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Recent advances in integrated biophysical and biochemical microfluidic methods for circulating tumor cells isolation and analysis

Ruiju Shi[a,c], *Yuyan Yue*[b], *Ziqi Liu*[b], *Hua Chai*[a,*], *Peng Miao*[a,*]

[a] Suzhou Institute of Biomedical Engineering and Technology, Chinese Academy of Sciences, Suzhou 215163, China

[b] Shandong Laboratory of Advanced Biomaterials and Medical Devices in Weihai, Weihai 264200, China

[c.] Jihua Institute of Biomedical Engineering and Technology, Ji Hua Laboratory, Foshan 528200, China

[*] Corresponding authors: chaihua@sibet.ac.cn (H. Chai); miaopeng@sibet.ac.cn (P. Miao).

ABSTRACT: Cancer is a worldwide public health problem. Circulating tumor cells (CTCs) are one of the most significant tumor biomarkers, reflecting critical information of disease transcriptome, genome, and proteome. Various microfluidic methods have been proposed to separate, detect and analyze CTCs, involving multiple processes. In this work, we aim to give a comprehensive overview on recent advances in integrated biophysical and biochemical microfluidic methods for CTCs separation and detection. First, early label-free biophysical methods are introduced and discussed, including basic passive and active approaches as well as active-passive integrated assays. Next, we summarize labelled methods with different biochemical reactions based on their unique working principles, such as immune affinity-based methods and magnetic nanoparticle-assisted methods. Moreover, the most dominating approaches combining biochemical and biophysical methods are discussed. Their applicability and unique features are compared. Finally, we offer the perspectives on integrated microfluidic approaches for CTCs biosensing and cancer diagnostics.

KEYWORDS: CTCs isolation; CTCs biosensing; magnetic nanoparticles; microfluidic chip; fluorescence; aptamer.

1. Introduction

Cancer is one of the most fatal diseases, which is a major public health burden worldwide [1]. Liquid biopsy is a powerful non-invasive method, which provides abundant information about tumor biomarkers [2,3]. Circulating tumor cells (CTCs) usually shed from primary tumor into peripheral blood, which reflect disease transcriptome, genome, and proteome information [4,5]. To capture living CTCs, numerous attempts have been made based on the physical properties of CTCs (e.g., size and density) or the recognition of specifically expressed proteins (e.g., epithelial cell adhesion molecule (EpCAM), E-cadherin (ECAD), cytokeratin (CK), and human epidermal growth factor receptor-2 (HER2)) [6]. The changes of biomarkers depict the heterogeneity of CTCs. Besides, due to a spatially different microenvironment in the blood and temporal changes in therapy response, CTCs are spatio-temporally heterogenous even in a single patient. The label-free technologies based on the biophysical properties provide another choice for the analysis of CTCs, which can be broadly classified as active and passive forms. The former uses external force fields (e.g., acoustic [7], optical [8], and dielectrophoretic [9]) to manipulate the particles. As for the passive techniques (e.g., microfluidic filters [10], deterministic lateral displacement (DLD) [11], inertial microfluidics [12], and viscoelastic [13]), the particle separation only depends on the channel geometry and inherent hydrodynamic forces in a simpler way without external forces (Fig. 1). The merits and limitations are summarized in Table 1.

In recent decades, integrated methods based on multiple label-free assays or combined label-free/labelled assays have attracted much attention. As an emerging technology, microfluidics shows the merits of unprecedented efficiency and precise control. In particular, integrated approaches in microfluidic platform could rely on different cell properties for the

isolation and analysis of cells. Compared with reported commercial platforms including CellSearch®, microfluidic platforms based on integrated technologies enable a rapid, sensitive, and streamlined analysis from low blood volumes. Moreover, they show the merits of simplified and miniaturized workflow, high level integration, low processing time (<5 hours) and sample volume [14]. In this paper, we aim to offer a comprehensive overview on recent advances in integrated technologies that couple multiple biophysical and biochemical methods by means of microfluidic platform for CTCs isolation and analysis. According to the combining principles of integrated technologies, they can be divided into three modalities: integrated biophysical, integrated biochemical, and integrated biophysical-biochemical microfluidic platform. Major parameters of some representative integrated microfluidic methods are summarized in Table 2.

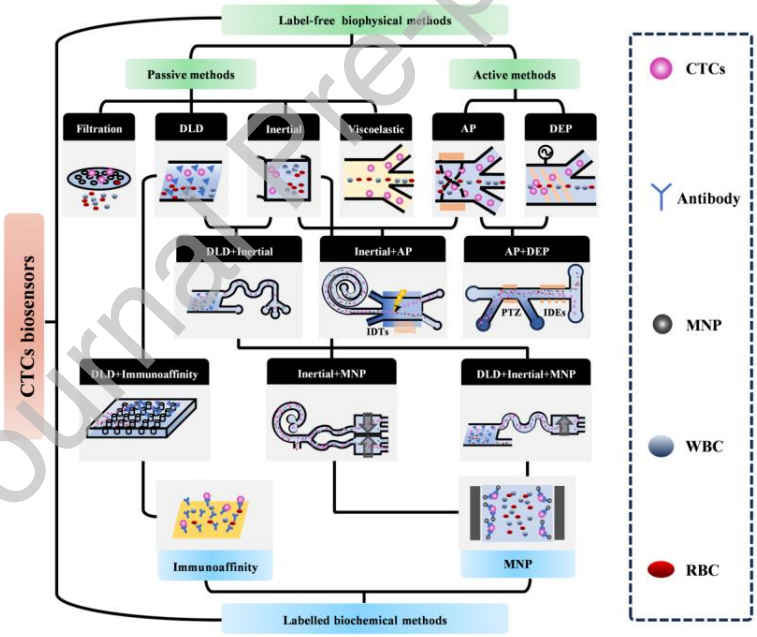

Fig. 1. An outline of biochemical and biophysical microfluidic platforms for CTCs assay. (MNP, magnetic nanoparticle; WBC, white blood cell; RBC, red blood cell).

Table 1. The advantages and limitations of methods for the detection of CTCs.

Methods		Advantages	Limitations
Integrated label-free biophysical methods	Passive-passive	High-throughput	Larger structure
	Active-active	Compact structure	Adverse effects on cell
	Passive-active	High tunability	Time-consuming
Integrated labelled biochemical methods	Antibody-assisted	High specificity	Non-destructive release process
	Aptamer-assisted	Cost-effectiveness	Low-throughput
	Peptide-assisted	High specificity and sensitivity	Relative weak interactions with target cells
	Integration of multiple ligands	High capture efficacy	Complex progress
Integrated biochemical and biophysical methods	Integration of biochemical and passive biophysical methods	Lower cellular damage, higher volume throughput, and higher separation yield requirements	Complicated modification process
	Integration of biochemical and active biophysical methods	High separation efficiency and purity	Low-throughput

Table 2. The major parameters of each integrated microfluidic method for the detection of particles.

Principles		Sample type	Throughput	Efficiency	Purity	Clinical application	Ref.
Integrated label-free biophysical methods	inertial-membrane filter	A549	4 mL/h	74.4%	N.A.	cancer patients	15
	elasto-inertial	MCF-7, MDA-MB-231, and A549 cells	240 μL/min	95%	61%	clinical MPPEs from patients	19
	DEP-AP	Submicrometer particles	0.75-3 μL/min	86%	95%	N.A.	25
	AP-inertia	Particles and microalgae cells	>100 μL/min	>94%	N.A.	N.A.	29
	DEP-inertia	Particles	300 μL/min	>93.8%	>96%	N.A.	40
Integrated labelled biochemical methods	Antibody-assisted	MCF-7	100 μL/min	91.0%	N.A.	N.A.	53
	Aptamer-assisted	Cancer cells	N.A.	84.3-91.3%	N.A.	Isolation of CTCs from patients	68
	Peptide-assisted	MCF-7	N.A.	97.4-106.0%	N.A.	N.A.	92
	Multiple ligands-assisted	MCF-7	N.A.	89%	90%	Isolation of CTCs from patients	97
Integrated biochemical and biophysical methods	Aptamer-inertia	MCF-7	60 μL/min	93.6%	90%	N.A.	105
	Antibody-AP	CTCs	N.A.	93.3%.	N.A.	Isolate CTCs from cancer patient	109

2. Integrated label-free biophysical methods for the detection of CTCs

Label-free methods (e.g., microfluidic filters and inertial microfluidics) with high-throughput and easy manipulation have been widely investigated for CTCs assay, which show the advantages of low cost and easy integration. However, the specificity and separation efficiency of single label-free method are inferior. By the integration of two or more techniques into one microfluidic chip, the separation efficiency can be effectively improved. According to the principles, these methods can be divided into three modalities: passive-passive, active-active and passive-active. Passive methods such as microfluidic filter, DLD, inertial microfluidic and viscoelastic separation show the merits of low cost and high volumetric flow rates. Thus, passive-passive modality integrates several passive methods into microfluidic chip for high-throughput isolation. However, separation at high volumetric flow rates often results in cell damage or reduced viability. By contrast, active label-free techniques (e.g., acoustophoresis (AP) and dielectrophoresis (DEP)), celebrated for their gentle operation without contact, are combined in active-active modality to enhance the separation efficiency. Compared with the former one, this modality requires additional devices to apply external force driving particles, resulting in a relatively large and complex structure. As for passive-active modality, both active and passive techniques are selected and integrated into microfluidic chip to minimize the drawbacks of each technology.

2.1. Passive-passive modality

Passive methods selected for this integration modality include microfluidic filter, DLD, inertial microfluidic, viscoelastic separation and so on. For example, Wang et al. developed an inertial-based cell sorter equipped with a membrane filter, which can be used for the enrichment of CTCs with efficiency as high as 74.4% [15]. Large CTCs were initially focused and separated through a double spiral channel. Then, it could be enriched by a membrane filter with uniformly

sized pores (Fig. 2a). Abdulla et al. presented an integrated chip for CTCs isolation and single-cell immunoblotting [16]. Besides the module of membrane filter, the chip included a thin layer of a photoactive polyacrylamide gel. Microwell arrays were placed at the bottom of the chamber for immunoblotting (Fig. 2b). Xiang et al. reported a two-stage inertial DLD sorter for size-based cell separation [17]. The microfluidic sorter was used to remove the background blood cells, and the separation efficiency could be improved to 99.9% by the second sorter with triangular posts. This two-stage sorter offered a high-throughput process without clogging, and the recovery of cells with high activity is easier. Liu et al. incorporated a filtration concept into a DLD structure to achieve one-step CTCs isolation [18]. The dimensions of the chips were relatively larger (Fig. 2c). The innovative filter-DLD structure exhibits excellent recovery of tumor cells with high efficiency and cell purity. During recent decades, viscoelastic separation has been successfully applied in the separation of spheres (e.g., microparticles, blood cells, tumor cells [19,20], and submicron particles [13,21]) owing to their extensive existence in normal life like in human body fluids, daily foods, etc. By integrating elasto-inertial focusing with a virtual fluidic channel, Zhou et al. provided a great hydrodynamic symmetrical force for inducing significant and homogeneous cell deformation and adjustable detection at a high throughput of up to 3000 cells per second [22].

2.2. Active-active modality

Active label-free methods involve the utilization of extra forces like acoustophoretic and dielectrophoretic forces to manipulate cell motions based on their intrinsic biophysical properties. AP technique employs sound waves to induce target migration. DEP is another active technique applying the interactions between a nonuniform electric field and induced unevenly distributed dipoles to manipulate the motion. AP is preferred to non-invasively sort biological samples due

to its minimal impact on cell viability and characteristics [23]. While DEP has a higher control efficiency with a relative compact structure, which has the merits of facile operation for manipulating cells [24]. Integrating acoustic/electric in a single microfluidic chip not only increases the throughput but also significantly improves the control of the manipulated targets. Currently, various integrated AP-DEP devices have been used to separate particles and even cells [25]. Utilizing remote DEP, Smith et al. demonstrated the separation of cells in a microfluidic channel coupled with an array of virtual electrodes, and the separation efficacy is over 98% without adverse effects on cell viability [26]. Unlike the cascade method, an acousto-dielectric tweezing mechanism was established for the characterization of a single cell through coupling physical fields [27]. This chip contained a pair of interdigital transducers on a $LiNbO_3$ wafer, which could generate shear-horizontal surface acoustic waves (SAWs). Meanwhile, AC electric fields within microchannel were induced by the parallel tri-port electrodes. However, these hybrid systems combining AP and DEP required special medium and generate more Joule heat that might cause adverse effects on cell viability [28]. Thus, more investigation is needed to adopt the devices in the applications of CTCs manipulation and analysis.

2.3. Passive-active modality

2.3.1. Passive-AP hybrid methods

Although AP is an ideal tool with the advantages of biocompatible and contactless, its sorting and flux with excellent performances cannot be guaranteed simultaneously. Inertial focusing force induced by serpentine and spiral microchannel can be integrated to focus the targets to improve the sorting performance of the chip. Compared with serpentine microchannel, spiral microchannel was more compact and had been chosen for prefocusing cells before acoustophoretic separation frequently. An inertia–acoustophoresis hybrid microfluidic device

was devised by Kim and coworkers [29]. Target could be pre-focused through the serpentine-shaped microchannel, and then separated based on size via acoustophoretic force. Utilizing traveling surface acoustic waves (TSAW), Mutafopulos et al. reported a microfluidic fluorescence activated cell-sorting (μFACS) device to sort labeled cells [30]. The TSAW pulses were generated to rapidly deflect cells into a separate channel upon fluorescence detection (Fig. 2d). Three types of cell lines were sorted at cell velocities exceeding 1 m/s, during which good sorting purity and satisfactory cell viability could be maintained. Using the same strategy, Zhou et al. presented another passive-AP hybrid microfluidic sorting approach for manipulating single cell [31]. The cells were pre-enriched by a reverse wavy channel, and the target cells with fluorescent dyes were continuously infused into a fluorescence interrogation region, in which a highly focused acoustic beam could be activated upon the detection of fluorescent labels (Fig. 2e). The above methods integrated different technologies according to cascade strategy, which required at least two steps for CTCs separation.

2.3.2. Passive-DEP hybrid methods

The efficiency of single DEP adopted for separating CTCs is reported to be low [32]. Many researchers have combined various passive technologies with DEP to achieve better performances including DLD-DEP and inertial-DEP. The drawbacks of the DLD can be minimized after properly integrated with DEP [33]. Utilizing nonlinear symmetrical post array, Kim et al. presented a DEP-based ratchet migration mechanism for sub-μm size particles separation [34]. Its separation capabilities of submicron particles including polystyrene particles, mitochondria and liposomes were well demonstrated. The deterministic ratchet mechanism induced significantly larger migration velocities (5 μm/s). With the advantages of the versatility of electrokinetics and the precision of DLD, Calero et al. described a novel separation technique

in a continuous flow [35]. Unlike ordinary DLD-DEP that only consisted of a pair of electrodes placed at both sides, Beech et al. embedded DEP electrodes inside a DLD device by using metal-coated microposts, which significantly decreased the DLD-based critical size for separation, and increased the dynamic range with switching times [36]. Recently, the increasing integrated DLD-DEP was applied for continuous separation of CTCs from peripheral blood cells. Aghaamoo et al. proposed a deterministic DEP method and utilized numerical modeling to investigate the different aspects of coupled DLD-DEP design [37]. The velocity field with the corresponding streamlines matched the expected flow profiles of DLD arrays. However, the device still suffered from clogging at flow rates lower than 1 μL/min. To address this issue, Rahmati and Chen extended the dielectrophoretic DLD method to the perpendicular configuration to cover a wider range of working frequency [38].

The flexibility and tunability of the device based on inertial microfluidics are usually too weak to accommodate particles with different sizes. Integration of this technology with DEP platform is a good idea to improve the throughput and enhance tunability. A microfluidic device with two-stage separation combining inertial sorting and DEP has been developed, which showed merits in automation, cost and versatility [39]. However, the indispensable two separation steps resulted in a time-consuming and a larger dimension compared with single chip. Coupling external DEP force field with inertial forces in one chip for particle tunable separation can eliminate those drawbacks effectively. Zhang et al. proposed a hybrid DEP-inertial microfluidic platform to separate various particle mixtures by adjusting electrical voltage [40]. As shown in Fig. 2f, the micro-electrodes were patterned on the bottom of serpentine microchannel, and the generated DEP force was effective for the specific region. All particles were enriched at the bottom owing to the top sheath flow at the inlet of the compact microfluidic

device. By the change of the electric voltage, the dimensions of target binary particle mixture could be easily tuned. Coupling DEP unit and inertial unit together in one microfluidic chip, Yao et al. reported three microchips [41], which were classified by the relative positions of three-dimensional (3D) sidewall electrodes and CE structure (Fig. 2g). Among them, the full-coupling microchip with contraction/expansion microstructures had highest cell sorting efficiency due to the synergistic effects between DEP/hydrodynamic sorting.

The integrated label-free methods show merits of relative simplicity and straightforward integration to a microfluidic configuration justifies their inclusion as a viable CTCs isolation tool. Specially, the DEP technique's high control capabilities and DLD's high precision separation result in a very efficient and effective separation platform. The main problem with such hybrid systems is that the common disadvantage of low-throughput cannot be eliminated by combining them on one hybrid platform. Replacing DLD with inertial microfluidics in the integrated platform, the throughput can be improved effectively. Since the compact structure and the controllable separation, more coupling DEP-inertial microfluidic devices have been applied for CTCs tunable separation, and have a promising wide range of applications in biomedicine.

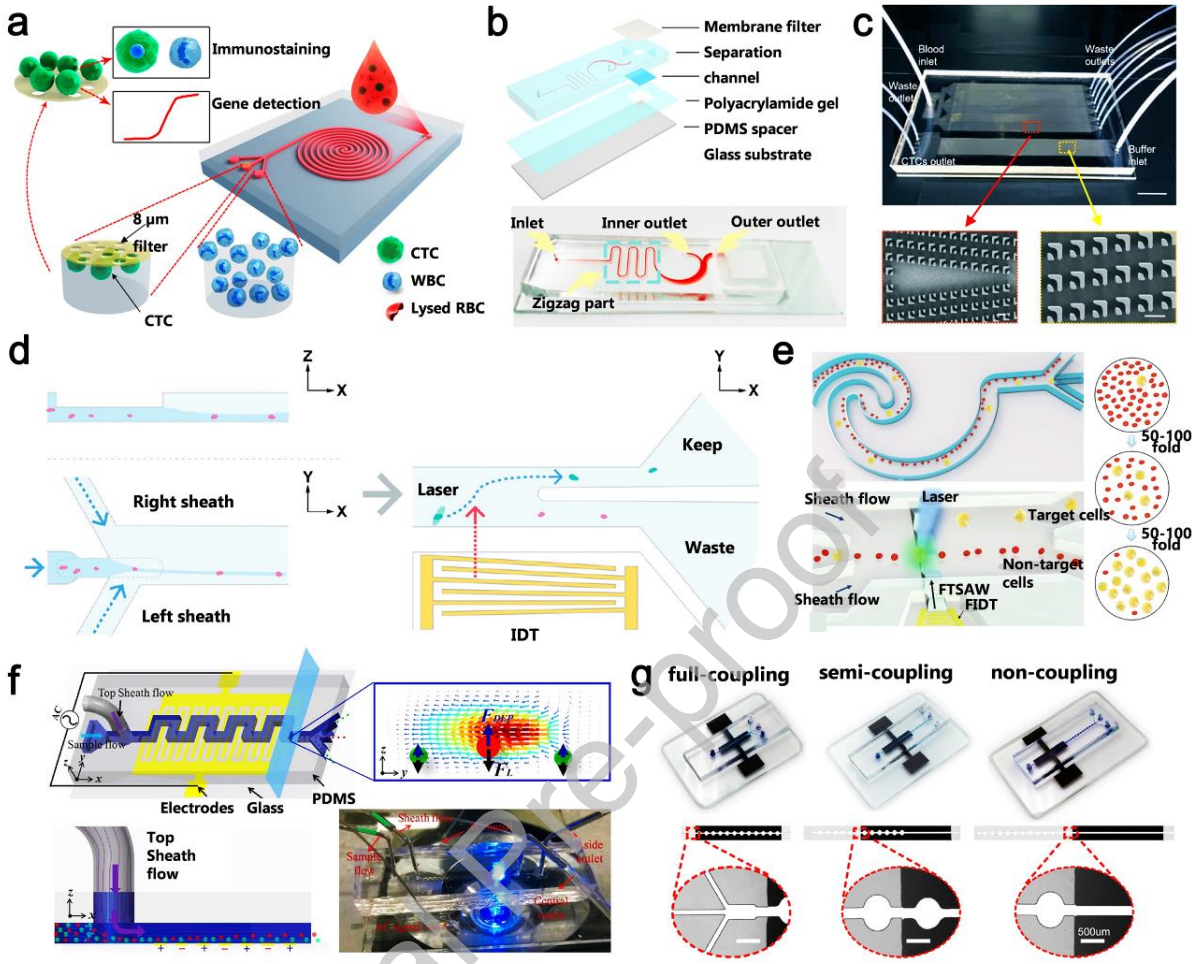

Fig. 2. (a) Schematic of the microfluidic cell sorter for size-based CTCs isolation and detection. [15] Copyright 2015, American Chemical Society. (b) The exploded diagram and physical illustration of the chip. [16] Copyright 2022, Springer Nature. (c) Photograph of the chip and SEM images of the filer-DLD array. [18] Copyright 2021, the Royal Society of Chemistry. (d) Scheme of the sorting device integrating a spiral channel for inertial flow focusing with a tapered IDT. [30] Copyright 2021, the Royal Society of Chemistry. (e) Hybrid sorting method combining inertial focusing and acoustic manipulation for cell isolation. [31] Copyright 2019, the Royal Society of Chemistry. (f) Scheme and the basic separation principle of the DEP-inertial

microfluidic chip. [40] Copyright 2018, Elsevier. (g) Photos of full-coupling, semi-coupling and non-coupling microchips. [41] Copyright 2019, Elsevier.

3. Integrated labelled biochemical methods for the detection of CTCs

Labelled methods based on biochemical features of CTCs show more specific capabilities like immunoaffinity assays. They can accurately distinguish CTCs from other blood components, which improves the purity and capture yield of target cells. Currently, increasing microfluidic platforms have been established for CTCs analysis due to advanced nanotechnology. The merits of the use of nanomaterials include good sensitivity, high purity, and fast characterization. For example, functionalized magnetic nanoparticles (MNPs) have been widely for the label and bulk cell separation. MNPs can be conjugated with various ligands (e.g., antibody [42], aptamer [43], peptide [44], or other small molecules [45]) which anchor on the CTCs with high specificity. Therefore, CTCs can be actively isolated from whole blood samples with an external magnetic field [46]. Additionally, the majority of CTCs detection based on a $DAPI^{positive}$, $CK^{positive}$, and $CD45^{negative}$ signature with numerous teams including EpCAM positivity in their CTCs definition [14]. Thus, labelled methods based on biochemical features of CTCs show merits of isolation and detection in a single microfluid chip. By patterning or microfabricating the channel, the methods of CTCs detection (e.g., Optical, electrochemical and physical) have been employed in integrated microfluidic platforms.

3.1. Antibody-assisted microfluidic platforms

Antibody-assisted technologies are developed based on the unique expressions of certain antigens on CTCs for identification such as EpCAM, N-cadherin, CD44, HER2, and MUC1 [47-49]. EpCAM is overexpressed in squamous cell carcinomas and widely applied for CTCs immune-based separation. By modifying the antibodies on the interface of various nanostructures

(e.g., microchannels and micropores), CTCs can be specifically captured. For example, Nagrath et al. pioneered an immunocapture chip to separate CTCs from peripheral blood sample of cancer patient by coating EpCAM antibodies on microposts [50]. The immunocapture efficiency is relatively low and significantly related to cell–substrate contact area and frequency. To realize highly efficient EpCAM-expressing CTCs capture, Wang et al. overlayed a polydimethylsiloxane (PDMS) chip with chaotic mixing channel on the ordinary patterned SiNP substrate with antibody coating [51]. Compared to a static setting, cell–substrate contact frequency was significantly enhanced due to the vertical flow induced by this kind chaotic mixer. Implanted cellular surface elements and extracellular matrix (ECM) scaffolds in 3D nanostructures provide a suitable environment for the recognition and analysis of cells. Wu et al. developed an electrochemical cytosensor based on electrospun nanofibers-deposited nickel micropillars [52]. It could offer CTCs a good microenvironment for adhesion and physiological functions, and greatly amplify the electrochemical response. Cheng et al. designed a 3D conductive scaffold microchip with breakable Au−S bond by immobilizing gold nanotubes on porous PDMS [53]. The effective capture by anti-EpCAM and release of CTCs by electro-mediated accurate breakage of Au−S bond are shown in Fig. 3a. The 3D scaffold generated chaotic migration of CTCs and the densely covered gold nanotubes improved the quantity of immobilized antibody. Although anti-EpCAM antibody is the golden standard for detecting CTCs, the expression levels vary in response to an external stimulus. Thus, other antibodies should be used to supplement for CTCs assay. Deliorman et al. developed a microfluidic device combining microfluidic–atomic force microscopy (AFM), for specific capture and the characterization of elasticity and adhesiveness [54]. The microfluidic device is assembly reversible and suitable for AFM measurement (Fig. 3b). The AFM measurements based only on

5 CTCs samples revealed that CTCs of localized cancer origin are stiffer (23.9 ± 2.2 kPa elasticity and 341 ± 11 nm deformability) than CTCs of metastatic cancer origin (6.2 ± 1.8 kPa elasticity and 502 ± 6 nm deformability). Accordingly, the nanomechanical properties of CTCs could be correlated to the disease status, which potentially opened a door in identifying metastatic biomarkers and drug resistance.

Immunocaptured CTCs are always difficult to be released with integrity and high activity, which cannot satisfy the downstream analysis. However, MNPs bound CTCs can be isolated and released from peripheral blood *in vitro* when applying or removing an external magnetic field. This noncontact manipulation provides solutions for not only possibility of *in vitro* but also *in vivo* applications [55,56]. Unlike conventional label-free methods, immunomagnetic methods realize the CTCs detection without the coupling of another technology and progress, which may integrate isolation, detection and further functional analyses. For example, Cui et al. encapsulated ZnS: Mn^{2+} quantum dots (QDs) and Fe_3O_4 nanoparticles into SiO_2 nanospheres. Anti-EpCAM antibodies were also modified at the surface [57]. The nanocomposites possessed magnetic, recognition and fluorescent properties. The release process is important and plays a key role for subsequent analysis, including electrochemical stimulation, electrochemical adsorption, and protease degradation. However, these methods are invasive and may cause damages and disturb cell microenvironment.

Currently, there are two major integrated strategies for combing immunomagnetic methods with microfluidic chips. First, using a rapid magnetic-field-assisted electrostatic self-assembly procedure, streptavidin-coated magnetic beads functionalized with ligands are directly patterned inside a microfluidic channel to capture CTCs as they flow through [58,59]. Second, CTCs labeled with immunomagnetic nanoparticles possesses magnetism and then will be separated in

microchannel within magnetic field [60]. Based on the first strategy, Sivagnanam et al. separated MCF-7 breast cancer cells by patterning self-assembled protein-coated magnetic beads inside a microfluidic chip [61]. The second strategy attracted much more interest. Hoshino et al. used a PDMS-based microfluidic chip to screen blood samples [62]. After incubating with Fe_3O_4 magnetic nanoparticles, the target cell could be labeled and then captured when the blood flowed through the microchannel. Xue et al. developed an integrated microfluidic system with a continuously moving Halbach array magnet for negative isolation CTCs [63]. As illustrated in Fig. 3c, an array magnet was settled in the inside of the ring-shaped microfluidic channel for generating a continuously moving magnetic field to negatively isolate CTCs without a sample loading volume limitation. To compensate for the drawbacks of positive and negative enrichment methods, Hyun et al. developed a two-stage microfluidic chip, which consisted of a magnetic-activated sorter (μ-MACS) and a geometrically activated surface interaction (GASI) chip [64]. The μ-MACS chip was applied for eluting the magnetic nanoparticle-coated white blood cells (WBCs). The GASI chip captured and classified heterogeneous CTCs (Fig. 3d).

As previously mentioned, antibody-based microfluidic platforms show the merits of high sensitivity, which have been widely used for the immunocapture of CTCs. But most of these platforms based on antibody (EpCAM) are highly dependent on the expression of CTCs, resulting in missing mesenchymal CTCs and EpCAM negative CTCs. Moreover, immunocaptured CTCs are difficult to be released with integrity and high activity, which cannot satisfy the downstream analysis. Although the utilization of magnetic particles can release captured cells though diverse release processes (e.g., electrochemical stimulation, electrochemical adsorption, and protease degradation), the structural integrity and the cell microenvironment can still be affected.

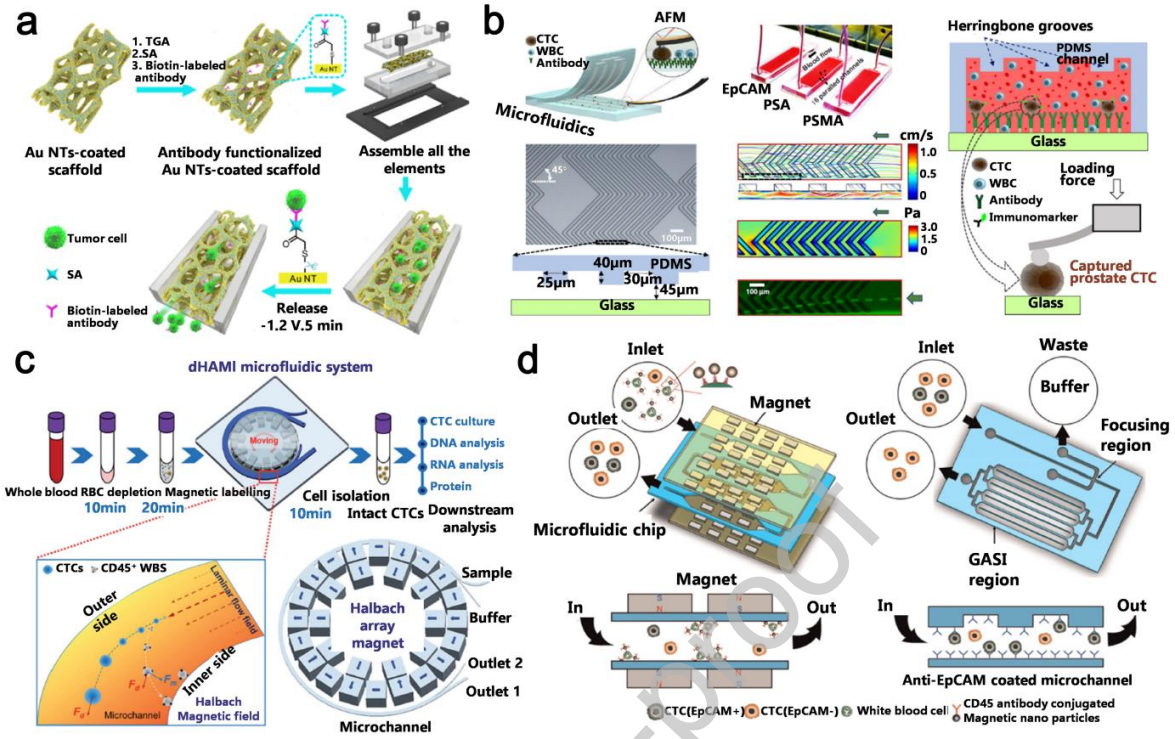

Fig. 3. (a) Schematic illustration of the capture and release of CTCs by the 3D conductive scaffold. [53] Copyright 2021, American Chemical Society. (b) The microfluidic-AFM set-up associated with the capture of CTCs from whole-blood samples. [54] Copyright 2020, Springer Nature. (c) Illustration of the dynamic Halbach array magnet integrated microfluidic system. [63] Copyright 2019, the Royal Society of Chemistry. (d) Schematic diagrams of μ-MACS chip and GASI chip. [64] Copyright 2015, Elsevier.

3.2. Aptamer-assisted microfluidic platforms

Aptamer is a type of small oligonucleotide, which acts as recognition biomolecules with high affinity [65,66]. Compared with antibody, it shows certain advantages such as cost-effectiveness, easy synthesis and functionalization [67,68], which is an excellent candidate for the recognition of CTCs. Aptamer-modified microfluidic interfaces possess great potential for liquid biopsy. In 2009, Phillips et al. pioneered an aptamer-based microfluidic method for the capture of CTCs with excellent capture efficiency (>80%) and purity (>97%) under optimized

parameters [69]. Flow velocity is among the most critical factors that affect the microfluidic device efficiency, which determines the contact time of aptamer and target cells [70]. To regulate the flow field, increasing microstructures and nanostructures (e.g., micropillars, nanoparticles, nanosubstrates and nanofiber) have been implanted in microfluidic chips. Although decreasing flow velocity enhances the probability of the interactions by increasing time, realizing the improvement of capture efficiency, the development of rapid capture and detection is significantly hindered. By implanted two different dimensioned micropillar arrays in two orthogonal microfluidic channels, Reinholt & Craighead presented a microfluidic method for the recognition of cancer cells and isolation of corresponding genomic DNA (gDNA) [71]. As illustrated in Fig. 4a, the cancer cells suspended in PBS buffer were specifically bound to aptamers immobilized on larger micropillars located at the intersection of the channels. Following cells lysing, gDNA within immobilized cells was separated by physical entanglement within another smaller-dimensioned micropillar array enabling amplification and analysis. This method offered a special strategy to monitor different mutations in the same small population of cancer cells. To increase the efficiency and throughput, Sheng et al. developed a microfluidic device utilizing multiple aptamer modified gold nanoparticle (AuNP) for cancer cells separation [72]. Up to 95 aptamers were attached onto a single nanoparticle, leading to improved molecular recognition capability, and an increase of 39-fold in binding affinity compared to a single aptamer (Fig. 4b). Fang's group introduced an aptamer-modified NanoVelcro Chip for the isolation and release of non-small cell lung cancer (NSCLC) CTCs [73]. As shown in Fig. 4c, CTCs capture efficiency was enhanced by combining the aptamer-modified silicon nanowire substrates (SiNWS) to improve CTCs capture efficiency. With enzyme-catalyzed digestion, specific release of nanosubstrate-immobilized CTCs was induced. Based on the poly (lactic-co-

glycolic acid) (PLGA) electrospun nanofibers, Wu et al. developed dual aptamer-assisted microfluidic devices (Fig. 4D) to analyze CTCs of ovarian cancer [74]. The microfluidic device successfully captured 1 to 13 cells in clinical patients' samples, which was confirmed by the immunofluorescence staining experiments.

Most current aptamer sensors focus on the strengthening of binding affinity. However, the aptamer orientation and entanglement on microfluidic interfaces are always ignored [75]. Different DNA nanostructures such as tetrahedral DNA (TDNA) and cubic DNA nanostructure (CDNA) have been applied to regulate the orientation of aptamer strands. Wang et al. constructed a microfluidic system by combining TDNA with aptamer-triggered hybridization chain reaction (HCR) in a herringbone channel chip for efficient CTCs capture and controlled release [76]. To achieve multivalent binding interaction, Peng et al. replaced TDNA with CDNA to control the orientation and valency of the aptamer on a microfluidic surface [77]. Due to the well-aligned orientation, CTCs could be successfully captured from patients' blood (Fig. 4e). Integrating aptamer-targeted magnetic nanoparticles with a microfluidic chip not only offers facile and efficient isolation of CTCs, but also provides additional functions such as real-time detection and phenotypic analysis. Poudineh et al. described a microfluidic approach that profiles along two independent phenotypic axes [78]. This microfluidic chip is clearly shown in Fig. 4f. Based on aptamer-based IMNs, cells were initially sorted according to the surface marker level. Then, various heterogeneous cells extracted from each zone were subjected to chemotactic phenotype detecting and sorting. This novel 2D phenotypic profiling is an effective strategy to monitor the heterogeneous phenotypes of CTCs and isolate phenotypically distinct cell subpopulations. By integrating biomimetic magnetosomes with microfluidic device, Zhang et al. developed a promising strategy for target tumor cells capture and detection [79]. After effective

capture reaction of CTCs (>90%) within 20 min, the microfluidic chip could conveniently quantify CTCs without leukocyte background (Fig. 4g). The aptamer and leukocyte membrane modified Fe_3O_4 magnetic nanoclusters (A-M/NCs) were arranged in the nickel square of microfluidic device, which significantly enhanced the local topographic interaction opportunities with the target CTCs, as well as endowed the on-line CTCs detection feasibility. Kajani et al. designed a specific microfluidic device consisting of mixing, sorting and separation modules (Fig. 4h) [80]. The direct exposure of Apt-MNPs with blood samples for 60 min was appropriate for the sensitive and effective capture of CTCs, which was confirmed by immunofluorescence detection method (Fig. 4h). Thanks to the high-affinity anti-MUC1 aptamer and glutaraldehyde linker, the CTCs capture yield exceeded 91% without cell damage under optimum condition. Obviously, the efficiency of CTCs capture from the clinical samples was comparable with the examined commercial kit, making it a suitable candidate for the subsequent culture and molecular analysis in clinical purposes.

Although aptamer-based microfluidic platforms are emerging as powerful tools for the detection of CTCs in a noninvasive, sensitive, and controlled target release, few aptamer-based microfluidic platforms are suitable for routine clinical utility [81,82]. Some obstacles must be addressed before it can be implemented as a real-world tool. First, the compromised stabilities and binding affinities of aptamer in complex body fluids. Second, the collision opportunities between circulating targets and capture substrates, and the capturing capability of substrates should be further enhanced. Third, integrated devices and standardized processing procedures are important factors for clinical application, which also need to be improved for aptamer-based microfluidic methods.

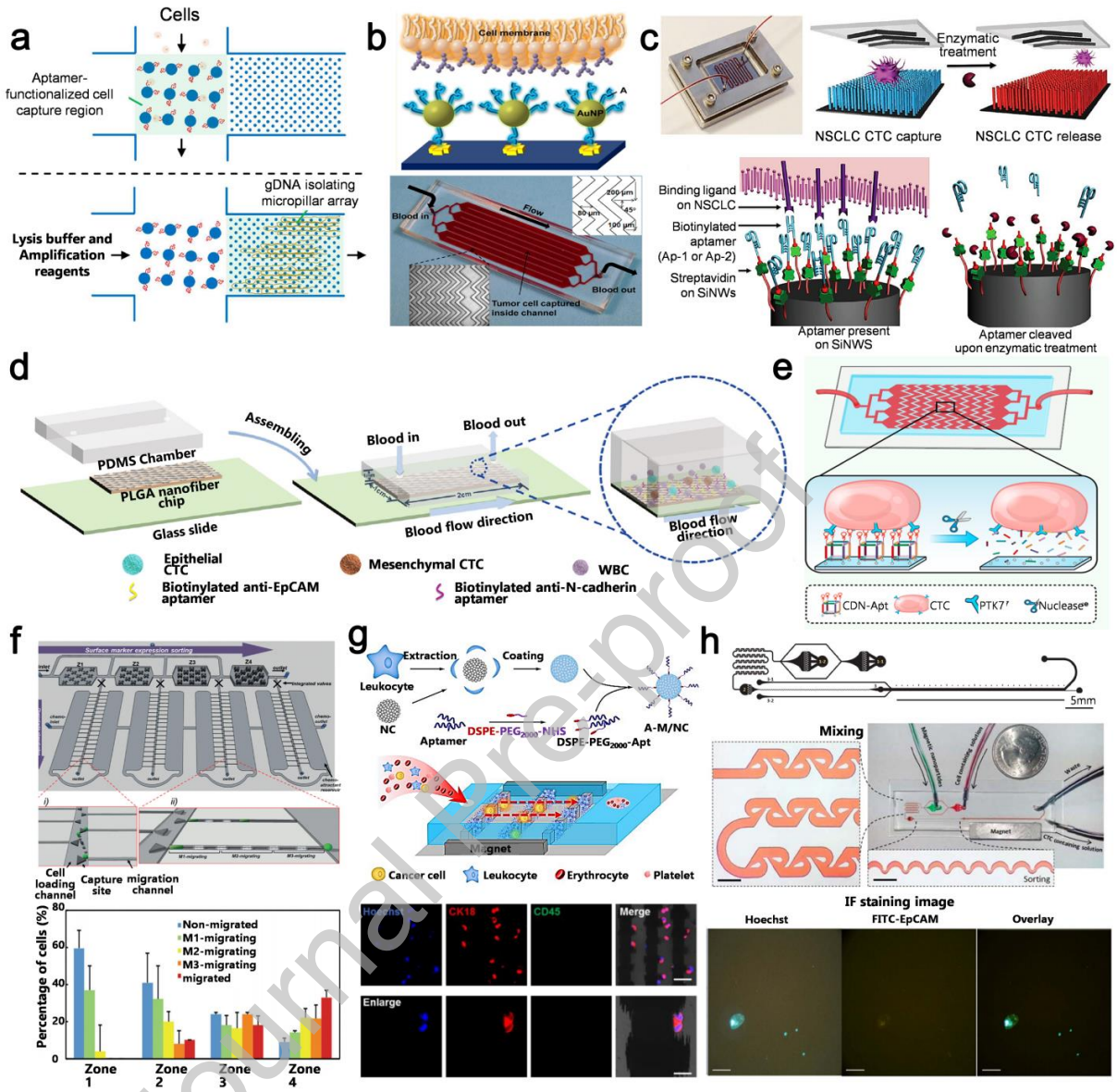

Fig. 4. (a) Scheme of the capture and release of CTCs by two micropillar arrays. [71] Copyright 2018, American Chemical Society. (b) Illustrations of enhanced cell capture using AuNP-aptamer-modified surface and the microfluidic device with herringbone mixing microstructures. [72] Copyright 2013, American Chemical Society. (c) Image of an aptamer-coated NanoVelcro Chip and scheme of the molecular mechanism governing the capture and enzymatic release of NSCLC CTCs. [73] Copyright 2013, John Wiley and Sons. (d) Schematic diagram of the dual

aptamer-modified PLGA nanofiber-based microfluidic device. [74] Copyright 2021, the Royal Society of Chemistry. (e) Scheme depicting the workflow of CDNA−Aptamer−Chip for capture and release of CTCs. [77] Copyright 2022, American Chemical Society. (f) Profiling functional and biochemical phenotypes of CTCs in microfluidic chip. [78] Copyright 2017, John Wiley and Sons. (g) Construction of biomimetic microfluidic system. [79] Copyright 2019, American Chemical Society. (h) Scheme of microfluidic device containing mixer module, sorting module and magnetic separation module. [80] Copyright 2022, the Royal Society of Chemistry.

3.3. Peptide-assisted microfluidic platforms

Compared with traditional antibody, peptide has the merits of small dimensions, excellent biocompatibility, high binding affinity, stability and are easier to be synthesized with various functionalization, so it tends to perform better in the detection of cancer biomarkers and therapeutics [83,84]. A variety of recognition peptides are key roles in ligand–receptor and protein–protein interaction, performing remarkably diverse functions (e.g., molecular recognition, surface modification and signal labeling) in microfluidic platform. The capture efficiency and purity of CTCs are above 70%, and some approaches can even exceed 90% [85-88].

Peptide can also be implanted into microfluidic device to efficiently and precisely capture and release CTCs with minimal effect on cell viability. Shen et al. established peptide-silicon nanowires' substrate (Pe-SiNWS), and integrated it into microfluidic device with herringbone structure [89]. The CTCs capture efficiency (95.6%) is significantly improved owing to the enhancement of interactions between cells and channel substrate by adopting unique staggered herringbone structure and nanowire. The peptide digestion has been used for the release of CTCs with advantages of simple operation, efficient release, and minimal effect on cell viability (93.5%). Chen et al. developed an analogous extracellular matrix's platform for the enrichment

and selective separation of CTCs by using asparagine-glycine-arginine (NGR) peptide modified TiO_2 nanofiber surfaces grafted by BSA [90]. The platform could capture PC-3 cancer cells from mimical blood samples with a high specificity and sensitivity. Aiming to culture CTCs isolated from metastatic breast cancer patients, Carmona-Ule et al. presented peptide-functionalized device by depositing Peptide-Functionalized Nanoemulsions (Pept-NEs) in a culture well-plate [91]. Since this technique avoided the need for multi-step procedures associated with off-chip re-culturing, Pept-NEs-functionalized chip appeared to be an interesting option for the development of a potential isolation and culture technology. Different proteins and cells lead to severe biofouling in blood, hence the direct detection of CTCs in real blood samples remains challenging. Han et al. designed a multifunctional peptide with antifouling capability and recognition ability in biological samples, and integrated it with the electrodeposited conducting polymer poly to devise a sensitive electrochemical assay with a two-step strategy [92]. It was able to perform directly in blood samples without complicated purification and separation.

The universal incomplete contact issue of the biological macromolecules and ligands caused by the steric hindrance of the antibody functioned magnetic nanoparticles is effectively addressed by using peptide-modified magnetic nanoparticles. Microfluidic platforms based on peptide functionalized magnetic nanocomposites are a promising technique for CTCs isolation and detection [92,93]. Liang et al. reported a method based on peptide-functionalized magnetic nanoparticles, which demonstrated superiority in non-small cell lung cancer (NSCLC) clinical applications [94]. After capturing CTCs, releasing captured CTCs without damage plays a crucial role for personalized cancer therapy. Besides isolation and release of CTCs, the culture of CTCs *in situ* is also important for understanding the biology of metastasis in cancer disease. Using peptide functionalized magnetic nanocomposites, *in situ* chemotherapy can be realized

while detecting CTCs. Liu et al. constructed a novel Fe_3O_4–FePt magnetic nanocomposites [95]. After modification with NH_2–PEG–COOH and the tumor-targeting molecule tLyP-1, Fe_3O_4–FePt could serve as a promising multifunctional nano separator for efficiently capturing CTCs and simultaneously inducing in situ chemotherapy.

Although peptide is a type of promising molecules to target cells with littler damage, their conformational flexibility and small structures occasionally lead to relative weak interactions with target cells. Besides, peptide is usually modified on MNPs to isolate CTCs, and the attachment of the immunoaffinity MNPs and cell surface might cause cytotoxicity in the subsequent culturing process. Thus, releasing isolated cells with viability by using platforms combined with peptide is worth deep exploration. Moreover, peptide-based microfluidic platform is still in its infancy, so further studies must be carried out to optimize the use of peptide-functionalized chips for the isolation and detection of CTCs in real blood sample of patients.

3.4. Integration of multiple ligands

The utilization of single recognition molecule always results in unsatisfactory capture efficiency due to the rarity and heterogeneity of CTCs. Many studies have assembled multiple molecular probes (e.g., antibody, aptamer, peptide, folic acid (FA), as well as materials (e.g., dendrimer and biotinylated polymer) to identify heterogeneous CTCs.

The “antibody-aptamer” strategy combines the advantages of both recognition elements. It also overcomes the limitations of microchips functionalized by either antibody (e.g., difficulty in releasing CTCs with high activity) or aptamer alone (e.g., low affinity, low specificity, more steric hindrance and less available biomarkers). Zhang et al. incorporated an optimized ensemble of aptamers and antibodies into a microfluidic device with micropillar arrays for the capture/isolation of rare cells [96]. Compared with the method using anti-PTK7 alone or sgc8

aptamer alone, antibody–aptamer approach significantly enhanced the capture efficiency especially at a higher flow rate. By introducing an aptamer-antibody (Apt-mAb), Chen et al. developed an ideal regenerative microchip in the separation and profiling of various cancer cells from blood samples [97]. The Fc6 aptamer indirectly linked the interface functionalization of the microfluidic channels to the monoclonal antibodies that specifically bound the target cell receptor. The Apt-mAb provided optimal orientation, flexibility, and reduced steric hindrance, allowing easy reprogramming for any mAb and functionalization of a variety of surfaces. Therefore, universality and regenerative capacity to the microchip for CTCs separation and profiling were promised. In addition to aptamers, other molecular probes (e.g., peptide) and materials (e.g., dendrimer and biotinylated polymer) have also been coupled with ordinary single antibody [98,99]. Lu et al. fabricated a decomposable immunomagnetic system based on the immobilization of peptide-tagged antibody on engineered protein-coated magnetic beads [100]. Over 70% of captured cells could be released by biotin addition, and the viability of released cells could reach 85%. This method was successfully applied to isolate and detect CTCs in cancer patients' peripheral blood samples, and 2–215 CTCs were identified with high purity. Using the analogous strategy, Yoshihara et al. constructed a superefficient cell-capture and release method by immobilizing antibodies on the peptides that bound to 3D microfiber fabrics [101]. By functionalizing fluorescence-visible magnetic capsules with folic acid and anti-EpCAM antibody, Ma et al. proposed the multi-targeting magnetic capsules for rare MCF-7 cells isolation, which enabled a more than 80% capture efficacy of CTCs [102].

As mentioned before, label methods for CTCs isolation exhibit higher specificity compared to label-free methods. The magnetic particles modified with targeting ligands enable the capture and release of CTCs in magnetic field. Due to the fact that the anchoring degree of ligand-

modified magnetic nanoparticles depends on the expression level of receptors on CTCs, the magnetic particle-based separation can achieve CTCs subpopulations profiling and clarifying. By combining multiple ligands on MNPs, the heterogeneous CTCs can be captured effectively. Moreover, the non-contact manipulation of magnetic particles offers the possibility of in vivo application, promising to become an adjuvant therapy for cancer by cleaning CTCs from the blood. Recently, advanced technologies, such as nanomaterials, QDs, and Raman spectroscopy, have been integrated with microfluidic chips, enabling the next generation isolation technologies and subsequent biological analysis of CTCs. However, endocytosis of these ligand-functionalized MNPs is unavoidable and seriously damage the viability, proliferative capacity, and differentiation of isolated CTCs. In addition, non-selective endocytosis by non-targeted cells also decreases the purity of the captured cells. Therefore, to ensure the purity and viability of isolated CTCs, endocytosis of MNPs must be minimized before its clinical application. Besides, the incubation progress or modification progress is necessary and time consuming, which restricts the rapid detection of CTCs.

4. Integrated biochemical and biophysical methods

As mentioned above, biochemical methods that utilize ligands possess great specificity and capture purity of CTCs, while label-free biophysical methods provide high throughput and easy manipulation. However, the efficacy is restricted by the rarity and heterogeneity of CTCs, resulting in poor or incorrect information. In recent decades, the combination of biochemical and biophysical methods has attracted plenty of researchers' attention. Based on the microfluidics technology, increasing integrated methods have been proposed and improved by means of microfluidic chip to isolate, detect, and analyze CTCs.

4.1. Integration of biochemical and passive biophysical methods

The most frequently used method is integrating ligand into passive biophysical assays, such as filtration, DLD, and inertial microfluidics. By modifying the surface of membrane filter or filtration-based microchannels with ligands (e.g., antibodies and aptamers), CTCs with larger size can adhere to the ligands. Meunier et al. modified the surface of a membrane filters of various diameters with antibodies to capture CTCs (Fig. 5a). Compared to the pristine filter without modification, the recovery rate of modified filter was improved from 80% to 95% in separating cancer cells from blood samples, while avoiding cell contamination [103]. A micro-aperture chip based on three methods was fabricated with immunomagnetics for the detection of large CTCs [104]. The micropore-arrayed filtration apertures with 8 μm diameter were characterized by detecting cancer cell lines spiked into healthy human blood, and the detection yield of CTCs were found to be consistently near 90% (Fig. 5b). Zhang et al. proposed an ultrasensitive single-cell recognition platform integrating an improved spiral separation unit with a hydrodynamic filtrating purification unit (Fig. 5c). The platform facilitated the recognition of single CTC with a sorting separation rate of 93.6% at a flow rate of 60 μL/min, which provided a promise solution for commercializing and industrializing various portable, low-cost rare cell isolating/detecting/phenotyping systems [105]. The capture mechanism of integrated microfluidic devices based on the combination of filter and ligand affinity is to restrict the target cell in channel, resulting in the difficulty in releasing CTCs with high activity. Huang et al. combined gelatin-coated silica microbeads with microfluidic filter to increase specific surface area to enhance capturing efficiency of CTCs (Fig. 5d). A laminar-like interface was used to realize continuous-flow CTCs purification. The degradation of gelatin using MMP-9 enzyme endowed biocompatible collection of precious CTCs samples [106].

The combination of multiple technologies can not only improve the purity, but also apply other functions (e.g., phenotypic profiling, cell culture and direct protein analysis). For instance, an integrated multifunctional microfluidic system developed by Abdulla et al. successfully identified a subgroup of apoptosis-negative cells, and its clinical application in personalized CTCs epithelial-to-mesenchymal transition (EMT) analysis was demonstrated (Fig. 5e) [16]. Because the expression level of the isolated CTCs marker can be directly determined at the single-cell level, and the therapeutic response to anticancer drugs can be monitored simultaneously, it provided a promising clinical translational tool for clinical drug response monitoring and personalized regimen development. By coupling micropore membrane filtration with aptamer-SERS bio-probe recognition, Lv et al. proposed a surface enhanced Raman spectroscopy (SERS) method capable of distinguishing isolated CTCs from residual WBCs in 10s [107]. Significantly, the synthesized process aptamer-SERS bio-probe is relatively efficient and convenient by binding SH-aptamer to 4-mercaptobenzoic acid (4-MBA) modified AuNPs with the help of ethyl acetate. Based on their Raman signal intensity difference at 1075 cm^{-1}, single human non-small cell lung cancer A549 cells could be distinguished by mapping its surface biomarkers that related to selected specific aptamer. This system could achieve above 90% recovery rate and recognition rate, even the quantity of A549 cells in blood solution simulating CTCs sample is low to 20. In addition to high efficiency, the merits of rapid, reliable and cost-effective detection indicate a good prospect in clinical application for CTCs detection.

Integrating passive biophysical methods (e.g., DLD and inertial focusing) and biochemical methods takes advantage of the high flow rate of the passive biophysical method and compensates for the low specificity and purity of the former by the biochemical methods. These combined approaches can assist the design and fabrication of the next generation of microfluidic

devices which fulfill lower cellular damage, higher volume throughput, and higher separation yield requirements. The majority of unitized microfluidic chips proposed for CTCs isolation and enrichment uses cascading connection strategy, requiring multiple independent steps and more progress time. So, the compact integrated microchip still lacks of investigation and remains challenging owing to the discrepant requirements of flow rate.

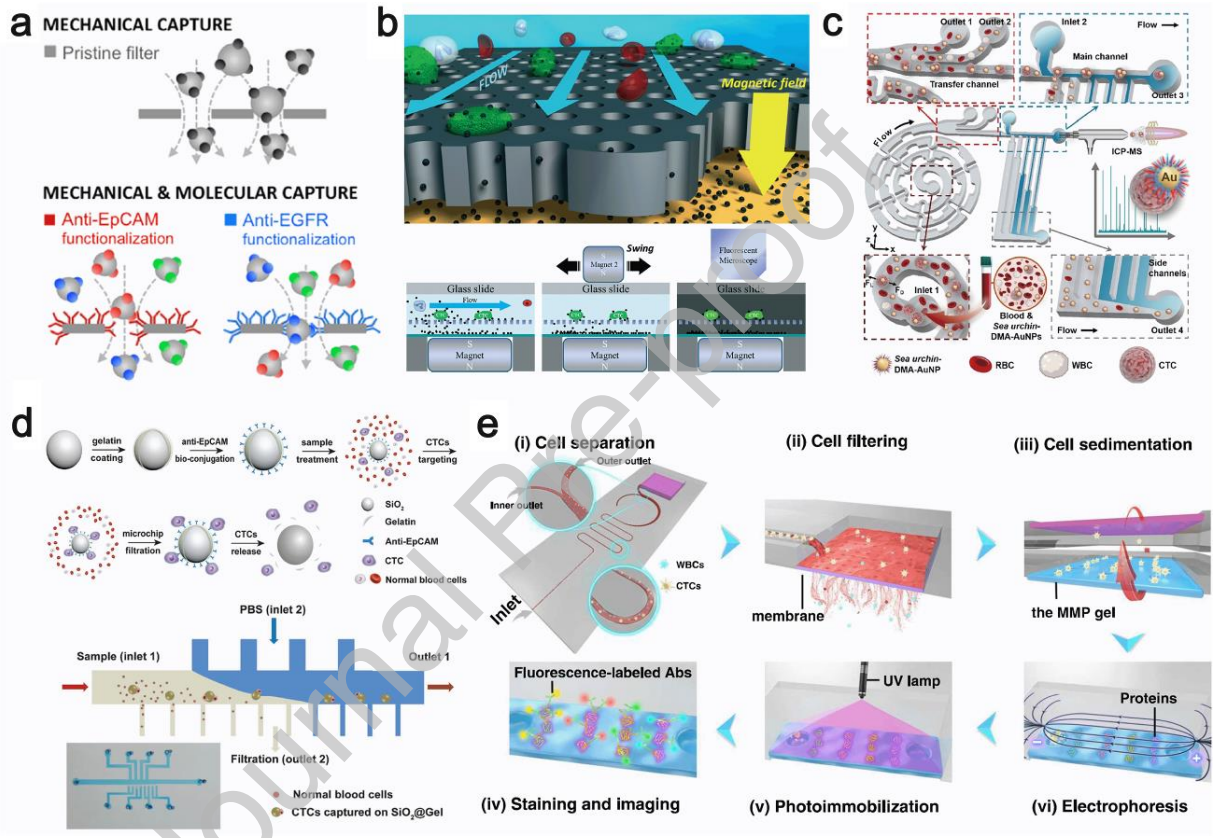

Fig. 5. (a) Illustration of the integrated method using filters with antibodies specific to a molecular receptor expressed on a cell. [103] Copyright 2016, American Chemical Society. (b) Illustration of the micro-aperture chip system and cross-section views of the fluidic chamber. [104] Copyright 2015, the Royal Society of Chemistry. (c) Scheme of the working principle of multifunctional microfluidic platform with an enhanced spiral separation unit and a hydrodynamic filtrating purification unit. [105] Copyright 2021 American Chemical Society. (d)

Scheme of microfluidic isolation of CTCs based on microbead-mediated size amplification. [106] Copyright 2016, John Wiley and Sons. (e) Schematic illustration of chip operation procedure. [16] Copyright 2022, Springer Nature.

4.2. Integration of biochemical and active biophysical methods

AP based on acoustic radiation forces is known as a typical high-throughput and non-contact technique to separate microparticles or cell populations. But these microfluidic methods for CTCs separation display challenges in yielding a significant WBC background in their separation products. Utilizing in-line magnetic particle-based leukocyte depletion to diminish significant background contamination of WBC in its separation progress is an attractive solution. Cushing et al. proposed an acoustophoretic immuno-affinity negative selection for the isolation of tumor cell from a WBC contamination. The WBC specifically bound to CD45-antibodies was selected and reduced by using negative acoustic contrast elastomeric particles (EPs) [108]. This method based on multi-step process enabled various tumor cells (DU145 and MCF-7) isolation with separation efficiencies of 99%. By modifying ZnO nanoflibers with antibody, Li et al. engineered a super efficient platform for single-cell capture/encapsulation [109]. Because the dense ZnO nanofibers provided additional binding sites, an increased capture efficiency reaches up to 93.3%.

DEP always produced high purities for cell separation. However, it could not achieve enough throughput and efficiency. Incorporation of DEP with immunomethods can facilitate the development of CTCs isolation and detection platform with excellent performances. Huang et al. reported a hybrid DEP-immuno system based on interdigitated electrodes which were designed in a Hele-Shaw flow cell. The specific recognition relied on the functionalized monoclonal antibody [110]. By precisely tuning the applied AC electric field frequency, the immunocapture of prostate cancer cell line (LNCaPs) and pancreatic cancer cell lines (Capan-1, PANC-1, BxPC-

3) could be enhanced and nonspecific adhesion of peripheral blood mononuclear cells was reduced with positive and negative DEP, respectively [111]. Recently, combining with a built-in capacitive sensor, a DEP microfluidic enrichment platform including a lollipop-shaped gold microelectrode structure under a polydimethylsiloxane chamber was presented for CTCs detection [112]. With the proposed platform, A549 non-small human lung carcinoma and S-180 sarcoma cell lines were firstly attracted to the center of the working chamber via DEP forces, and then captured by an electrode immobilized by anti-EGFR. After the cell concentration process, the differential capacitance can be read directly to detect the presence of the target cells, which is suitable for rapid cancer diagnoses and other metabolic disease applications. Zhao et al. proposed a novel method combining DEP and magnetophoresis in a microfluidic chip for the separation of CTCs from cell. The effect of the flow rate, the AC electric potential and the ferromagnet structure on the separation were investigated based on simulation [113].

The above integrated methods provide contactless operation progresses with a significant purity of captured CTCs. Nevertheless, incorporation of active sorting elements requires additional equipment, and the interaction of generated multiple force field is complicated. Therefore, multiple independent microfluidic chips equipped with active sorting elements are connected by cascaded stages, resulting in an increase in dimensions and produce cost. Moreover, the viability of isolated cells is inferior owing to the Joule heat generated by electric field.

5. Conclusion and future perspective

With the development of microfluidic technology, increasing hybrid-modality methods have been developed for CTCs isolation and detection. Based on hybrid strategy and operating mechanism, they can be divided into label-free assays, labelled assays and integrated biochemical and biophysical methods. Microfluidic systems not only benefit automation and

scaling up, but also improve the separation performances. The combination of multiple label-free separation approaches potentially refines the separation resolution. However, the purity of CTCs is inferior. Integrated labelled biochemical methods have the limitations of time-consuming and throughput procedures. Therefore, the most frequently reported hybrid-modality is integration of biophysical and biochemical methods, which takes advantage of the high flow rate and compensates for the low specificity. Specifically, the integration of ligand affinity with filtration, inertial focusing, and DEP permits the capture of CTCs with an improved specificity. To achieve an integrated microfluidic detection platform, multiple functionalities (sorting, sensing and analytics) based on micromaterials or nanomaterials can be integrated to enable efficient isolation and precise analysis. However, the majority of unitized microfluidic platforms for CTCs isolation and enrichment are based on cascading connection strategy, which requires off-chip sample incubation with MNPs, increasing processing time and the potential for cell loss with manual transfer. Thus, it is still needed to develop more intimately integrated derivatives in order to improve the yield and the recovery efficiency. The proposal of commercial integrated microfluidic platform for clinical application is facing the following issues and challenges, requiring further investigation.

First, integration of single modality methods in microfluidic chip is a challenging task. In addition to the increased complexity in chip design and instrumentation, the flow rate disparity between disparate modules, passive sorting module and marker detection module in particular, should be considered and balanced in a single chip. Second, incorporation of active sorting elements (AP and DEP) may need additional equipment and the miniaturization for point-of-care testing should be designed. Additionally, the effect of specific medium conditions (e.g., conductivity and viscosity) on the cytoactive of cell should be addressed for facilitating cell

culture, downstream analysis and phenotypic evaluation. Third, with the increasing demand for efficient capture and active release in clinical applications, the binding ability between CTCs and capture objects during capture procedure, as well as the destruction of conjunctions during release process, requires in-depth investigation. The capturing capability of substrates that modified with various MNPs should be enhanced by increasing the associativity of capture biomolecules and the collision opportunities between circulating targets and capture substrates. Meanwhile, more studies are needed on the release of CTCs, satisfying rapidly, high efficiency release with minimal activity impact. Last, although hybrid-modality methods combine various single-modality methods using a sequenced strategy with great flexibility, challenges still remain in reducing monolithic dimensions, decreasing the probability of pollution and realizing automatic operation. The integrated devices and standardized processing procedures is in a chaotic situation. Unified and standardized procedure is urgently needed to meet clinical applications.

Authorship contributions

Ruiju Shi: Conceptualization, Investigation, Writing - original draft; Yuyan Yue: Writing - original draft; Ziqi Liu: Writing - original draft; Hua Chai: Writing - review & editing; Peng Miao: Conceptualization, Supervision, Writing - review & editing.

Declaration of competing interest

The authors declare that they have no conflicts of interest in this work.

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References

[1] M.Y. Wang, M. Sperrin, M.K. Rutter, A.G. Renehan, Cancer is becoming the leading cause of death in diabetes, The Lancet, 401 (2023) 1849.

[2] F.L. Li, H.Y. Xu, Y.F. Zhao, Magnetic particles as promising circulating tumor cell catchers assisting liquid biopsy in cancer diagnosis: a review, TrAC, Trends Anal. Chem., 145 (2021) 116453.

[3] H. Wang, P. Liang, L. Zhang, L. Shi, Y. Ge, L. Zhang, X. Qiao, A soluble pH-responsive host-guest-based nanosystem for homogeneous exosomes capture with high-efficiency, Chin. Chem. Lett., 34 (2023) 108129.

[4] N. Yu, G.F. Ma, Y.Y. Chen, S. Huang, Y.L. Gong, S.S. Li, H.Q. Gu, H.L. You, P. Miao, MnO2 nanosheets and gold nanoparticles supported electrochemical detection of circulating tumor cells, Colloids Surf., B, 229 (2023) 113482.

[5] M. Wang, D. Li, C. Duan, J. Jiao, Y. Gong, X. Wang, Z. Wang, Y. Xiang, Enrichment and analysis of circulating tumor cells by integrating multivalent membrane nano-interface and endogenous enzyme-signal amplification, Chin. Chem. Lett., 34 (2023) 107388.

[6] D.F. Lin, L.S. Shen, M. Luo, K. Zhang, J.F. Li, Q. Yang, F.F. Zhu, D. Zhou, S. Zheng, Y.D. Chen, J.J. Zhou, Circulating tumor cells: biology and clinical significance, Signal Transduction Targeted Ther., 6 (2021) 404.

[7] D.J. Collins, Z.C. Ma, Y. Ai, Highly localized acoustic streaming and size-selective submicrometer particle concentration using high frequency microscale focused acoustic fields, Anal. Chem., 88 (2016) 5513-5522.

[8] X.J. Hu, D.M. Zhu, M. Chen, K.K. Chen, H.L. Liu, W. Liu, Y. Yang, Precise and non-invasive circulating tumor cell isolation based on optical force using homologous erythrocyte binding, Lab Chip, 19 (2019) 2549-2556.

[9] I.F. Cheng, W.L. Huang, T.Y. Chen, C.W. Liu, Y.D. Lin, W.C. Su, Antibody-free isolation of rare cancer cells from blood based on 3d lateral dielectrophoresis, Lab Chip, 15 (2015) 2950-2959.

[10] Y.P. Liu, T.Y. Li, M.X. Xu, W. Zhang, Y. Xiong, L.G. Nie, Q. Wang, H.C. Li, W. Wang, A high-throughput liquid biopsy for rapid rare cell separation from large-volume samples, Lab Chip, 19 (2019) 68-78.

[11] K. Loutherback, J. D'Silva, L.Y. Liu, A. Wu, R. Austin, J. Sturm, Deterministic separation of cancer cells from blood at 10 ml/min, AIP Adv., 2 (2012) 42107.

[12] B. Kwak, S. Lee, J. Lee, J. Lee, J. Cho, H. Woo, Y.S. Heo, Hydrodynamic blood cell separation using fishbone shaped microchannel for circulating tumor cells enrichment, Sens. Actuators, B, 261 (2018) 38-43.

[13] Y. Meng, Y. Zhang, M. Bühler, S. Wang, M. Asghari, A. Stürchler, B. Mateescu, T. Weiss, S. Stavrakis, A.J. deMello, Direct isolation of small extracellular vesicles from human blood using viscoelastic microfluidics, Sci. Adv., 9 (2023) eadi5296.

[14] S.M. Abusamra, R. Barber, M. Sharafeldin, C.M. Edwards, J.J. Davis, The integrated on-chip isolation and detection of circulating tumour cells, Sens. Diagn., 3 (2024) 562-584.

[15] J.D. Wang, W.J. Lu, C.H. Tang, Y. Liu, J.S. Sun, X. Mu, L. Zhang, B. Dai, X.Y. Li, H.L. Zhuo, X.Y. Jiang, Label-free isolation and mrna detection of circulating tumor cells from patients with metastatic lung cancer for disease diagnosis and monitoring therapeutic efficacy, Anal. Chem., 87 (2015) 11893-11900.

[16] A. Abdulla, T. Zhang, S.H. Li, W.K. Guo, A.R. Warden, Y.F. Xin, N. Maboyi, J.T. Lou, H.Y. Xie, X.T. Ding, Integrated microfluidic single-cell immunoblotting chip enables high-throughput isolation, enrichment and direct protein analysis of circulating tumor cells, Microsyst. Nanoeng., 8 (2022) 13.

[17] N. Xiang, J. Wang, Q. Li, Y. Han, D. Huang, Z.H. Ni, Precise size-based cell separation via the coupling of inertial microfluidics and deterministic lateral displacement, Anal. Chem., 91 (2019) 10328-10334.

[18] Z.B. Liu, Y.Q. Huang, W.L. Liang, J. Bai, H.T. Feng, Z.H. Fang, G. Tian, Y.J. Zhu, H.B. Zhang, Y.X. Wang, A.X. Liu, Y. Chen, Cascaded filter deterministic lateral displacement microchips for isolation and molecular analysis of circulating tumor cells and fusion cells, Lab Chip, 21 (2021) 2881-2891.

[19] C. Ni, D. Wu, Y. Chen, S. Wang, N. Xiang, Cascaded elasto-inertial separation of malignant tumor cells from untreated malignant pleural and peritoneal effusions, Lab Chip, 24 (2024) 697-706.

[20] N. Xiang, Z. Ni, D. Wu, High-throughput and high-purity separation of malignant tumor cells in pleural and peritoneal effusions using interfacial elasto-inertial microfluidics, Sens. Diagn., 2 (2023) 929-937.

[21] E. Cho, J.-a. Kim, M.K. Aslan, Y. Meng, S. Stavrakis, A. deMello, Tunable viscoelastic size-based particle separation in straight microchannels with triangular cross-sections, Sens. Actuators, B, 414 (2024) 135892.

[22] Z. Zhou, C. Ni, Z. Zhu, Y. Chen, Z. Ni, N. Xiang, High-throughput adjustable deformability cytometry utilizing elasto-inertial focusing and virtual fluidic channel, Lab Chip, 23 (2023) 4528-4539.

[23] S. Karthick, P.N. Pradeep, P. Kanchana, A.K. Sen, Acoustic impedance-based size-independent isolation of circulating tumour cells from blood using acoustophoresis, Lab Chip, 18 (2018) 3802-3813.

[24] V. Nerguizian, I. Stiharu, N. Al-Azzam, B. Yassine-Diab, A. Alazzam, The effect of dielectrophoresis on living cells: crossover frequencies and deregulation in gene expression, Analyst, 144 (2019) 3853-3860.

[25] M. Tayebi, D.H. Yang, D.J. Collins, Y. Ai, Deterministic sorting of submicrometer particles and extracellular vesicles using a combined electric and acoustic field, Nano Lett., 21 (2021) 6835-6842.

[26] A.J. Smith, R.D. O'Rorke, A. Kale, R. Rimsa, M.J. Tomlinson, J. Kirkham, A.G. Davies, C. Wälti, C.D. Wood, Rapid cell separation with minimal manipulation for autologous cell therapies, Sci. Rep., 7 (2017) 41872.

[27] L. Shen, Z.H. Tian, J.X. Zhang, H.D. Zhu, K.C. Yang, T. Li, J. Rich, N. Upreti, N.J. Hao, Z.C. Pei, G. Jin, S.J. Yang, Y.S. Liang, W. Chaohui, T.J. Huang, Acousto-dielectric tweezers for size-insensitive manipulation and biophysical characterization of single cells, Biosens. Bioelectron., 224 (2023) 115061.

[28] A. Al-Ali, W. Waheed, E. Abu-Nada, A. Alazzam, A review of active and passive hybrid systems based on dielectrophoresis for the manipulation of microparticles, J. Chromatogr. A, 1676 (2022) 463268.

[29] U. Kim, B. Oh, J. Ahn, S. Lee, Y. Cho, Inertia-acoustophoresis hybrid microfluidic device for rapid and efficient cell separation, Sensors, 22 (2022) 4709.

[30] K. Mutafopulos, P. Spink, C.D. Lofstrom, P.J. Lu, H. Lu, J.C. Sharpe, T. Franke, D.A. Weitz, Traveling surface acoustic wave (tsaw) microfluidic fluorescence activated cell sorter (μfacs), Lab Chip, 19 (2019) 2435-2443.

[31] Y.N. Zhou, Z.C. Ma, Y. Ai, Hybrid microfluidic sorting of rare cells based on high throughput inertial focusing and high accuracy acoustic manipulation, RSC Adv., 9 (2019) 31186-31195.

[32] X.L. Liu, X. Chen, Z.W. Yang, H. Xia, C.Y. Zhang, X.Y. Wei, Surface acoustic wave based microfluidic devices for biological applications, Sens. Diagn., 2 (2023) 507-528.

[33] W. Waheed, A. Alazzam, B. Mathew, N. Christoforou, E. Abu-Nada, Lateral fluid flow fractionation using dielectrophoresis (lfff-dep) for size-independent, label-free isolation of circulating tumor cells, Journal of Chromatography B, 1087-1088 (2018) 133-137.

[34] D. Kim, J.H. Luo, E.A. Arriaga, A. Ros, Deterministic ratchet for sub-micrometer (bio)particle separation, Anal. Chem., 90 (2018) 4370-4379.

[35] V. Calero, P. Garcia-Sanchez, C. Honrado, A. Ramos, H. Morgan, Ac electrokinetic biased deterministic lateral displacement for tunable particle separation, Lab Chip, 19 (2019) 1386-1396.

[36] J.P. Beech, K. Keim, B.D. Ho, C. Guiducci, J.O. Tegenfeldt, Active posts in deterministic lateral displacement devices, Adv. Mater. Technol, 4 (2019) 1900339.

[37] M. Aghaamoo, A. Aghilinejad, X.L. Chen, J. Xu, On the design of deterministic dielectrophoresis for continuous separation of circulating tumor cells from peripheral blood cells, Electrophoresis, 40 (2019) 1486-1493.

[38] M. Rahmati, X.L. Chen, Separation of circulating tumor cells from blood using dielectrophoretic DLD manipulation, Biomed. Microdevices, 23 (2021) 49.

[39] X.H. Li, J.P. Duan, J.Y. Wang, Z. Qu, M.M. Ji, B.Z. Zhang, Continuous particle separation of microfluidic chip with integrated inertial separation and dielectrophoresis separation, AIP Adv., 12 (2022).

[40] J. Zhang, D. Yuan, Q.B. Zhao, S. Yan, S.Y. Tang, S.H. Tan, J.H. Guo, H.M. Xia, N.T. Nguyen, W.H. Li, Tunable particle separation in a hybrid dielectrophoresis (DEP)- inertial microfluidic device, Sens. Actuators, B, 267 (2018) 14-25.

[41] J. Yao, J.X. Chen, X.D. Cao, H. Dong, Combining 3d sidewall electrodes and contraction/expansion microstructures in microchip promotes isolation of cancer cells from red blood cells, Talanta, 196 (2019) 546-555.

[42] C.-H. Chu, R.X. Liu, T. Ozkaya-Ahmadov, M. Boya, B.E. Swain, J.M. Owens, E. Burentugs, M.A. Bilen, J.F. McDonald, A.F. Sarioglu, Hybrid negative enrichment of circulating tumor cells from whole blood in a 3d-printed monolithic device, Lab Chip, 19 (2019) 3427-3437.

[43] P. Miao, Y.G. Tang, Gold nanoparticles-based multipedal dna walker for ratiometric detection of circulating tumor cell, Anal. Chem., 91 (2019) 15187-15192.

[44] H.F. Zhong, C.W. Yuan, J.Y. He, Y. Yu, Y.L. Jin, Y.Y. Huang, R. Zhao, Engineering peptide-functionalized biomimetic nanointerfaces for synergetic capture of circulating tumor cells in an epcam-independent manner, Anal. Chem., 93 (2021) 9778-9787.

[45] C.C. Chen, Z.L. Wang, Y.W. Zhao, Y. Cao, P. Ding, H. Liu, N. Su, R.J. Pei, A folic acid modified polystyrene nanosphere surface for circulating tumor cell capture, Anal. Methods, 11 (2019) 5718-5723.

[46] D. Qiao, H. Li, W. Zhu, L. Zhu, D. Zhao, H. Li, Microfluidic separation of particles by synergistic effect of geometry-induced hydrodynamics and magnetic field, Chin. Chem. Lett., (2023) 108646.

[47] Y.J.Y. Xiang, C. Hu, G. Wu, S.L. Xu, Y. Li, Nanomaterial-based microfluidic systems for cancer biomarker detection: recent applications and future perspectives, TrAC Trends in Anal. Chem., 158 (2023) 116835.

[48] L.H. Guo, C. Liu, M.L. Qi, L. Cheng, L. Wang, C.X. Li, B. Dong, Recent progress of nanostructure-based enrichment of circulating tumor cells and downstream analysis, Lab Chip, 23 (2023) 1493-1523.

[49] Q. Zeng, Q. Wei, J. Luo, Y. Qian, M. Yang, Y. Zou, L. Lu, Novel photoelectrochemical immunosensor for MCF-7 cell detection based on n-p organic semiconductor heterojunction, Chin. Chem. Lett., 33 (2022) 2954-2958.

[50] S. Nagrath, L.V. Sequist, S. Maheswaran, D.W. Bell, D. Irimia, L. Ulkus, M.R. Smith, E.L. Kwak, S. Digumarthy, A. Muzikansky, P. Ryan, U.J. Balis, R.G. Tompkins, D.A. Haber, M. Toner, Isolation of rare circulating tumour cells in cancer patients by microchip technology, Nature, 450 (2007) 1235-1239.

[51] S.T. Wang, K. Liu, J. Liu, Z.T.F. Yu, X.W. Xu, L.B. Zhao, T. Lee, E.K. Lee, J. Reiss, Y.-K. Lee, L.W.K. Chung, J.T. Huang, M. Rettig, D. Seligson, K.N. Duraiswamy, C.K.F. Shen, H.-R. Tseng, Highly efficient capture of circulating tumor cells by using nanostructured silicon substrates with integrated chaotic micromixers, Angew. Chem., Int. Ed., 50 (2011) 3084-3088.

[52] X. Wu, T.Y. Xiao, Z. Luo, R.X. He, Y.P. Cao, Z.Z. Guo, W.Y. Zhang, Y. Chen, A micro-/nano-chip and quantum dots-based 3D cytosensor for quantitative analysis of circulating tumor cells, J. Nanobiotechnol, 16 (2018) 65.

[53] S.B. Cheng, M.M. Chen, Y.K. Wang, Z.H. Sun, Y. Qin, S. Tian, W.G. Dong, M. Xie, W.H. Huang, A three-dimensional conductive scaffold microchip for effective capture and recovery of circulating tumor cells with high purity, Anal. Chem., 93 (2021) 7102-7109.

[54] M. Deliorman, F.K. Janahi, P. Sukumar, A. Glia, R. Alnemari, S. Fadl, W.Q. Chen, M.A. Qasaimeh, AFM-compatible microfluidic platform for affinity-based capture and nanomechanical characterization of circulating tumor cells, Microsyst. Nanoeng, 6 (2020) 20.

[55] B.Y. Li, H.W. Shen, Q. Liu, X. Liu, J. Cai, L. Zhang, D. Wu, Y.X. Xie, G.M. Xie, W.L. Feng, A three-site recognition cytosensor based on multi-active auIrPt polyhedral nanozymes for detection of CTCs, Sens. Actuators, B, 386 (2023) 133762.

[56] W.X. Xia, H.D. Li, Y.Q. Li, M. Li, J.L. Fan, W. Sun, N. Li, R.J. Li, K. Shao, X.J. Peng, In vivo coinstantaneous identification of hepatocellular carcinoma circulating tumor cells by dual-targeting magnetic-fluorescent nanobeads, Nano Lett., 21 (2021) 634-641.

[57] H. Cui, R. Li, J. Du, Q.F. Meng, Y. Wang, Z.X. Wang, F.F. Chen, W.F. Dong, J. Cao, L.L. Yang, S.S. Guo, Rapid and efficient isolation and detection of circulating tumor cells based on zns:mn2+ quantum dots and magnetic nanocomposites, Talanta, 202 (2019) 230-236.

[58] M. Tang, C.Y. Wen, L.L. Wu, S.L. Hong, J. Hu, C.M. Xu, D.W. Pang, Z.L. Zhang, A chip assisted immunomagnetic separation system for the efficient capture and in situ identification of circulating tumor cells, Lab Chip, 16 (2016) 1214-1223.

[59] W.T. Shi, S.Q. Wang, A. Maarouf, C.G. Uhl, R. He, D. Yunus, Y.L. Liu, Magnetic particles assisted capture and release of rare circulating tumor cells using wavy-herringbone structured microfluidic devices, Lab Chip, 17 (2017) 3291-3299.

[60] C.M. Earhart, C.E. Hughes, R.S. Gaster, C.C. Ooi, R.J. Wilson, L.Y. Zhou, E.W. Humke, L. Xu, D.J. Wong, S.B. Willingham, E.J. Schwartz, I.L. Weissman, S.S. Jeffrey, J.W. Neal, R. Rohatgi, H.A. Wakelee, S.X. Wang, Isolation and mutational analysis of circulating tumor cells from lung cancer patients with magnetic sifters and biochips, Lab Chip, 14 (2014) 78-88.

[61] V. Sivagnanam, B. Song, C. Vandevyver, J.-C.G. Bünzli, M.A.M. Gijs, Selective breast cancer cell capture, culture, and immunocytochemical analysis using self-assembled magnetic bead patterns in a microfluidic chip, Langmuir, 26 (2010) 6091-6096.

[62] K. Hoshino, Y.Y. Huang, N. Lane, M. Huebschman, J.W. Uhr, E.P. Frenkel, X.J. Zhang, Microchip-based immunomagnetic detection of circulating tumor cells, Lab Chip, 11 (2011) 3449-3457.

[63] M. Xue, A. Xiang, Y.H. Guo, L. Wang, R. Wang, W.W. Wang, G. Ji, Z.F. Lu, Dynamic halbach array magnet integrated microfluidic system for the continuous-flow separation of rare tumor cells, RSC Adv., 9 (2019) 38496-38504.

[64] K.-A. Hyun, T.Y. Lee, S.H. Lee, H.-I. Jung, Two-stage microfluidic chip for selective isolation of circulating tumor cells (CTCs), Biosens. Bioelectron., 67 (2015) 86-92.

[65] Y. Jiang, X.F. Chen, N.H. Feng, P. Miao, Electrochemical aptasensing of sars-cov-2 based on triangular prism dna nanostructures and dumbbell hybridization chain reaction, Anal. Chem., 94 (2022) 14755-14760.

[66] H.G. Sun, W.H. Tan, Y.L. Zu, Aptamers: versatile molecular recognition probes for cancer detection, Analyst, 141 (2016) 403-415.

[67] J. Wang, W.B. Cheng, F.Y. Meng, M. Yang, Y. Pan, P. Miao, Hand-in-hand RNA nanowire-based aptasensor for the detection of theophylline, Biosens. Bioelectron., 101 (2018) 153-158.

[68] L.L. Wu, H.M. Ding, X. Qu, X.N. Shi, J.M. Yang, M.J. Huang, J.L. Zhang, H.M. Zhang, J. Song, L. Zhu, Y.L. Song, Y.Q. Ma, C.Y. Yang, Fluidic multivalent membrane nanointerface enables synergetic enrichment of circulating tumor cells with high efficiency and viability, J. Am. Chem. Soc., 142 (2020) 4800-4806.

[69] J.A. Phillips, Y. Xu, Z. Xia, Z.H. Fan, W.H. Tan, Enrichment of cancer cells using aptamers immobilized on a microfluidic channel, Anal. Chem., 81 (2009) 1033-1039.

[70] Y. Wan, J.F. Tan, W. Asghar, Y.-t. Kim, Y.L. Liu, S.M. Iqbal, Velocity effect on aptamer-based circulating tumor cell isolation in microfluidic devices, J. Phys. Chem. B, 115 (2011) 13891-13896.

[71] S.J. Reinholt, H.G. Craighead, Microfluidic device for aptamer-based cancer cell capture and genetic mutation detection, Anal. Chem., 90 (2018) 2601-2608.

[72] W.A. Sheng, T. Chen, W.H. Tan, Z.H. Fan, Multivalent dna nanospheres for enhanced capture of cancer cells in microfluidic devices, ACS Nano, 7 (2013) 7067-7076.

[73] Q.L. Shen, L. Xu, L.B. Zhao, D.X. Wu, Y.S. Fan, Y.L. Zhou, W.H. OuYang, X.C. Xu, Z. Zhang, M. Song, T. Lee, M.A. Garcia, B. Xiong, S. Hou, H.-R. Tseng, X.H. Fang, Specific capture and release of circulating tumor cells using aptamer-modified nanosubstrates, Adv. Mater., 25 (2013) 2368-2373.

[74] Z. Wu, Y. Pan, Z.L. Wang, P. Ding, T. Gao, Q. Li, M.C. Hu, W.P. Zhu, R.J. Pei, A PLGA plga nanofiber microfluidic device for highly efficient isolation and release of different phenotypic circulating tumor cells based on dual aptamers, J. Mater. Chem. B, 9 (2021) 2212-2220.

[75] Y.L. Liu, H.M. Zhang, Y.H. Du, Z. Zhu, M.X. Zhang, Z.H. Lv, L.L. Wu, Y.Y. Yang, A. Li, L. Yang, Y.L. Song, S.L. Wang, C.Y. Yang, Highly sensitive minimal residual disease detection by biomimetic multivalent aptamer nanoclimber functionalized microfluidic chip, Small, 16 (2020) 2000949.

[76] C.G. Wang, Y. Xu, S.N. Li, Y. Zhou, Q.L. Qian, Y.F. Liu, X.Q. Mi, Designer tetrahedral DNA framework-based microfluidic technology for multivalent capture and release of circulating tumor cells, Mater. Today Bio, 16 (2022) 100346.

[77] J. Peng, Y.L. Liu, R. Su, L.Q. Zeng, Z.X. Huo, R.X. Peng, X.Y. Yu, H.M. Zhang, C.Y. Yang, L. Yang, Z. Zhu, Dna-programmed orientation-ordered multivalent microfluidic interface for liquid biopsy, Anal. Chem., 94 (2022) 8766-8773.

[78] M. Poudineh, M. Labib, S. Ahmed, L.N.M. Nguyen, L. Kermanshah, R.M. Mohamadi, E.H. Sargent, S.O. Kelley, Profiling functional and biochemical phenotypes of circulating tumor cells using a two-dimensional sorting device, Angew. Chem., Int. Ed., 56 (2017) 163-168.

[79] F. Zhang, L.L. Wu, W.D. Nie, L.L. Huang, J.F. Zhang, F. Li, H.Y. Xie, Biomimetic microfluidic system for fast and specific detection of circulating tumor cells, Anal. Chem., 91 (2019) 15726-15731.

[80] A.A. Kajani, L. Rafiee, M. Samandari, M.A. Mehrgardi, B. Zarrin, S.H. Javanmard, Facile, Rapid and efficient isolation of circulating tumor cells using aptamer-targeted magnetic nanoparticles integrated with a microfluidic device, RSC Adv., 12 (2022) 32834-32843.

[81] C. Cruz, A. Miranda, T. Santos, 17 - aptamer-based microfluidics for circulating tumor cells, in: p. kesharwani (ed.) aptamers engineered nanocarriers for cancer therapy, Woodhead Publishing2023, pp. 403-432.

[82] D. Sun, Y. Ma, M. Wu, Z. Chen, L. Zhang, J. Lu, Recent progress in aptamer-based microfluidics for the detection of circulating tumor cells and extracellular vesicles, J. Pharm. Anal., 13 (2023) 340-354.

[83] Y. Cao, L. Zhou, Z.K. Fang, Z.H. Zou, J. Zhao, X.L. Zuo, G.X. Li, Application of functional peptides in the electrochemical and optical biosensing of cancer biomarkers, Chem. Commun., 59 (2023) 3383-3398.

[84] P. Miao, J. Yin, L.M. Ning, X.X. Li, Peptide-based electrochemical approach for apoptosis evaluation, Biosens. Bioelectron., 62 (2014) 97-101.

[85] J. Bu, W.-j. Jeong, R. Jafari, L.J. Kubiatowicz, A. Nair, M.J. Poellmann, R.S. Hong, E.W. Liu, R.H. Owen, P.A. Rawding, C.M. Hopkins, D. Kim, D.J. George, A.J. Armstrong, P. Král, A.Z. Wang, J. Bruce, T. Zhang, R.J. Kimple, S. Hong, Bimodal liquid biopsy for cancer immunotherapy based on peptide engineering and nanoscale analysis, Biosens. Bioelectron., 213 (2022) 114445.

[86] F. Jia, Y. Wang, Z. Fang, J. Dong, F. Shi, W. Zhang, Z. Wang, Z. Hu, Novel peptide-based magnetic nanoparticle for mesenchymal circulating tumor cells detection, Anal. Chem., 93 (2021) 5670-5675.

[87] T. Li, N. Li, Y. Ma, Y.-J. Bai, C.-M. Xing, Y.-K. Gong, A blood cell repelling and tumor cell capturing surface for high-purity enrichment of circulating tumor cells, J. Mater. Chem. B, 7 (2019) 6087-6098.

[88] H. Zhong, C. Yuan, J. He, Y. Yu, Y. Jin, Y. Huang, R. Zhao, Engineering peptide-functionalized biomimetic nanointerfaces for synergetic capture of circulating tumor cells in an epcam-independent manner, Anal. Chem., 93 (2021) 9778-9787.

[89] Q. Shen, H. Yang, C. Peng, H. Zhu, J. Mei, S. Huang, B. Chen, J. Liu, W. Wu, S. Cao, Capture and biological release of circulating tumor cells in pancreatic cancer based on peptide-functionalized silicon nanowire substrate, Int. J. Nanomed., 14 (2019) 205-214.

[90] C.C. Chen, Z. Wu, P. Ding, N. Sun, H. Liu, Y. Chen, Z.L. Wang, R.J. Pei, Peptide ngr modified tio2 nanofiber substrate for circulating tumor cells capture, Adv. Fiber Mater., 2 (2020) 186-193.

[91] N. Carmona-Ule, N. Gal, C. Abuín Redondo, M. De La Fuente Freire, R. López López, A.B. Dávila-Ibáñez, Peptide-functionalized nanoemulsions as a promising tool for isolation and ex vivo culture of circulating tumor cells, bioengineering (basel), 9 (2022) 380.

[92] R. Han, Y. Li, M. Chen, W.T. Li, C.F. Ding, X.L. Luo, Antifouling electrochemical biosensor based on the designed functional peptide and the electrodeposited conducting polymer for ctc analysis in human blood, Anal. Chem., 94 (2022) 2204-2211.

[93] J. Peng, Q. Zhao, W. Zheng, W. Li, P. Li, L. Zhu, X. Liu, B. Shao, H. Li, C. Wang, Y. Yang, Peptide-functionalized nanomaterials for the efficient isolation of her2-positive circulating tumor cells, ACS Appl. Mater. Interfaces, 9 (2017) 18423-18428.

[94] N. Liang, L. Liu, P. Li, Y. Xu, Y. Hou, J. Peng, Y. Song, Z. Bing, Y. Wang, Y. Wang, Z. Jia, X. Yang, D. Li, H. Xu, Q. Yu, S. Li, Z. Hu, Y. Yang, Efficient isolation and quantification of circulating tumor cells in non-small cell lung cancer patients using peptide-functionalized magnetic nanoparticles, J. Thorac. Dis., 12 (2020) 4262-4273.

[95] C. Liu, B. Yang, X. Chen, Z. Hu, Z. Dai, D. Yang, X. Zheng, X. She, Q. Liu, Capture and separation of circulating tumor cells using functionalized magnetic nanocomposites with simultaneous in situ chemotherapy, Nanotechnology, 30 (2019) 285706.

[96] J. Zhang, W. Sheng, Z.H. Fan, An ensemble of aptamers and antibodies for multivalent capture of cancer cells, Chem. Commun., 50 (2014) 6722-6725.

[97] Y.L. Chen, Y.K. Yang, J.L. Feng, A.J. Carrier, D. Tyagi, X. Yu, C.G. Wang, K.D. Oakes, X. Zhang, A universal monoclonal antibody-aptamer conjugation strategy for selective non-

invasive bioparticle isolation from blood on a regenerative microfluidic platform, Acta Biomater., 152 (2022) 210-220.

[98] J. Fan, Y. Fan, Z. Wei, Y. Li, X. Li, L. Wang, H. Wang, Transformable peptide nanoparticles inhibit the migration of n-cadherin overexpressed cancer cells, Chin. Chem. Lett., 31 (2020) 1787-1791.

[99] P. Maturavongsadit, X. Bi, T.A. Gado, Y.-Z. Nie, Q. Wang, Adhesive peptides conjugated pamam dendrimer as a coating polymeric material enhancing cell responses, Chin. Chem. Lett., 27 (2016) 1473-1478.

[100] N.N. Lu, M. Xie, J. Wang, S.W. Lv, J.S. Yi, W.G. Dong, W.H. Huang, Biotin-triggered decomposable immunomagnetic beads for capture and release of circulating tumor cells, ACS Appl. Mater. Interfaces, 7 (2015) 8817-8826.

[101] A. Yoshihara, R. Sekine, T. Ueki, Y. Kondo, Y. Sunaga, T. Nakaji-Hirabayashi, Y. Teramura, M. Takai, Rapid and highly efficient capture and release of cancer cells using polymeric microfibers immobilized with enzyme-cleavable peptides, Acta Biomater., 67 (2018) 32-41.

[102] S.H. Ma, X.X. Zhou, Q. Chen, P.P. Jiang, F. Lan, Q.Y. Yi, Y. Wu, Multi-targeting magnetic hyaluronan capsules efficiently capturing circulating tumor cells, J. Colloid Interface Sci., 545 (2019) 94-103.

[103] A. Meunier, J.A. Hernández-Castro, K. Turner, K.B. Li, T. Veres, D. Juncker, Combination of mechanical and molecular filtration for enhanced enrichment of circulating tumor cells, Anal. Chem., 88 (2016) 8510-8517.

[104] C.L. Chang, W.F. Huang, S.I. Jalal, B.D. Chan, A. Mahmood, S. Shahda, B.H. O'Neil, D.E. Matei, C.A. Savran, Circulating tumor cell detection using a parallel flow micro-aperture chip system, Lab Chip, 15 (2015) 1677-1688.

[105] X. Zhang, X. Wei, X. Men, C.X. Wu, J.J. Bai, W.T. Li, T. Yang, M.L. Chen, J.H. Wang, Dual-multivalent-aptamer-conjugated nanoprobes for superefficient discerning of single circulating tumor cells in a microfluidic chip with inductively coupled plasma mass spectrometry detection, ACS Appl. Mater. Interfaces, 13 (2021) 43668-43675.

[106] Q.Q. Huang, B. Cai, B.L. Chen, L. Rao, Z.B. He, R.X. He, F. Guo, L.B. Zhao, K.K. Kondamareddy, W. Liu, S.S. Guo, X.Z. Zhao, Efficient purification and release of circulating tumor cells by synergistic effect of biomarker and sio2@gel-microbead-based size difference amplification, Adv. Healthcare Mater., 5 (2016) 1554-1559.

[107] W. Lv, B. Fu, W. Liu, W. Huang, M. Li, Y. Liu, Y. Kang, J. Wang, S. Bai, C. Lu, X. Dai, Efficient detection of single circulating tumor cell in blood using raman mapping based on aptamer-sers bio-probe coupled with micropore membrane filtration, Talanta, 267 (2024) 125220.

[108] K. Cushing, E. Undvall, Y. Ceder, H. Lilja, T. Laurell, Reducing wbc background in cancer cell separation products by negative acoustic contrast particle immuno-acoustophoresis, Anal. Chim. Acta, 1000 (2018) 256-264.

[109] R. Li, Z.Y. Gong, K.Z. Yi, W. Li, Y.C. Liu, F.B. Wang, S.S. Guo, Efficient detection and single-cell extraction of circulating tumor cells in peripheral blood, ACS Appl. Bio Mater, 3 (2020) 6521-6528.

[110] C. Huang, S.M. Santana, H. Liu, N.H. Bander, B.G. Hawkins, B.J. Kirby, Characterization of a hybrid dielectrophoresis and immunocapture microfluidic system for cancer cell capture, Electrophoresis, 34 (2013) 2970-2979.

[111] C. Huang, J.P. Smith, T.N. Saha, A.D. Rhim, B.J. Kirby, Characterization of microfluidic shear-dependent epithelial cell adhesion molecule immunocapture and enrichment of pancreatic cancer cells from blood cells with dielectrophoresis, Biomicrofluidics, 8 (2014) 044107.

[112] L.Q. Do, H.T.T. Thuy, T.T. Bui, V.T. Dau, N.-V. Nguyen, T.C. Duc, C.-P. Jen, Dielectrophoresis microfluidic enrichment platform with built-in capacitive sensor for rare tumor cell detection, BioChip J., 12 (2018) 114-122.

[113] K. Zhao, P. Zhao, J. Dong, Y. Wei, B. Chen, Y. Wang, X. Pan, J. Wang, Implementation of an integrated dielectrophoretic and magnetophoretic microfluidic chip for CTC isolation, Biosensors (Basel), 12 (2022) 757.

Author Biography

Hua Chai (BRID: 06957.00.78712)

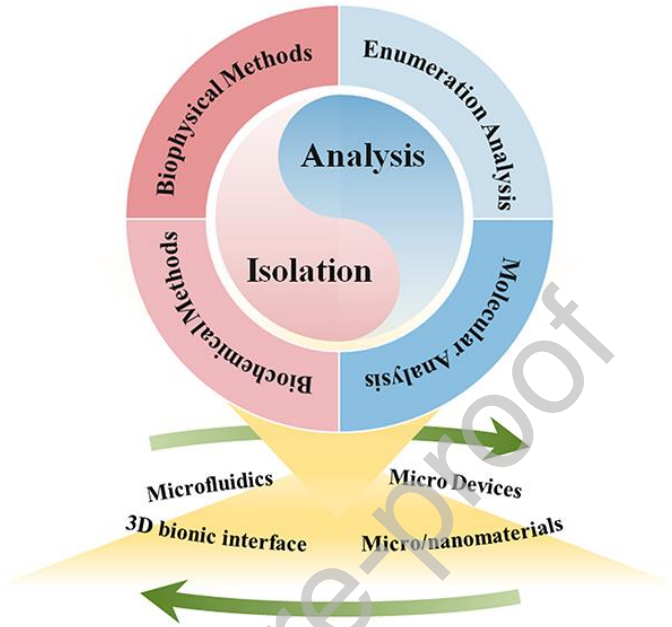
Hua Chai received her B.S. and Ph.D. degrees from Jilin University and University of Science and Technology of China in 2017 and 2022, respectively. Afterward, she worked at Suzhou

Institute of Biomedical Engineering and Technology, Chinese Academy of Sciences. Her research interests focus on bioanalytical chemistry and DNA materials.

Peng Miao (BRID: 03522.00.61319)

Peng Miao received his B.S. and M.S. degrees from Nanjing University in 2008 and 2011. Afterwards, he received his Ph.D. degree from University of Chinese Academy of Sciences in 2016. He is now a full professor at Suzhou Institute of Biomedical Engineering and Technology, Chinese Academy of Sciences. His research interests focus on DNA nanotechnology.

Graphical Abstract



Conflict of Interest

The authors have declared no conflict of interest.