) The Royal Society of Chemistry. (C) Reprinted with the permission from [231], copyright (2012) John Wiley and Sons. (D) Reprinted with the permission from [236], copyright (2011) American Chemical Society.

a proper chromatography, there are several disadvantages associated with this method, including being time-consuming, and requiring a large sample volume [223]. Therefore, on-chip electrokinetic-based methods have been recently used for protein purification from biofluids because of their simple, rapid, small sample volume requirements, and multifunctional properties.

5.1 On-chip electrokinetic based methods for isolation of cancer-associated proteins

Although DEP has been discovered by Pohl in 1951 [38], the pioneering work on protein manipulation with DEP was reported during the 1990s and early 2000s by Washizu and Morgan groups [224–226]. The late development of DEP application for manipulating macromolecules was due to the belief that the dielectrophoretic effect on submicron particles is impossible because of the dominant Brownian motion. In 1994, Washizu's group fabricated micrometer-sized electrodes and manipulated the avidin (68 kD) and other biomolecules by DEP forces [227]. They observed that DEP occurred at the electric field of 0.4×10^6 to 1×10^6 V/m, which was substantially lower than the DEP strength that was theoretically predicted. Therefore, the DEP force on submicron particles was substantially underestimated, and it was possible to manipulate macromolecules utilizing the DEP force. A stable protein entrapment with DEP was demonstrated by Morgan's group in 1998, in which the microfabricated electrodes were introduced to generate high electric field gradients [228]. The electric field was generated using the quadrupole "polynomial" electrodes design that was suitable for observing both positive and negative DEP forces. In this design, four electrodes centro-symmetrically arrayed on the same plane that leaved a gap area in the center with a diameter of 6 μm. The highest field intensity occurred at the edge of the electrodes, and the lowest intensity at the center of the electrode array. This study for the first time reported, the observation of negative DEP induced on macromolecules which can be used to manipulate and entrap them.

In the past two decades, more interests in protein manipulation with DEP have been emerged because of the increasing demand for rapid protein analysis tools as well as advancement in micro-and nano-fabrication techniques [128,129,229–231]. For instance, Abdallah et al. developed a microfluidic device to sort membrane protein crystals based on their size using a DEP device (Fig. 5A) [128]. They also demonstrated the sorting performance of their approach utilizing a numerical simulation which was in excellent agreement with their empirical results. Nakano et al. developed an iDEP device for protein isolation with nanometer-size features (Fig. 5B) [129,232]. This work showed that β-galactosidase proteins and immunoglobulin G (IgG) micelles suspended in a phosphate buffer solution with a conductivity of 0.01 S/m could be concentrated at the inlet of nano-constrictions by an induced negative DEP force on macromolecules. They also investigated the voltage dependence of β-galactosidase protein concentration, and the results indicated that the maximum protein concentration at the inlet of nano-constrictions could be obtained by applying a 200V potential.

In addition, Liao et al. reported a nano-constriction device for rapid protein pre-concentration in physiological media (conductivity: 1.6 S/m) through a balance of electrokinetic

forces (Fig. 5C) [231]. They found that the addition of a DC field offset (1.5 V/cm) to the negative DEP force (~200 V_{pp}/cm at 1 MHz) resulted in the tilt of the potential profile to cause deep potential energy wells for rapid protein concentration. In the following study, they further decreased the constriction gap distance from 50 nm to 30 nm and quantitatively studied the enrichment efficiency of proteins using the same scheme (DC-offset + negative DEP) [230]. They also reported that a protein enrichment factor of $>10^5$ was achieved in 20 s, which was orders of magnitude faster than most of the reported methods. In another study by Rohani et al., a similar device was developed to selectively enrich PSA from PBS media with conductivity of 1.6 S/m containing anti-mouse immunoglobulin (IgG) protein [233]. In this device, PSA was selectively enriched $\sim 10^3$-fold in 30 s as a DC field offset (1.5 V/cm) added to the AC field (70 V_{rms}/cm and 4–6 MHz).

In recent years, Woolley's group developed a microfluidic system coupled with immunoaffinity purification module and a rapid microchip electrophoresis separation module in a laser-induced fluorescence detection system to isolate and quantify an aforementioned cancer-associated protein (AFP) [234]. In conjunction with the laser-induced fluorescence detection, the microfluidic system could quantify AFP at 1 ng/mL level from ~10 μL of human serum in tens of minutes. Later on, the microfluidic system was improved by the same group, which could simultaneously quantify multiple cancer-associated proteins from human serum [235]. The simultaneous detection of multiple markers enabled more sensitive and accurate cancer screening with higher throughput. Likewise utilizing capillary electrophoresis, Yu et al. reported a multilayer poly(methyl methacrylate) (PMMA) microchip comprised of an array of capillary electrophoresis system with integrated on-chip fluorescence detection function (Fig. 5D) [236]. On this microchip, there are eight parallel lanes, allowing up to eight different samples to be labeled and separated simultaneously. All parallel electrophoresis units share the same electrophoresis reservoirs (buffer inlet, buffer waste, and separation waste) and a dye labeling reservoir, offering a scalable solution to integrate a high-throughput analysis. They also determined the detection limit of the device for detection of a heat shock protein 90 (HSP90) which is a gastric-cancer-related protein. The results showed that the detection limit was 600 ng/mL, which was about a twofold improvement when compared to their previous work [237]. They claimed that the detection limit could be further improved to at least ten-fold to reach the normal serum HSP90 levels (~ 20 ng/mL) by optimizing their design and experimental set-up.

6 Concluding remarks

Currently, the early-stage cancer diagnosis relies on either an invasive tissue biopsy or expensive imaging techniques; and thus, liquid biopsy has gained a great deal of attention. Tumor-derived exosomes, circulating tumor cells, cell-free DNAs and RNAs, and cancer-related protein biomarkers have all provided essential information with regards to cancer diagnosis and prognosis in liquid biopsy-based approaches. However, the conventional isolation methods of these circulating biomarkers, including ultracentrifugation, immuno-affinity assay, and chromatography, usually have the drawbacks of long processing time, complex procedures, and large sample volume requirements. On the contrary, the use of on-chip electrokinetic-based devices has been on the rise due to the advantage of rapid, label-free, much smaller sample volume requirement, and minimal damage to the biomarkers. Therefore, here we focused on advancement in emerging on-chip electrokinetic technologies for isolation and purification of cancer-related biomarkers from biofluids which are summarized in Table 2.

Although many attempts have been made to develop on-chip electrokinetic-based devices for the purification of cancer-related biomarkers, technologies that can isolate the circulating biomarkers with a high recovery rate and purity from low sample volume still are in demand. In addition, there are a few other research directions in the field that the authors consider worthy of future studies. Firstly, to further simplify the whole procedure and give a quick diagnosis result, a discrete module for biomarker detection is expected to be combined with the isolation module. Also, since one biomarker may be related to multiple types of cancer and the co-isolated biomolecules may cause false positive or convoluted results, simultaneous isolation and detection of multiple biomarkers with a multiple channel design could potentially improve the diagnostic accuracy and efficiency. Moreover, a low electric field is required to keep the integrity of biomarkers. Current electrokinetic-based, especially iDEP-based devices, often require a very high electric field (hundreds to thousands V/cm) for an effective isolation, which could potentially cause the target biomarkers denaturation. Thus, a promising solution could be to consider novel methods that enhance the spatial extent of the high field region, such as using nanopipettes to confine the electric field in a small pore region (e.g., Ref. [23]), using dispersed propelling electrokinetic traps [238], or combining ion concentration polarization methods in nanochannels with AC electrokinetics (e.g., Refs. [231] and [233]). Besides, it is important to isolate biomarkers directly from the biofluid samples. Currently, most of the DEP-based approaches were carried out in the suspending medium with a very low conductivity ($\ll$0.1 S/m) to obtain a positive DEP response (as shown in Table 2). As a result, the important clinical samples such as whole blood, plasma, serum, urine, and saliva with high conductivities (0.5–1.2 S/m) require to be significantly diluted before the DEP trapping can be carried out, which represents a serious limitation for using these techniques as a viable diagnostic tool. One of the promising solutions could be combining different electrokinetic strategies (such as AC-DEP, iDEP, EOF, and EP) on a single chip to trap biomarkers with multiple electrokinetic force balance (Refs. [23] and [230,231]) rather than relying on a single force such as positive DEP. Furthermore, to improve the purity of the markers, the electrokinetic-based methods

Table 2. Summary of on-chip electrokinetic methods for isolation and purification of cancer-associated biomarkers

Sample Type	Technology	Voltage (V_{pp}) @ Frequency range (kHz)	Medium conductivity (S/m)	Medium Composition	Other results	Ref.
MDA231 breast cancer cell	AC DEP	5 @ 200	0.01	8.5% sucrose, 0.3% dextrose, and EDTA	Sorting rate: $\sim 10^3$ cells/sec	[80]
PC-3 prostate cancer cells	ODEP	2–10 @ 100	3.4×10^{-4}	250 mM sucrose	Purity: 100%; Recovery yield: 41.5%	[81]
PC-3 prostate cancer cells	ODEP	5 @ 100	3.4×10^{-4}	250 mM sucrose	Purity: ~94.9%; Recovery yield: ~54%	[21]
Daudi and NCI-H929 cancer cells	DEP, TWD, and ROT	5 @ >1000	$5.7–26.2\times10^{-3}$	5% dextrose and PBS	The membrane capacitance and membrane permittivity of cancer cell were reported	[82]
THP-1 and MCF-7 cancer cells	cDEP	~231 @ 50–90	$10–11.5\times10^{-3}$	8.5% sucrose, 0.3% glucose, and 0.725% RPMI	The device can continuously sort human leukemia cells from diluted blood samples	[84–86]
MDA-MB-231 breast cancer cells	cDEP	300 @ 600	1.2×10^{-2}	Diluted plasma	Throughput: 0.2 mL/hr; Recovery rate: 96%	[87]
MDA-MB-435, 468, 231tumor cells	DEP-FFF	10 @ 60	0.03	9.5% sucrose, 0.3% dextrose, and 0.1% Pluronic F68	Isolation is completed within ~15 min; Recovery yield: >90%	[88,89]
MDA-MB-231, HL-60 cancer cells	DEP-FFF	2.8 @ 15–200	0.03	Sucrose buffer and KCl	Throughput: $>10^6$ cells/min; Recovery yield: 92%	[90,91]
MCF-7 breast cancer cell	MOFF and DEP	10 @ 2000; 10 @ 900	0.057	8.5% sucrose, 0.3% dextrose, and PBS	Enrichment factor: 162.4; Recovery yield: 75.81%	[92]
SW620 and HT29 colorectal cancer cell	Antibody selection, EOF and EP	100 V/cm @ DC	NA	50mM PBS and Tris-glycine buffe	Enrichment factor: 500; Purity: 100%; Recovery yield: 96%	[93]
K562 human chronic myelogenous leukemia cell	Software controlled DEP	6 @ 100	NA	280 mM mannitol	nDEP and pDEP frequency range for different cells were reported	[94]
SKOV3 ovarian cancer cell, MDA-MB-231 breast cancer cell	DEP in a microfluidic flow chamber	2–4.5 @ 45–85	0.03	9.5% sucrose, 0.01% dextrose, 1mM PBS, and PBS	Viability: >97.1%; Recovery yield: >70%	[95]
*ct*DNA from CLL patient blood samples	AC electrokinetic	11 @ 10; 12 @15	0.7–1.2	Unprocessed blood; plsama	Processing time: < 10 min	[116–118]
Double stranded DNA and single-stranded DNA	Electrodeless DEP (eDEP)	5 @ 0.2–1	NA	0.5× TBE buffer	The origin of the low-frequency DEP force in DNA was discussed	[127]
DNA with size of 2 kbp	eDEP	10 V/cm @ DC	>1	500 mM NaCl	The trapping mechanism of DNA was discussed	[131]
Linear DNA and cccDNA (7–164 kbp)	AC iDEP	150–420 @ 0.03–0.2	NA	10 mM phosphate solution	DNA separation based on their length was reported	[132]
Linear DNA with length of 10.0, 8.0, 6.0, and 5.0 kbp; cccDNA	Arc-shaped insulating ridge DEP	50–900 @ 0.05–0.7	NA	1 mM phosphate buffer	Size difference of 16.7% of DNA sample separation was reported	[135]
λ (48.5 kbp) and T4GT7 (165.6 kbp) DNA	Curved microchannel DEP	10 V/cm @ DC	NA	Purified water mixed with 2% 2-mercaptoethanol	Mechanism of confined DNA dielectrophoresis was discussed	[137]

(*Continued*)

Table 2. (Continued)

Sample Type	Technology	Voltage (V_{pp}) @ Frequency range (kHz)	Medium conductivity (S/m)	Medium Composition	Other results	Ref.
1 μg/ml DNA-spiked plasma sample	EP	9 V @ DC	NA	1% agarose gel	On-chip quantification was completed in 5 min	[138]
Nucleic acids	ITP assay	NA	NA	Blood mixed with lysis buffer and trailing electrolyte buffer	A clinically relevant limit of detection of 500 parasites per microliter was achieved	[139]
λ DNA (48.5 kbp)	Polyelectrolyte migration and EP	250 @ DC	NA	0.25× Tris-EDTA	Trapping is completed within 2 hours; Recovery yield: ∼100%	[140]
Glioblastoma exosomes	AC electrokinetic	10 @ 15	0.7–1.2	0.5× PBS and plasma	Total processing time: < 30min	[178]
Exosomes harvested from glioblastoma, metastatic, and meningioma cell lines	AC electrokinetic	14 @ 15	0.7–1.2	Plasma	Tumor exosomes were detected by on-chip immunofluorescence; Recovery yield: 60–70%	[179]
Exosomes harvested from the blood of pancreatic cancer patient	AC electrokinetic	14 @ 15	0.7–1.2	0.5× PBS and plasma	Cancer patient samples could be distinguished from healthy subject with 90% sensitivity and 82% specificity	[180]
Exosomes harvested from A549 lung cancer cell line	AC DEP	2–10 @ 2–10	NA	Diluted plasma	Purity is higher than ultracentrifugation (UC); Recovery yield:>83%	[181]
Exosome from plasma, serum, saliva, and cell culture media	iDEP	10V/cm @ DC	0.5–1.2	Plasma, serum, saliva, and cell culture media	Trapping yield is higher than DU; Processing time < 30 min	[23]
MCF-7 breast adenocarcinoma exosomes	iDEP	2000 @ DC	0.0014	Bi-distilled water	DEP size-based separation was observed	[183,184]
Exosomes from the whole blood of mice	EP and filtration	10 @ DC	NA	Mouse blood mixed with TAE buffer	Purity is similar to UC	[143]
β-galactosidase proteins and immunoglobulin G (IgG) micelles	iDEP	200 @ DC	0.01	Phosphate buffer	DEP behavior of protein in dependence of the applied potentials was investigated	[129]
Fluorescently labeled streptavidin protein	AC DEP with DC offset	∼200 V_{pp}/cm @ 1000; 1.5 V/cm @ DC	1.6	∼150 mM salt	The mechanism of DC field offset under AC negative DEP was discussed	[231]
Alexa-488-labeled streptavidin, and goat anti-human IgG protein	AC DEP with DC offset	214 V_{pp}/cm @ 1000; 1.5 V/cm @ DC	1.6	10 mM PBS mixed with 150 mM NaCl	Protein enrichment factor of $>10^5$ was achieved in 20s	[230]
PSA protein	AC DEP with DC offset	70 V_{rms}/cm @ 4000–6000; 1.5 V/cm @ DC	1.6	Supporting electrolyte of PBS (0.1 M)	PSA was selectively enriched $\sim 10^3$-fold in 30s	[233]

(*Continued*)

Table 2. (Continued)

Sample Type	Technology	Voltage (V_{pp}) @ Frequency range (kHz)	Medium conductivity (S/m)	Medium Composition	Other results	Ref.
AFP protein	Microchip EP separation	600–2000 @ DC	NA	Dimethyl sulfoxide (DMSO) mixed with serum	AFP can be quantified at ~1 ng/mL levels in ~10 μL of human serum in a few tens of minutes	[234]
Fluorescently labeled AFP, CytC, HSP90, and CEA protein	Microchip EP separation	400 @ DC	NA	DMSO mixed with serum	The capability of simultaneously quantifying four protein biomarkers with low concentration was reported	[235]
HSP90 protein	Microchip capillary EP separation	1000–1600 @ DC	NA	2.5 mM borate buffer containing 1 mM CTAB	A detection limit of 600 ng/mL was obtained for heat shock protein 90	[236]

can be integrated with other isolation methods, such as size exclusion chromatography, filtration, immuno-affinity, and acoustic techniques. With the consideration of all these directions, the on-chip electrokinetic-based device would have more systematic clinical relevance and would make a way for more reliable means to understand and defeat cancer.

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The authors have declared no conflict of interest.

Data availability statement

Data sharing is not applicable to this article as no new data were created or analyzed in this study.

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