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Toward Personalized Cancer Treatment: From Diagnostics to Therapy Monitoring in Miniaturized Electrohydrodynamic Systems

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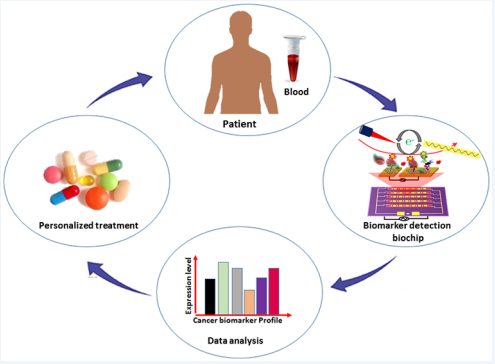
CONSPECTUS: Historically, cancer was seen and treated as a single disease. Over the years, this image has shifted, and it is now generally accepted that cancer is a complex and dynamic disease that engages multiple progression pathways in each patient. The shift from treating cancer as single disease to tailoring the therapy based on the individual's characteristic cancer profile promises to improve the clinical outcome and has also given rise to the field of personalized cancer treatment. To advise a suitable therapy plan and adjust personalized treatment, a reliable and fast diagnostic strategy is required. The advances in nanotechnology, microfluidics, and biomarker research have spurred the development of powerful miniaturized diagnostic systems that show high potential for use in personalized cancer treatment. These devices require only minute sample volumes and have the capability to create instant cancer snapshots that could be used as tool for cancer risk indication, early detection, tumor classification, and recurrence.



Miniaturized systems can combine a whole sample-to-answer workflow including sample handling, preparation, analysis, and detection. As such, this concept is also often referred to as "lab-on-a-chip". An inherit challenge of monitoring personalized cancer treatment using miniaturized systems is that cancer biomarkers are often only detectable at trace concentrations present in a complex biological sample rich in interfering molecules, necessitating highly specific and sensitive biosensing strategies. To address the need for trace level detection, highly sensitive fluorescence, absorbance, surface-enhanced Raman spectroscopy (SERS), electrochemical, mass spectrometric, and chemiluminescence approaches were developed. To reduce sample matrix interferences, ingenious device modifications including coatings and nanoscopic fluid flow manipulation have been developed. Of the latter, our group has exploited the use of alternating current electrohydrodynamic (ac-EHD) fluid flows as an efficient strategy to reduce nonspecific nontarget biosensor binding and speed-up assay times. ac-EHD provides fluid motion induced by an electric field with the ability to generate surface shear forces in nanometer distance to the biosensing surface (known as nanoshearing phenomenon). This is ideally suited to increase the collision frequency of cancer biomarkers with the biosensing surface and shear off nontarget molecules thereby minimizing nonspecific binding.

In this Account, we review recent advancements in miniaturized diagnostic system development with potential use in personalized cancer treatment and monitoring. We focus on integrated microfluidic structures for controlled sample flow manipulation followed by on-device biomarker interrogation. We further highlight the progress in our group, emphasis fundamentals and applications of ac-EHD-enhanced miniaturized systems, and outline promising detection concepts for comprehensive cancer biomarker profiling. The advances are discussed based on the type of cancer biomarkers and cover circulating tumor cells, proteins, extracellular vesicles, and nucleic acids. The potential of miniaturized diagnostic systems for personalized cancer treatment and monitoring is underlined with representative examples including device illustrations. In the final section, we critically discuss the future of personalized diagnostics and what challenges should be addressed to make these devices clinically translatable.

1. INTRODUCTION

Therapeutic success in cancer management is one of the least fruitful areas among all chronic diseases. Such poor treatment outcome can be attributed to the fact that cancer is heterogeneous and adopts multiple mechanisms for disease progression and immune evasion during the metastatic spread of the disease.[1−3] Until now, more than 200 forms of cancer have been reported with signature molecular characteristics that underpin the need for patient specific treatment strategy

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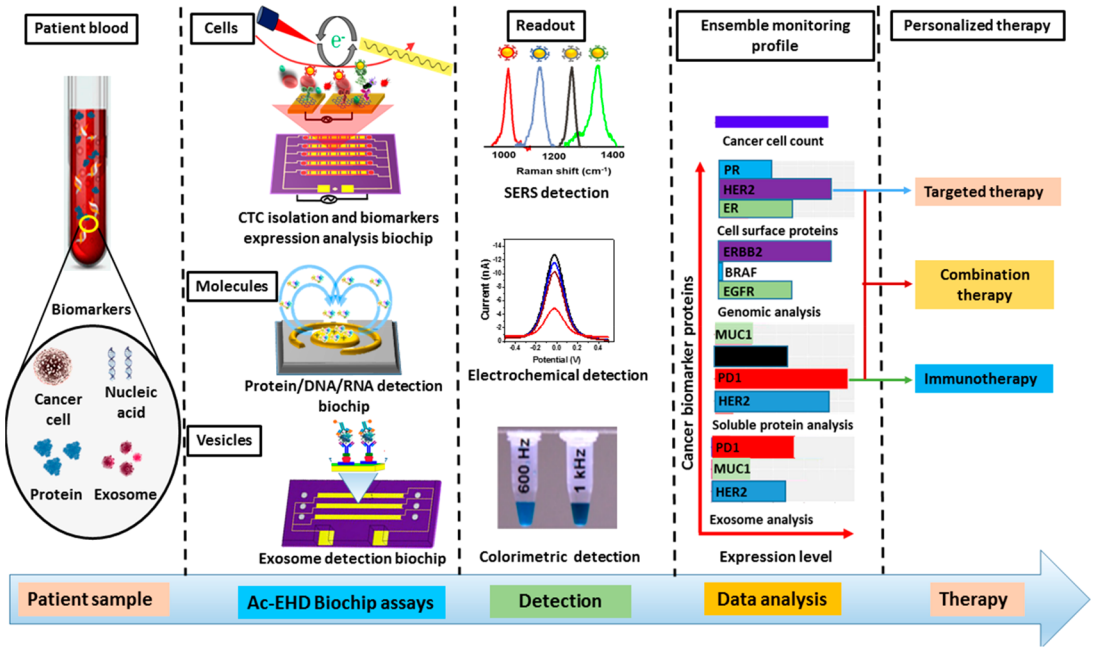

Figure 1. Overview of ac-EHD miniaturized systems for personalized cancer treatment. Cancer biomarkers (cells, proteins, DNA/RNA, exosomes, etc.) are obtained from a minimally invasive liquid biopsy sample and are analyzed using a suite of multiplexed platforms with informative detection read-outs that facilitate the creation of a patient-specific cancer profile and personalized treatment plan.

formulation.[4] Even though multiple molecular targets have been identified for cancer treatment, selection of most effective therapeutic strategy is still challenging.[2,5−7] This is largely because the expression of cancer specific molecular signatures differs patient to patient and sensitive technology for characterizing rare key molecular biomarkers is still short of the need.[8,9]

Current cancer diagnosis techniques are invasive, requiring tissue dissection and large sample volume, which are painful and not suitable for regular monitoring purposes. Over the years, a number of candidate biomarkers have been identified circulating in blood that carry critical information about the disease and offer attractive means of liquid biopsy for cancer management.[10,11] These cancer biomarkers include circulating tumor cells (CTCs), receptor expression profile of tumor cells, proteins, DNA/RNA, exosomes, etc.[12] Although the presence of these biomarkers in blood can provide important information about disease status and personalized therapy selection, their extreme rarity in patient blood make their isolation many fold more challenging.[13,14] Conventional flow cytometry and ELISA based methods are the gold standards for cell and protein analysis, but they lack the sensitivity to decipher rare molecular events, which is essential for decoding cancer.[15,16] Cell Search is a FDA approved immunoaffinity based method for CTC analysis, which detects and quantify CTCs by targeting epithelial cell adhesion molecule (EpCAM) expression from them.[17] However, biomarker expression in cancer cells is heterogeneous; thus only screening cells for their single biomarker expression is insufficient to realize the true picture of disease progression cascade.[3] Next generation sequencing is an extremely sensitive technique for detecting genetic and epigenetic aberrations, but the complexity of analysis limits its application in clinics.

Recent advancement in nanobiotechnology and microfluidics has significantly contributed to notable improvements of diagnostic technologies and enables detection of a minute amount of clinically relevant molecular biomarkers in patient samples.[18,19] The major advantages of microfluidic platforms include (1) controlled manipulation of fluid flow inside microfluidic capture zone, which maximizes the diffusion of target molecules toward the capture zone, (2) ability to handle low sample volume, (3) simple and low cost platform, and (4) easy integration with highly sensitive optical (e.g., fluorescence, SERS, SPR) and electrochemical readout techniques.[20] Until now, a number of potential microfluidic techniques have been reported for cancer specific biomarker isolation and analysis from patient blood samples.[21,22] Even though all the reported techniques have their own advantages for rare molecule analysis, one of the major issues associated with most of the miniaturized techniques involves nonspecific adsorption of molecules, which leads to poor sensitivity and specificity.[23,24] To minimize nonspecific adsorption, alternating current electrohydrodynamic (ac-EHD) fluidic flow inside a microfluidic biosensor is one of the most recent additions in advanced biosensor development and has shown its potential to reduce nonspecifically adsorbed molecules significantly.[25] Further, the integration of fluorescence and SERS based detection with ac-EHD microfluidic platforms immensely increases its potential for profiling multiple biomarkers in a single target cell, as well as screening several cellular and molecular biomarkers in patient samples.[26,27]

Here, we review the utilization of ac-EHD in microfluidic platforms (Figure 1), aiming toward its application for clinically relevant rare biomarker analysis for cancer diagnosis and personalized therapy. More specifically, we explore the integration of ac-EHD based target isolation with highly sensitive fluorescence and SERS based readout techniques within a miniaturized device to demonstrate its potential for multilevel (i.e., CTC, protein, DNA, exosome, etc.) biomarker analysis. A detailed overview of the mechanism of ac-EHD system and its potential advantages for biomolecule analysis followed by current challenges are also highlighted in this Account. Finally, discussions of challenges and opportunities in developing an ac-EHD based next generation miniaturized diagnostic platform is presented.

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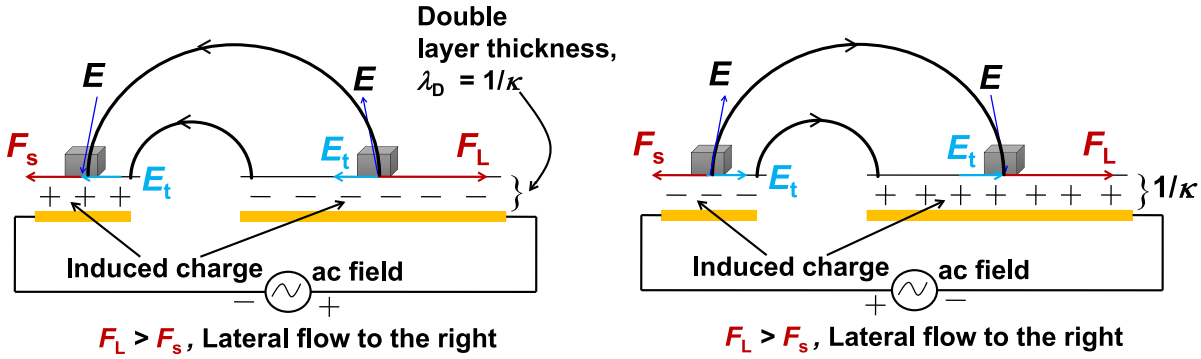

Figure 2. ac-EHD mechanism showing charge accumulation toward the larger electrodes (independent of polarity directions) under alternating current electric field. Reproduced with permission from ref 40. Copyright 2014 American Chemical Society.

2. FUNDAMENTALS OF AC-EHD

Electrokinetic manipulation of fluidic movement in microscopic scale has long been studied, and more recently its potential for developing highly sensitive and specific immune sensors is explored in several studies.[28,29] In principle, the application of electric potential on electrodes causes charge accumulation from the bulk solution on electrode surfaces that results in variation in charge density near the electrodes and formation of an electrical double layer (EDL). Because of EDL formation and its interaction with the tangential component of the electric field, a net force generates on the double layer of the electrodes that triggers the fluid flow. By changing the electrode geometry, ionic strength of the fluid, and electric field conditions, it is possible to engender controlled fluidic mixing and directional fluid flow. In their pioneering study, Brown et al. showed the critical advantages of an asymmetric pair of electrodes for controlled fluidic micromixing and unidirectional fluid flow under electric potential.[30] In brief, the application of an electric field (E) on an asymmetric pair of electrodes results in the formation of charged double layers (nanometer thickness) on both smaller and larger electrodes. Under this condition, the resultant force, F ($F = \rho E_t$, where ρ = charge density), generated on both of the electrodes triggers fluid flow in the direction of broken asymmetry (Figure 2). Such fluidic manipulation is highly desirable for microfluidic immunosensors where slow diffusion of targets on the sensor surface is a critical bottleneck for ultrasensitive detection and analysis. Furthermore, the resultant gentle force could also facilitate removal of nonspecific molecules from the sensor surface.

2.1. ac-EHD Assay

Disease biomarkers in biological fluids are present in minute amounts within a background of nonspecific molecules. While conventional gold standard techniques fall short for assays of rare biomarkers due to poor sensitivity and large sample requirements, miniaturized immune assays are continuously getting attention for this purpose. Major strengths of miniaturized immunoassay platforms are (1) low sample volume requirement, (2) minimal reagent requirement, (3) minimal processing, and (4) ability to detect rare target analytes. Until now, several microfluidic immunosensors have been reported for rare cancer biomarker detection from complex biological samples.[31−35] Despite their potential in rare biomolecule detection, nonspecific adsorption remains an ongoing problem for most of the miniaturized assay. By realizing the potential of ac-EHD and its application in microfluidic immune assay, Trau et al. has recently coined a new phenomenon called "tunable nanoshearing" for ultrasensitive molecular analysis.[26,36,37] The application of ac-EHD in a microfluidic platform provides two major advantages, (i) enhanced capture efficiency and sensitivity due to the increased number of antibody−target collisions and (ii) enhanced specificity due to the ability to tune nanoscopic fluid shear forces at the electrode interface to shear away loosely bound, nonspecific species present in biological samples. Utilizing custom-built ac-EHD miniaturized devices, our group has successfully isolated and analyzed several biomarkers (e.g., cancer cells, proteins, DNA, RNA, or exosome) from simulated and patient blood samples.[38−40]

For cell, protein, and genomic target capture and analysis, we have investigated biochips (i.e., microfluidic and circular sensors) with different gold electrode designs (e.g., planner, microtip, or ring electrodes) as these parameters define the electrokinetic phenomena inside a biochip.[39] In our custom-made microfluidic biochips, the application of an ac electric field on electrode surfaces generates a net force that triggers controlled fluidic nanomixing, which is highly desirable for rare biomolecule capture on detection surfaces and pushes fluid from inlet to outlet without the need for an external pump.[39]

To perform ac-EHD assay, the isolation domain of an ac-EHD biochip (e.g., microfluidic and electrochemical sensors) is initially functionalized with antibodies of interest. This antibody immobilization is achieved either by standard biotin−streptavidin chemistry for antibody attachment on gold or by EDC-NHS coupling of antibodies on graphene oxide modified gold electrode surfaces. Postfunctionalization, target molecules in sample fluid are captured on the antibody array under an optimized ac electric field, which facilitates increased collision between targets and capture antibodies by generating fluidic nanomixing. Furthermore, the produced force from the application of the ac field on the asymmetric planner of electrodes (in case of ac-EHD microfluidic chips) enables pushing the sample fluid through the microchannel, thereby avoiding the need for an external fluid pump, and assists nonspecific biomolecule removal. Postcapture detection and characterization of targets are carried out by secondary antibody labeling and any of the standard detection protocols, for example, fluorescence detection, colorimetric readout, or SERS. Such profiling is essential to understand biomarker expressional heterogeneity in rare tumor cell populations and could convey important information for personalized therapy.

To obviate the requirement for secondary detection antibodies without compromising the sensitivity and specificity of rare biomolecule analysis and to improve the detection speed (i.e., 3

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Table 1. Existing and Emerging Technologies for Cancer Biomarker Analysis

biomarkers	platform	commercial availability	sensitivity	limitations	ref
cells	flow cytometry	yes	can sort millions of cells in few seconds	not suitable for small cell numbers	42
	cell search	yes	can detect CTCs from 7.5 mL blood sample (FDA approved)	EpCAM dependent, no multiplexing capability, no downstream analysis	17
	SERS	proof of concept	single cell sensitivity, high signal multiplexing capability	nanoparticle associated toxicity	43
	ac-EHD	proof of concept	single cell sensitivity, high specificity with negligible biofouling	whole blood processing is challenging	44
protein	ELISA (Luminex)	yes	concentration sensitivity up to pg/mL, multiple analyte detection in a small volume of sample	relatively poor sample handling in the clinical environment	16
	electrochemical	proof of concept	can detect 50 ng of protein	biofouling and limited multiplexing capability	45
	SERS	no	concentration sensitivity up to 10 fg/mL	biofouling, and cross-reactions among multiple encoded nanoprobes	46
	ac-EHD	proof of concept	concentration sensitivity up to 10 fg/mL, high specificity with negligible biofouling	limited multiplexing capability	25
nucleic acids	sequencing	yes	single base sensitivity and high multiplexing capability	PCR amplification of the sample and large bioinformatics analysis	47
	SERS	no	attomolar level DNA biomarker detection with multiple targets	nanoparticle stability in biological fluids	48
	electrochemical	proof of concept	high sensitivity, 10 ng/μL to 100 fg/μL concentration, detect many cancer types	limited multiplexing capability	41
	ac-EHD	proof of concept	amplification-free multi-RNA-type profiling	Limited multiplexing capability	49

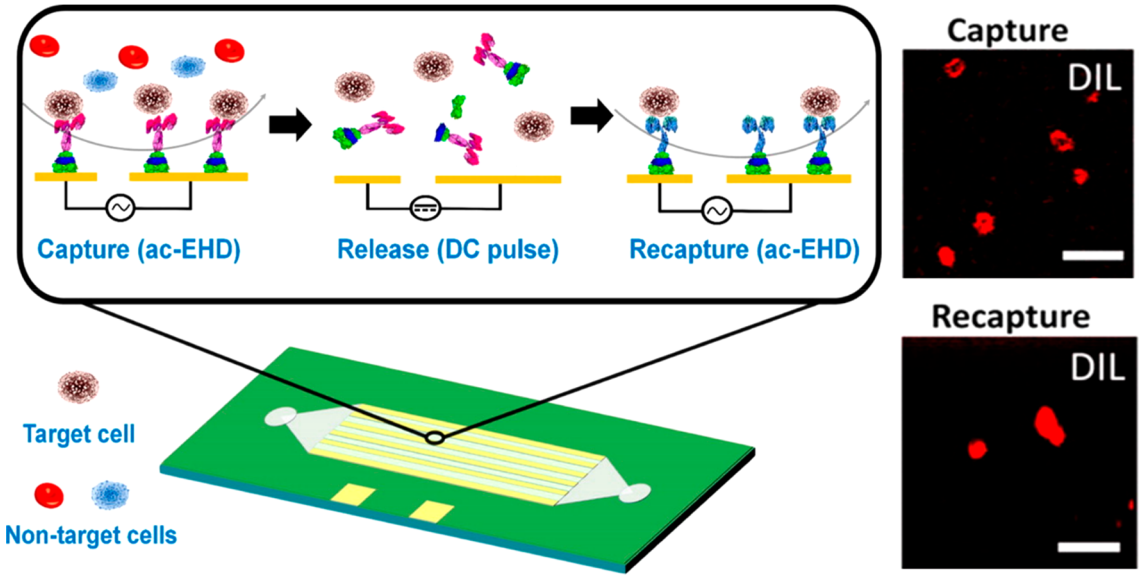

Figure 3. Schematic shows cell capture, release, and recapture mechanism on ac-EHD chip. Representative fluorescence images of capture and recapture of prestained SKBR-3 cells (DIL-red) spiked in blood. Scale bar = 50 μm. Reproduced with permission from ref 52. Copyright 2016 American Chemical Society.

min detection), we developed ac-EHD biochips that incorporate electrochemical readout as a label free detection method. Herein, the biochip designs contain a central circular electrode (working electrode) in proximity to a ring electrode (counter electrode) both of which act as an asymmetric electrode pair for fluid nanomixing under the applied electric field. The central circular electrode is functionalized with antibodies for target capture, and concentration of captured target is measured as a function of current reduction generated by the $[Fe(CN)_6]^{3-/4-}$ system in the electrolyte solution. This biochip system has also been employed for the detection of DNA/RNA biomarkers using interfacial biosensing that utilizes direct interaction of biomolecules with a bare metal surface (e.g., gold) to identify disease associated biomolecules. In this case, ac induced nanomixing is applied for expediting DNA/protein adsorption on a bare gold surface by the virtue of gold−DNA affinity.[41]

Table 1 compares different existing and emerging technologies for cancer biomarker analysis.

3. APPLICATIONS OF AC-EHD IMMUNOASSAY

3.1. Analysis of Cancer Cells

Tumor cells circulating in blood (CTCs) carry important biomolecular information for understanding disease progression pathways and hold great potential for treatment selection and patient outcome monitoring.[50] However, the extreme rarity of CTCs (a few to hundreds per milliliter of whole blood) makes their isolation challenging and highlights the need of an exceedingly sensitive and specific technique to detect CTCs

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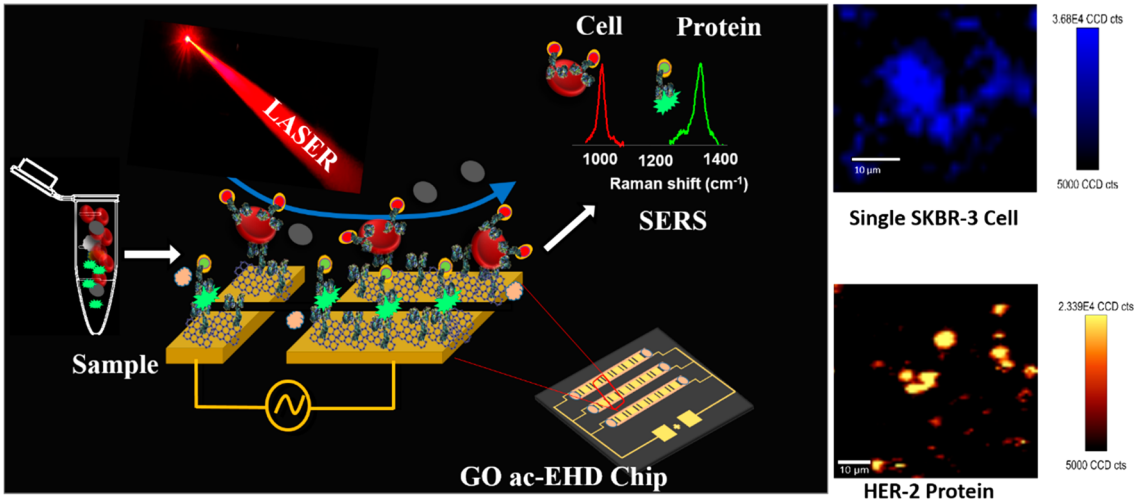

Figure 4. Schematic illustration of multiple cell and protein profiling on GO functionalized ac-EHD chip. False color SERS images of a single SKBR3 cell and HER2 proteins. Scale bar = 10 μm. Reproduced with permission from ref 44. Copyright 2018 Royal Society of Chemistry.

from a background of billions of blood cells.[50] Furthermore, the inherent heterogeneity in biomarker (i.e., intracellular and extracellular) expression among CTCs makes the task even more difficult. Commercially available Cell Search is an EpCAM based immunomagnetic isolation and detection platform for CTCs, which falls short in terms of multiplexed analysis of the targets. Until now several miniaturized biosensors (e.g., microfluidic sensors) have been reported for both labeled (i.e., immunoaffinity) and label free (e.g., size based separation) isolation and analysis of CTCs.[51] For example, the Lim group has developed a spiral microfluidic device for label free isolation of CTCs, which allows viable CTC retention for further downstream analysis.[21] The isolation principle is based on deterministic lateral displacement and streamline focusing of CTCs from other blood components within a spiral microfluidic device. Another example of a miniaturized CTC analysis chip includes the CTC-iChip, which incorporates both negative and positive immunomagnetic separation of target CTCs within a microfluidic device.[22] Despite the significant advancements in miniaturized platform development for CTC analysis, the rarity of CTCs in blood and nonspecific adsorption of biomolecules have rendered their isolation and analysis a major challenge and affect the detection sensitivities and specificities.

In order to achieve high sensitivity and minimize nonspecific adsorption of molecules on a microfluidic sensor, we have introduced ac-EHD induced fluid flow within microfluidic sensors to facilitate improved target cell capture and remove nontargets that stick loosely to the surface.[26] This strategy facilitated a 4-fold reduction of nonspecific blood cells on sensor surfaces and resulted in high CTC capture efficiency of 87% when analyzing simulated blood samples.[40] Furthermore, by realizing the clinical importance of screening multiple biomarkers on CTC surfaces and subgrouping them accordingly, we have developed a gentle cell release method from microfluidic sensor surfaces simply by applying a mild dc (direct current) potential (Figure 3).[52] The application of a dc pulse across the electrode surfaces triggers the oxidative desorption of the thiol bond, which acts as a linker between capture antibodies and gold surfaces and eventually results in efficient cell release. By controlling the sequence of ac and dc pulses, we also demonstrated the utility of cell capture, release, and recapture.

Parallel to subgrouping cancer cells, multiple biomarker analysis at single cell resolution would enable in depth profiling of disease conditions and drug response monitoring. However, this is an extremely difficult task that requires sensitive detection. To address this unmet need, Dey et al. have recently reported an integrated ac-EHD bioassay that permits cell capture and release and highly sensitive SERS based analysis of cell surface protein biomarker expression level in both bulk and single cell resolution.[52] Using this approach, we successfully captured T-cells on a biochip under an ac electric field and labeled them with cell surface target specific SERS nanotags and then released them with a dc pulse and interrogated them under Raman scattering to identify multiple T-cell subgroups of diverse TCR distributions.[53] Inspired by the success in cell surface biomarker expression level analysis, we endeavored to develop an integrated SERS−ac-EHD biochip with capability to simultaneously isolate cancer cells and soluble protein targets from biological samples and to profile cell surface protein biomarker expression level within a biochip (Figure 4).[44] One of the significant improvements of this biochip includes graphene oxide (GO)based functionalization, which removes the conventional biotin−streptavidin steps for antibody attachment to the electrode surfaces and enhances loading efficiency. Furthermore, the multiplexed device design enables interrogation of individual target cells and proteins in dedicated microchannels under the Raman microscope, which nullifies the need for cell release from the microfluidic chip. Using this approach, Reza et al. successfully characterized HER2 protein expression levels on breast cancer cell surfaces and at the same time detected two soluble protein biomarkers (i.e., HER2 and MUC16) from simulated biological fluid.[44] To gain insights into the disease progression mechanisms and facilitate therapy selection, this platform technique has potential for application in multiple surface biomarker expression level analysis by adding different antibody nanotags.

With significant advancements in molecular biology research, it is well understood that disease progression (e.g., cancer metastasis) is associated with multiple molecular pathways, which vary patient to patient. This finding highlights the need for patient specific therapy selection, and it is perceived that, the present "one-size-fits-all" treatment strategy will soon be replaced by targeted antibody therapy, immunotherapy, or a

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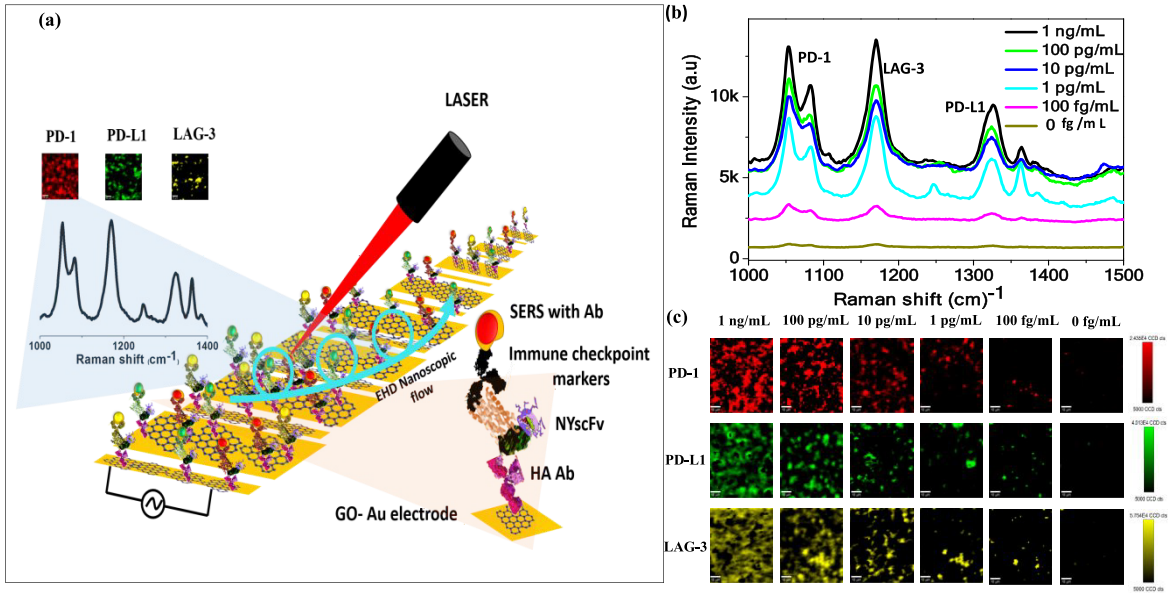

Figure 5. (a) SERS GO ac-EHD immunoassay platform for targeting multiple immuno checkpoint blockade (PD-1, PD-L1, and LAG-3) biomarkers. (b) Typical SERS spectra of the parallel detection of three targets in serum samples under ac-EHD field. (c) False color SERS images (scale bar is 10 μm) of PD-1, PD-L1, and LAG-3 detection for different concentrations. Reproduced with permission from ref 38. Copyright 2019 Elsevier.

combination of both. However, to achieve best outcomes for these personalized treatments, it is imperative to develop a diagnostic platform with multiplexing capability for identifying signature biomarkers in individuals both for therapy selection and for response monitoring. For this, the integrated ac-EHD microfluidic devices developed by Trau et al. could be of great importance for disease detection, treatment selection, and therapy monitoring.[39,44]

3.2. Protein Biomarker Analysis

The identification of different disease related protein molecules circulating in blood offers attractive alternatives to CTCs for disease diagnosis because (1) protein biomarkers are more abundant than CTCs, (2) proteins are accessible in blood plasma, which is comparatively less complex than whole blood, and (3) multiple disease related proteins can be analyzed in a single assay.[25] Although more available than CTCs, concentrations of different protein biomarkers in blood are often very low, particularly at the early stage of cancer.[13] This makes conventional detection techniques (e.g., ELISA) not applicable for protein analysis due to sensitivity issues. Moreover, ELISA based techniques are designed to analyze a single protein at a time and thus are not suitable for multiplexed analysis of protein biomarkers. Furthermore, conventional Western blotting has insufficient sensitivity to detect rare protein biomarkers. Over the years, several miniaturized diagnostic platforms have been developed for rapid and sensitive analysis of disease specific protein biomarkers in biological fluid.[54] For example, Volpetti et al. developed a microfluidic device containing 384 unit cells each of which can be functionalized with pairs of capture and detection antibodies.[55] Using this approach, they have successfully detected five different protein targets from biological samples. Similarly, Ali et al. reported a label-free microfluidic immunosensor for electrochemical detection of epidermal growth factor receptor 2 (EGFR2 or ErbB2) proteins down to femtomolar sensitivity.[56] Although, these assay techniques have shown high sensitivity for protein analysis, nonspecific adsorption of biomolecules still remains a major challenge for most of the techniques and limits their application for clinical use.

Our research group has been active in developing a highly sensitive and specific protein detection platform and reported multiple ac-EHD biochips for rapid, sensitive, and parallel detection of multiple cancer-specific protein biomarkers from complex biological samples.[25,38] For instance, we have devised custom-made three-electrode gold biochips for rapid isolation and electrochemical detection of target proteins (e.g., *Entamoeba histolytica* antigen, $BRAF^{V600E}$) in 3 min.[39,57] Using this biochip platform, we successfully detected several clinically relevant protein biomarkers (e.g., $BRAF^{V600E}$) obtained from as low as 10 cells of biological samples. Although the method is rapid, it can process only a few microliters of sample (<10 μL) at a time. Furthermore, multiple biomarker detection is not permitted with this biochip.

To enable large sample volume and multiplexed analysis, we have further developed multiple ac-EHD microfluidic platforms to process and analyze ~1 mL of initial sample in a single experiment and enable multiple biomarker analysis. For example, Vaidyanathan et al. have reported an ac-EHD microfluidic biochip for naked eye detection of rare protein biomarkers from patient serum.[58] Herein, the isolation process involves capture of target proteins on an antibody functionalized microfluidic domain under ac-EHD flow to increase interaction between proteins and capture antibodies. Postcapture, colorimetric naked eye detection is carried out via labeling of targets with HRP secondary antibodies and subsequent catalytic oxidation of 3,3′,5,5′-tetramethylbenzidine (TMB), which yielded a high detection sensitivity of as low as 100 fg mL^{-1} of three soluble protein biomarkers (i.e, HER2, PSA, and IgG) from a spiked human serum sample. However, concentration of protein biomarkers in blood during early stages of disease could be much lower than 100 fg mL^{-1} and thus this system might not be applicable for trace concentrations of target molecules in patient samples at early cancer stages.

To surmount the problem, we have introduced highly sensitive SERS based readout within our ac-EHD microfluidic

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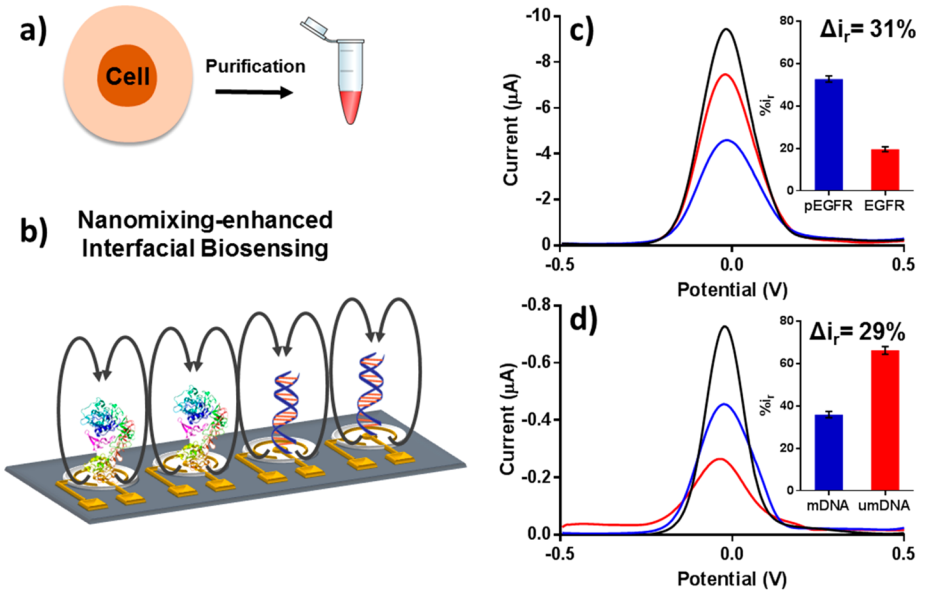

Figure 6. Simultaneous protein and DNA detection on a single ac-EHD chip using electric field-induced interfacial nanomixing. (a) The target protein and DNA extracted from cells followed by (b) ac-EHD nanomixing to enhance target adsorption for their electrochemical detection. (c) Protein phosphorylation status in EGFR protein and (d) DNA methylation. Reproduced with permission from ref 61. Copyright 2018 Royal society of Chemistry.

capture platform and achieved 10-fold increase in detection sensitivity.[37] The integration of SERS has also significantly enhanced the multiplexing capability of our miniaturized platform, which is evident from the simultaneous detection of four cancer related protein biomarkers from patient samples.[25] Although the technique is simple, it required antibody functionalization using biotin−streptavidin chemistry. To circumvent the use of comparatively expensive antibodies with limited shelf life, we further improved our device functionalization protocol by replacing biotin−streptavidin steps with graphene oxide modified electrodes and utilized novel recombinant nanoyeast single chain variable fragment (NY-scFv) affinity reagents as capture molecules instead of monoclonal antibodies.[38,57] Using this approach we successfully detected multiple immune checkpoint proteins (PD1, PDL1, etc.) from human serum available in very low concentration (i.e., 100 fg/mL).[38] This approach could be translated for multiple disease related protein biomarker analysis in clinical settings, which in turn would facilitate treatment selection for better patient outcome (Figure 5).

3.3. Genomic Analysis

Genetic abberation is one of the fundamental mechanisms that allows cancer cells to escape different regulatory processes (e.g., host's immune system) and develop cancer.[41] Identification and characterization of the key abberations are of immense importance for understanding disease progression pathways and determining the choice of therapy. For instance, harmful methylation states are found to be associated with cancer progression and could be utilized for treatment selection. Genetic analysis is performed from solid biopsies and more recently liquid biopsies. Liquid biopsies contain circulating tumor DNA/RNA (ctDNA/ctRNA), which are released from primary tumor cells into circulation and can be accessed for diagnostics. According to Newman et al., levels of ctDNA are highly correlated with tumor volume and measurement of ctDNA levels allowed for earlier response assessment than radiographic approaches.[59] Although important, conventional genetic analysis using quantitative polymerase chain reaction (qPCR) and DNA sequencing suffers from relatively long analysis times, nontarget interference, and the need for highly specific and costly fluorescence probes.[47,60]

Microfluidics have gained significant attention for their application in the analysis of abberant genetic makeup.[62] In their pioneering work, Macosko and co-workers encapsulated single cells with bar-coded microbeads in nanoliter droplets inside microfluidic channels to enable high-throughput single cell mRNA analysis.[63] In a nanochannel array, genome mapping was performed using fluorescently labeled DNA probes, and this miniaturized strategy achieved accurate, haplotype-resolved, sequence motif maps of 95 bacterial artificial chromosomes of hundreds of kilobases in length.[64] In another approach, Docherty et al. demonstrated simultaneous detection of three oligonucleotides using SERS in a microfluidic chip.[65] Although these methods have their merits, complex experimental procedures and nonspecific adsorption of biomolecules often leads to compromises in assay performance.

To overcome current limitations associated with genetic aberration analysis of cancer cells, our group has developed a highly sensitive and specific biochip by integrating the ac-EHD phenomenon with a label free electrochemical detection scheme to facilitate rapid adsorption of target biomolecules (i.e., interfacial biosensing) on the sensor surface for their detection.[61] The biochip was equipped with DNA methylation and protein phosphorylation detection zones. For DNA methylation analysis, the purified sample was added to the detection zone where rapid and controlled nanomixing under ac potential enabled maximum collisions of DNA molecules with the sensing surface. The higher affinity of unmethylated DNA resulted in a stronger surface adsorption compared to that with methylated DNA, which was electrochemically measured. The enhanced target adsorption decreased sensing time to 3 min and provided detection sensitivity down to pg/μL level (Figure 6).

Further, we have designed and fabricated an integrated biochip for on-chip RNA target isolation from complex

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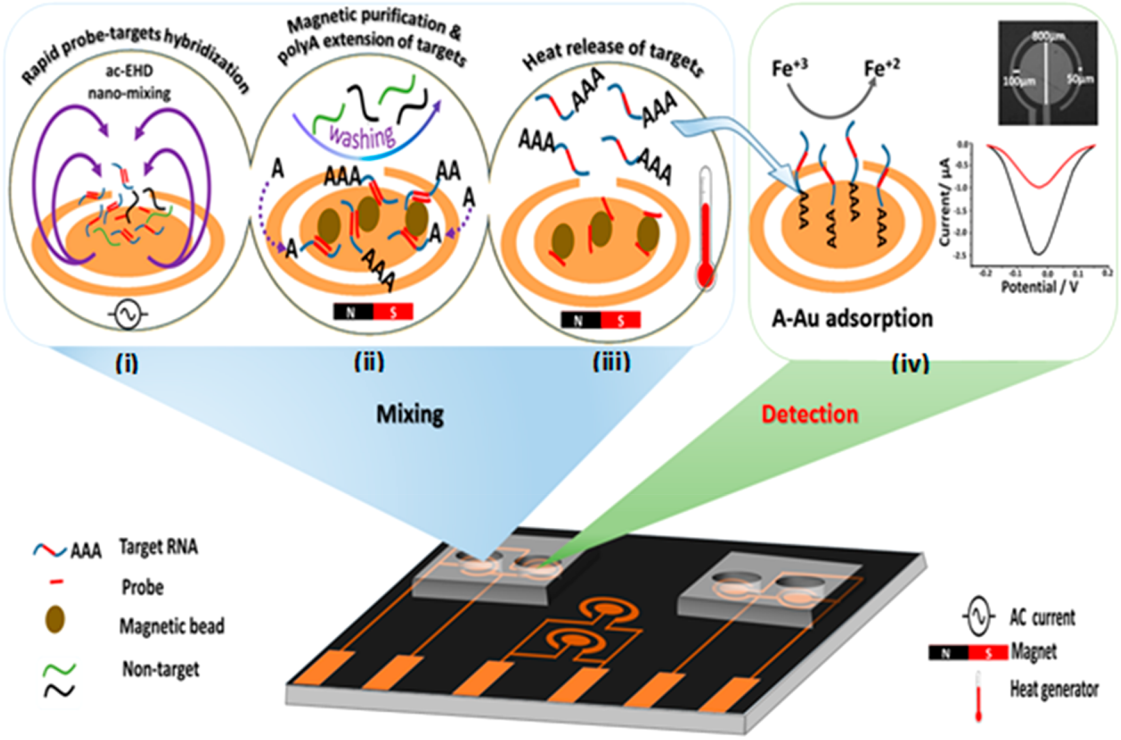

Figure 7. Biochip for genomic target purification and detection. (i) ac-EHD nanomixing to hasten the probe−target hybridization; (ii) subsequent addition of streptavidin−magnetic beads to bind biotinylated probe−target molecules for magnetic target purification and subsequent polyA extensions of purified targets; (iii) heat release of targets from capture probes; (iv) polyA sequences facilitated rapid target adsorption onto bare gold microelectrode surface for electrochemical detection. Reproduced with permission from ref 66. Copyright 2018 Wiley.

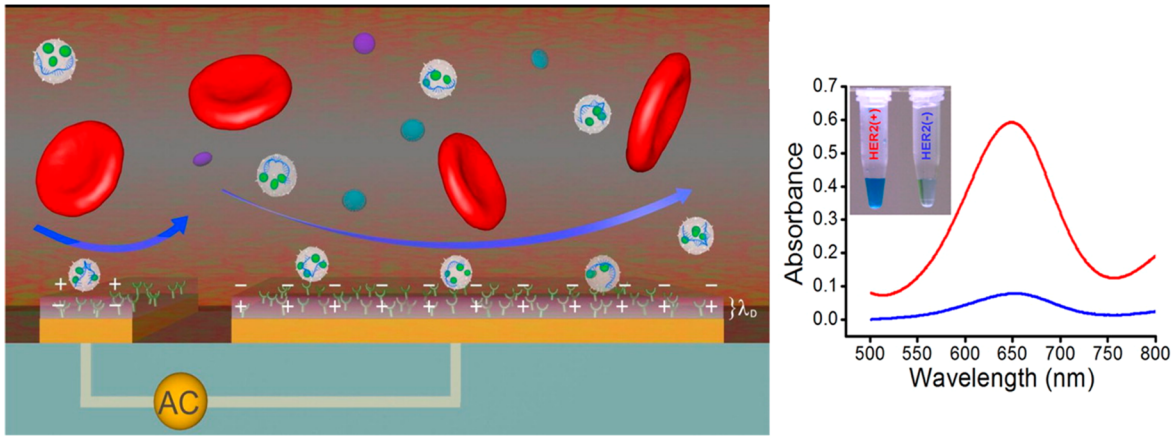

Figure 8. Multiexosome detection on ac-EHD system (exosomes represented as white spherical particles). UV−vis absorption spectra and colorimetric detection of HER2 (+) (patient A; red) and HER2 (−) (patient B; blue) breast cancer patient serum. Reproduced with permission from ref 36. Copyright 2014 American Chemical Society.

biological samples and their subsequent electrochemical detection within a single miniaturized platform.[66] Herein, within the RNA purification microcompartment, fluidic nanomixing under an ac electric potential significantly improves target RNA hybridization on complementary strand immobilized magnetic beads and facilitates on chip target isolation using simple magnet (Figure 7). Following isolation, heat release of target RNAs from magnetic beads enables transfer of target molecules to the detection zone where under ac-EHD, purified RNA targets can be adsorbed on a gold surface for label free electrochemical detection. This integrated method is rapid, avoids off chip sample preparation, and enables target isolation to detection within same platform, which could be an ideal platform for routine analysis of patient samples in clinical settings.

3.4. Extracellular Vesicle Analysis

Extracellular vehicles (EVs) are semispherical structures composed of a fluid core encapsulated by a lipid bilayer and carry functional biomolecular cargoes (e.g., transmembrane or cytoplasmic proteins, DNA or RNA, and lipids).[67] In contrast to rare CTCs, EVs are present in plasma at relatively high concentrations (>10^{11} EVs per milliliter), which renders their interrogation promising for early detection and treatment monitoring in cancer.[53] Conventional interrogation methods are based on EV isolation using physical separation (e.g.,

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ultracentrifugation, ultrafiltration) and affinity-based methods (e.g., magnetic pull-down strategies). Following isolation, EV analysis includes Western blotting, immunoassays, particle tracking analysis, transmission electron microscopy, flow cytometry, and mass spectrometry. While these techniques have their merits, they all require relatively large quantities of EVs, making interrogation laborious, time-consuming, and impractical for clinical settings.

Miniaturized systems have addressed some of these challenges and provided a suite of ingenious solutions including microfluidic immunoassays with plasmonic, electrochemical, and fluorescence detection schemes.[68,69] For instance, Kanwar et al. reported a microfluidic "ExoChip" device for on-chip isolation, quantification, and characterization of exosomes from pancreatic cancer patients using sensitive fluorescent tags.[70] Despite these improvements, the specific capture of exosomes from biological samples is complicated, relatively slow, and prone to errors.

To improve EV analysis, our group developed a miniaturized system for highly specific capture and detection of multiple exosome targets.[31] Nanoscopic flow stimulation by ac-EHD enabled capture of multiple exosomes extracted from breast cancer cells expressing HER2 protein and prostate cancer cells expressing prostate specific antigen. To facilitate a simple naked-eye read out, we applied the catalytic oxidation of peroxidase (e.g., from horseradish peroxidase conjugated detection antibody) in the presence of tetramethylbenzidine that resulted in a color change from transparent to blue. The ac-EHD enhanced EV captured improved the assay sensitivity three times (compared to hydrodynamic flow assays) and provided a visible color change at as low as 2760 EVs per milliliter (Figure 8).

4. FUTURE TRENDS

The future of miniaturized systems is being explored toward point of care miniaturized devices that improve cancer treatment by providing patient-specific cancer profiles to support the clinician in selecting appropriate treatment. Although the progress in material sciences and technology development enriched the diagnostic field with a suite of promising and powerful miniaturized systems, most of the works were proof-of-concept studies that required highly trained personnel. Studies on larger patient cohorts, in particular of longitudinal treatment monitoring, were scarce, further limiting the technology translation. Future miniaturized systems development should aim to bridge this gap by promoting assay automation with minimal hands-on processing and comprehensive clinical validation. A prime example of successful miniaturized system translation is the FDA-approved i-STAT Portable Clinical Analyzer that is capable of performing a wide variety of point-of-care tests on the same instrument and delivers rapid diagnostic information.

In the context of future miniaturized systems development, efforts should be geared toward integrated and portable multitask platforms that facilitate biomarker isolation and downstream analysis directly from clinical samples. For instance, in CTC-based diagnostics, such a system would empower investigation of individual rare malignant cells with multiple clinically relevant biomarkers with high precision and enable better disease diagnosis and management. Similarly, a multitask system that can simultaneously quantify multiple biomarkers (e.g., protein and DNA) of the same target could improve the diagnostic outcome by reducing false positive or false negative results. For instance, we have recently laid the foundation of an integrated multimolecular sensor that performs an entire sample-to-answer workflow of capturing melanoma cells, on-chip cell lysis, and quantification of the clinically actionable $BRAF^{V600E}$ DNA and protein using an electrochemical detection system.[39] We believe that this concept of multimolecular lab-on-chip biosensors could become a driver for more accurate and in-depth liquid biopsy diagnostics that support personalized treatment.

5. CONCLUSION

Miniaturized systems combining ac-EHD and sensitive detection techniques (e.g., fluorescence, SERS, electrochemical methods etc.) are promising for biomolecule analysis and have shown exceptional sensitivity for rare biomolecule detection. Particularly, the application of ac-EHD flow along with SERS based readout has significantly improved the detection specificity and allowed for the multiplexed detection of molecular targets simultaneously with high precision. However, the methods reported in this Account are evaluated in proof-of-concept experiments, and it is imperative to do longitudinal clinical trials for its integration in disease diagnosis and personalized therapeutic applications. Such in-depth clinical trials would facilitate improvement of the current protocol and expedite its transformation for real life application.

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