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Microfluidic-assisted analysis of extracellular vesicles for advanced biomedical applications

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Extracellular vesicles are lipid bilayer-enclosed nanoparticles that play a crucial role in intercellular communication by transporting bioactive molecules across cells, tissues, and even organisms. Although certain aspects of EV biogenesis, function, and metabolic pathways are yet to be fully elucidated, their distinctive features such as cargo protection, structural stability, and the capacity to cross biological barriers render them highly promising for medical applications, especially as novel biomarkers for disease diagnosis. However, the ultrasmall size and complex surface properties pose substantial challenges to their analysis. Microfluidic technology has emerged as a powerful tool for EV isolation and analysis. It offers remarkable advantages, including high throughput, efficiency, and minimal sample consumption, providing innovative solutions for isolating and analyzing EVs with unparalleled precision. Moreover, the integration of artificial intelligence and advanced digital analytics with microfluidic platforms has opened new avenues for enhanced data processing and diagnostic accuracy. In this review, we comprehensively summarize recent advances in EV research, beginning with an in-depth analysis of EVs' biogenesis mechanisms and the formation of disease-specific biomarkers. Then EV-mediated pathological processes with diagnostic significance are discussed, followed by a comprehensive evaluation of microfluidic-based approaches for isolation, purification, and single-vesicle analysis of EVs. The clinical diagnostic potential of EVs is assessed across major disease categories including cancers, cardiovascular disorders, neurological conditions, infectious diseases, and autoimmune pathologies, highlighting their emerging role as next-generation biomarkers. Finally, the current advances in EV-based diagnostics are discussed, along with the future perspectives on microfluidic-based EV analysis towards various biomedical applications and clinical translation.

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1. Introduction

Extracellular vesicles (EVs) are lipid bilayer membrane vesicles actively secreted by almost all types of living cells.[1] These vesicles serve as nanocarriers to transport proteins, nucleic acids and other bioactive substances originated from parental cells, and have therefore been recognized as critical mediators of intercellular communication, which enable transfer between cells, tissues, organs and even organisms.[2] It has been proved that EVs play essential roles in both physiological processes and pathological mechanisms.[3]

EVs are enriched with surface-specific proteins that are stably incorporated during biogenesis through physicochemical interactions, maintaining structural integrity during circulatory transport.[4,5] For small molecules encapsulated inside EVs such as nucleic acids, the presence of lipid bilayers can protect them from the interference by other molecules.[6] Therefore, EVs are regarded as potential biomarkers for diagnosis of various conditions including tumours.[7] Compared to conventional tissue biopsy, liquid biopsy using bodily fluid derived EVs has gained increasing attention due to its advantages in non-invasive detection and sensitive treatment monitoring. However, the detection of EVs is complicated due to their diminutive size and the technical challenges associated with their isolation and subtyping.[8] In this case, the microfluidic technique has emerged as a transformative methodological approach,[9] which provides an interdisciplinary platform capable of manipulating small volumes of fluid, with applications in detection, analysis, synthesis, and biological processing.[10] An important feature of microfluidic technology is the unique fluid properties, such as laminar flow and droplets, in microscale environments. Additionally, upon using external forces such as acoustic waves and electric fields, the accurate manipulation of microfluids is easy to achieve, providing unprecedented opportunities for the isolation and detection of EVs derived from various clinical samples.[11] By integrating immunoaffinity-based immobilization with a variety of detection methods, microfluidic platforms can analyse EVs at the level of a single entity with high sensitivity and high specificity.[12]

In this review, we comprehensively summarize the latest research progress in microfluidic-assisted analysis of extracellular vesicles for advanced biomedical applications. Following the discussion on unique biological characteristics of EVs as promising biomarkers for liquid biopsy, we provide a detailed classification of microfluidic technologies, including conventional platforms, next-generation digital microfluidics,

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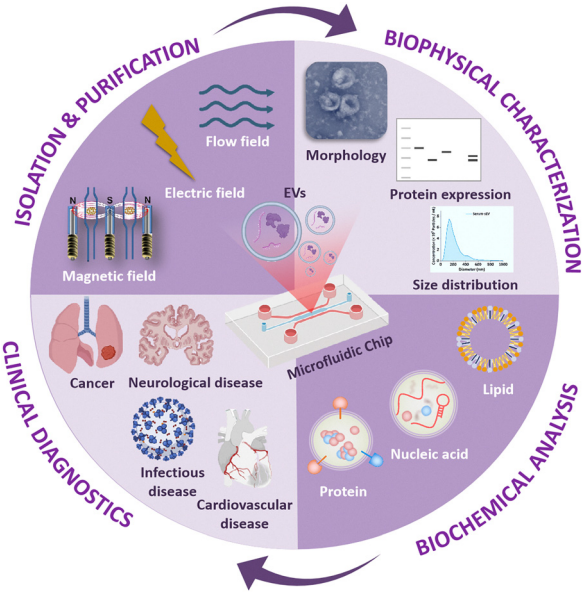

Scheme 1 Schematic illustration of microfluidic-assisted EV isolation, detection and biomedical applications.

and AI-integrated microfluidic systems. Furthermore, we discuss the synergistic potential of microfluidic technologies and EV detection, emphasizing their complementary advantages. The clinical applications of EVs are critically evaluated across various conditions, including cancers, cardiovascular disorders, neurological conditions, infectious and tropical diseases, and autoimmune pathologies. Finally, we outline future perspectives on the use of microfluidic-based EV detection for clinical diagnosis and therapeutic monitoring.

2. Extracellular vesicles: nature and biological functions

Although the biogenesis of EVs and the mechanisms underlying their biological characteristics require further investigation, their currently established properties demonstrate unique advantages, positioning them as promising next-generation biomarkers for clinical applications.

2.1 The biogenesis of EVs and the biomolecular crowns

EVs can be categorized into three main types based on their distinct origin and size: exosomes that are generated by the endocytosis pathway (30–200 nm), microvesicles that originate *via* membrane budding and fusion (100–1000 nm), and apoptotic bodies that generate during cell apoptosis (500–2000 nm).[13] Exosomes are regarded as the smallest EVs, and the process of exosome biogenesis is closely related to the endocytosis pathway. Microvesicles originate from outward budding and fusion of the plasma membrane (Fig. 1(a)).[14] Apoptosomes are apoptosis-derived vesicles characterized by surface-exposed phosphatidylserine.[15] Due to the limited function of apoptosomes in disease diagnosis, the item EVs described here mainly refer to exosomes and microvesicles which come from live cells. The surface of EVs is enriched with adhesion molecules (including tetraspanins and integrins), transporters, and receptors, whereas their luminal cargo carries various small nucleic acids—microRNAs (miRNAs), small interfering RNAs (siRNAs), messenger RNA (mRNA), transfer RNA (tRNA) and trace amounts of DNA.[16] Recent studies have revealed that EVs can adsorb substantial quantities of bioactive molecules due to their high surface-to-volume ratio and surface free energy,[17] forming distinctive surface layers termed "biomolecular corona" (Fig. 1(b)).[4] Since it is energetically challenging to totally remove the biomolecular corona from an EV without altering or destroying the nanoparticle, the proteins adsorbed on the EV surface during corona formation can be regarded as EV components and therefore be potentially exploited as biomarkers.[18]

2.2 The vital biological function of EVs to be biomarkers

Despite the complex nature of EVs and the need for further mechanistic studies, certain EV characteristics have demonstrated significant potential for clinical diagnostic applications. Characteristics such as cargo protection mechanisms,[19] structural stability,[20] and biofilm penetration capabilities[21] collectively support the potential of EVs as novel biomarkers.

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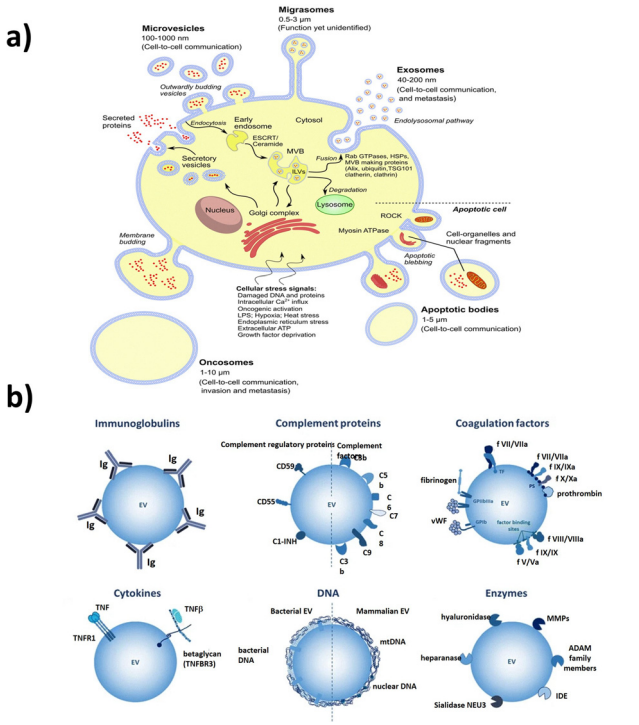

Fig. 1 The occurrence and biological characteristics of EVs. (a) The cellular origin and formation process of EVs. Adapted with permission from ref. 14. Copyright 2024 Royal Society of Chemistry. (b) Molecular composition and classification systems of EV surface markers. Adapted with permission from ref. 4. Copyright 2018 Springer Nature.

Cargo protection properties. EVs transfer various cargos inherited from parent cells, including proteins, nucleic acids, lipids and so on.[22] These bioactive components are efficiently transported by EVs, which are ubiquitously distributed in biological fluids and exhibit phagocytosis-resistant properties. Generally, the lipid bilayer structure of EVs can protect bioactive contents from extracellular enzymatic degradation and hemodynamic shear forces.[23] However, recent studies have revealed that the substances in EVs also exhibit variation in response to changing external cellular conditions.[24] For instance, tumour cells release more EVs with different components under hypoxia conditions,[25] which makes EVs potential biomarkers to reflect pathological alterations.

Stability. Increasing evidence demonstrates that EVs maintain structural integrity and cargo stability *in vivo* and *in vitro* even when exposed to physiologically challenging conditions.[26] Moreover, EVs exhibit remarkable stability in structure and contents during long-term cryopreservation.[27] Recent advances in bio-preservation technologies have led to the development of optimized EV stabilization systems, significantly enhancing their structural integrity and functional retention. For instance, a novel polyelectrolyte-based cryoprotectant system has been developed to significantly enhance the stability of EVs.[28] This advancement provides a critical foundation for establishing EVs as reliable diagnostic biomarkers by ensuring their structural and functional integrity during storage.

Biological-barrier-crossing properties. Living organisms possess multiple biological barriers, with the blood–brain barrier (BBB) representing the most selective endothelial interface due to its tight junction complexes and efflux transport systems.[29] The BBB severely restricts the passage of neurological disease biomarkers into peripheral circulation, while cerebrospinal fluid (CSF) sampling remains clinically invasive and high-risk. Consequently, there is a critical need for minimally invasive, blood-based biomarkers to diagnose neurological pathologies. EVs offer a promising solution, as they can cross the BBB and carry central neural system (CNS)-derived molecules. Studies have preliminarily suggested the mechanisms of the BBB-crossing process involve endocytic pathways, transcytosis, and receptor-mediated trafficking.[30] Therefore, EVs have been exploited to exert diagnostic and therapeutic function in neurodegenerative disorders and CNS tumours such as glioblastoma.[31]

3. Microfluidic-based isolation and analysis of EVs

Despite EVs' attractive physicochemical properties and unique clinical application potential, in the process of their clinical application, there are still significant problems including extraction, purification and specific detection that need to be solved. Microfluidic technology offers ideal tools for these challenges, since it is a technology that can precisely manipulate microfluids on the micrometre scale, enabling fluid mixing, separation, reaction and analysis through a network of microchannels.[32] Microfluidic platforms offer transformative advantages for EV analysis, combining ultra-low sample consumption (μL-scale volumes), high-density functional integration (multiplexed detection modules), and precise microenvironmental control (laminar flow regimes). These properties are particularly critical for EV research. Furthermore, microfluidic systems enable scalable parallel processing (high-throughput screening) and portable form factors (point-of-care compatibility), addressing key gaps in conventional bulk-analysis techniques.[33]

3.1 Integrated microfluidic technologies for biomedical applications

Microfluidic-assisted EV isolation is based on the differences in EVs' physical or biochemical properties like size, density, and immunoaffinity. A variety of advanced isolation approaches have been rapidly evolving upon integrating with external forces. Therefore, microfluidic chips can be categorized by their fluid control mechanisms, including pressure-driven, electrokinetic (electric-driven), and magnetohydrodynamic (magnetic-driven) systems.

Pressure-driven microfluidic chips. This type of microchip constitutes the most widely used microfluidic systems, and employs either positive pressure sources (*e.g.* syringe pumps or air compressors) or negative pressure sources (*e.g.* vacuum pumps) for fluid propulsion.[34] This actuation mechanism offers significant advantages in terms of operational simplicity and precise flow control.[35] In biomedical applications, numerous pressure-driven microfluidic platforms have been successfully developed for various target detection purposes, demonstrating

their versatility in diagnostic and analytical applications. For instance, using a polymer microfluidic platform, He and colleagues achieved simultaneous detection of five thyroid biomarkers in whole blood, demonstrating a rapid, miniaturized alternative to conventional immunoassays.[36]

Electrically driven microfluidic chips. This type of microchip mainly works based on electrophoresis, dielectric electrophoresis or electric wetting.[37] Electro-driven microchips display significant advantages in biomedical applications, which enables precise manipulation of nanoparticle and achieves millisecond-scale response times under electric field control. However, the inherent characteristics of electric field-driven mechanisms imposes specific limitations on sample properties. Therefore, adequate buffer solution is required to maintain optimal performance of fluids. Consequently, these systems exhibit prominent applications in single particle isolation and EV content analysis with high precision and rapid response properties.[38]

Magnetically driven microfluidic chips. This type of microchip mainly relies on precise control of fluids under a magnetic field.[39] Magnetic-driven microchips enable precise manipulation of micro-scale fluids or magnetic particles through external magnetic fields, offering key advantages such as non-contact operation, high spatial resolution, and excellent biocompatibility. These characteristics make them particularly valuable for biomedical applications including targeted drug delivery, high-precision cell sorting, and point-of-care diagnostic systems.[40] However, the technology faces several limitations including dependence on magnetic labelling of target analytes, stringent requirements for maintaining uniform magnetic fields, and challenges in scaling up for high-throughput processing. Moreover, further optimization is needed to address biocompatibility concerns and large-scale clinical applications.

Based on the above-mentioned technologies, microfluidic chips have shown revolutionary application potential in biomedical fields, in terms of tumour marker screening, disease diagnosis, portable point-of-care testing (POCT) *etc.*

3.2 Microfluidic-assisted extraction and purification of EVs

Advances in microfluidic technology have enabled significant improvements in EV analysis, overcoming key challenges in quantification, isolation, subpopulation sorting, and detection. For biomedical applications, EV isolation demands even stricter criteria, including target-specific enrichment, high yields, and seamless integration of separation with downstream characterization.[41] Conventional approaches such as ultracentrifugation and ultrafiltration lack the potential to isolate specific EV subpopulations, limiting their utility for targeted applications. However, microfluidic technology offers unique advantages in EV extraction. Categorized by the treatment of EVs, microfluidic-based isolation can be broadly categorized into two groups: label-free separation and label-dependent isolation (Table 1).[42]

Label-free isolation of EVs. Label-free isolation refers to the separation of EVs without immunological labelling, mainly relying on the size differences of EVs. When applied to microfluidic systems, external forces such as electric or magnetic fields exert their function, as mentioned above. The primary advantages of label-free methods lie in their simplicity, minimal equipment requirements, and high-throughput capabilities.[43] Numerous innovative designs have been developed for label-free EV isolation. For instance, Liu *et al.* designed a bidirectional tangential flow filtration-based microfluidic device for exosome isolation from cell supernatants and human serum. The device comprises two modules, each containing symmetrical serpentine channels fabricated in polymethyl methacrylate (PMMA) plates and a nanoporous membrane. By enhancing the fluid-membrane contact area through tangential flow, this system achieves efficient exosome isolation (Fig. 2(a)).[44] Yusuke *et al.* developed a straightforward yet efficient approach for the direct isolation and subsequent detection of target EVs using engineered superparamagnetic nanocomposites. Multifunctional magnetic carriers were surface-modified with a universal tetraspanin antibody, which were dispersed in sample solution, serving as "dispersible nanocarriers" to achieve high-yield exosome capture. The separation specificity and efficiency were rigorously validated through targeted detection assays.[45] Cho *et al.* developed an "Exodisc" system incorporating dual nanofillers, which allows for the automated enrichment of EVs (20–600 nm) from biological samples such as cell culture supernatants or cancer patient urine within 30 min using a compact centrifugal microfluidic platform. This approach facilitates rapid, label-free, and highly sensitive EV isolation.

Table 1 Summary of various isolation methods of EVs using microfluidic technology

Isolation of EVs	Control mode of microfluidics	Time	Samples	Advantage	Disadvantage	Ref.
Label-free isolation	Pressure-driven	<2 h	Serum	High efficiency & integration density	Less marker specificity	44
	Pressure-driven	30 min	Urine	High efficiency, readily available samples	Less marker specificity	46
	Electrically-driven	100 min	Plasma	High throughput	Expensive and time-consuming	47
	Electrically-driven	15 min	Plasma	Rapid and high throughput	Less marker specificity	48
	Magnetically-driven	4.5 h	Blood	High throughput and automation	Expensive and time-consuming	50
Labelling-based isolation	Pressure-driven	1 h	Serum	High maker specificity	Expensive	54
	Magnetically-driven	100 min	Plasma	Rapid & high maker specificity	Expensive and time-consuming	55
	Magnetically-driven	1 h	Plasma	High throughput High maker specificity	Complicated operation	56
	Pressure-driven	2 h	Blood	High maker specificity High automation	Time consuming	57
	Pressure-driven Magnetically-driven	2 h	Serum	Integrated isolation and detection	Expensive	60

Additionally, microfluidic chips incorporating electric or magnetic fields have been widely applied for EV separation. (Fig. 2(b)).[46] Son *et al.* proposed a DEP-based microfluidic chip for efficient separation of EVs and lipoproteins. Their protocol involves initial filtration of human plasma to collect EVs and lipoproteins of similar sizes, followed by buffer exchange and subsequent injection into a microfluidic chip featuring inclined comb-shaped microelectrodes for high-resolution separation (Fig. 2(c)).[47] Heller *et al.* introduced an alternating current electrokinetic microarray chip capable of rapidly isolating glioblastoma-derived EVs from undiluted human plasma samples. This system requires only 30–50 μL of plasma and concentrates EVs in the high-field regions around microelectrodes within 15 min.[48] In the realm of magnetic field applications, Peng *et al.* designed a magnetic nanoparticle-based microfluidic platform that enriches EVs from cell culture medium and plasma in 29 min without manual pipetting or off-chip incubation (Fig. 2(d)).[49] Ferraro *et al.* further advanced this field with a droplet microfluidic platform for magnetic bead-based affinity capture of EVs, enabling high-throughput processing (2 mL samples in 4.5 hours) with significant automation (Fig. 2(e)).[50] Despite the rapid and high-throughput separation with simple instrumentation, label-free EV isolation still faces challenges in isolating EV subpopulations with high specificity.

Labelling-based isolation of EVs. To improve the specific purification of EVs, strategies incorporating EV surface biomarkers on microfluidic chips have significantly improved isolation specificity.[51] Based on the membrane protein biomarkers such as CD63 and CD81, EVs can be targeted and isolated through immunoaffinity-based techniques.[52,53] Ye *et al.* developed an integrated system combining affinity-based capture with passive microfluidic particle trapping. Their method employs streptavidin-functionalized microbeads (20 μm diameter) conjugated with biotinylated antibodies, enabling efficient EV immobilization through antigen–antibody binding (Fig. 3(a)).[54] He *et al.* advanced this field by creating a novel microfluidic platform that simultaneously performed immunocapture of circulating exosomes and target protein detection. This system could selectively isolate EVs directly from minute plasma samples (30 μL) within 100 min (Fig. 3(b)).[55]

Immunomagnetic separation has also shown significant promise. Chen *et al.* utilized immunomagnetic EV isolation from cell culture supernatants under continuous flow in microchips, achieving high-throughput, high-efficiency separation of EVs rapidly.[56] Furthermore, researchers have developed highly integrated systems for EV isolation, for instance, Lee and colleagues engineered an integrated microfluidic platform incorporating antibody-conjugated magnetic beads for efficient isolation of EVs directly from whole blood, and this system combines continuous hydrodynamic focusing with immunomagnetic separation to

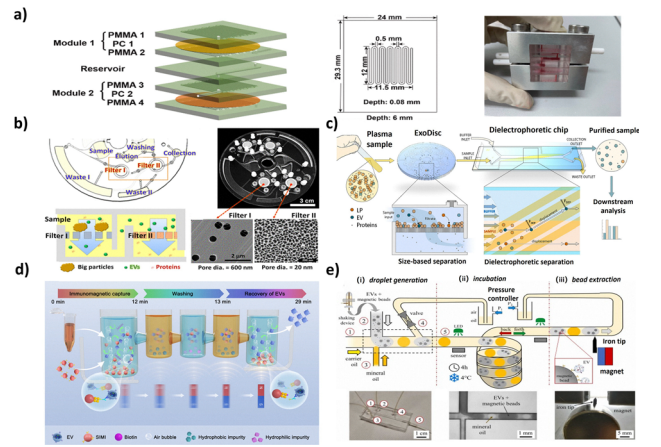

Fig. 2 Label-free microfluidic technologies for EV isolation. (a) Serum-compatible bidirectional tangential flow filtration microdevice for clinical EV isolation. Adapted with permission from ref. 44. Copyright 2023 Elsevier. (b) The Exodisc: an integrated microfluidic platform for high-efficiency extracellular vesicle enrichment from patient urine samples. Adapted with permission from ref. 46. Copyright 2017 American Chemical Society. (c) DEP-based microfluidic platform for high-resolution separation of lipoproteins and extracellular vesicles in human biofluids. Adapted with permission from ref. 47. Copyright 2024 American Chemical Society. (d) High-throughput microfluidic system utilizing magnetic nanoparticles achieves efficient EV extraction from cell culture supernatants in 29 min. Adapted with permission from ref. 49. Copyright 2024 American Chemical Society. (e) High-yield EV extraction from cell culture supernatants *via* magnetic bead-functionalized droplet microfluidics. Adapted with permission from ref. 50. Copyright 2024 Elsevier.

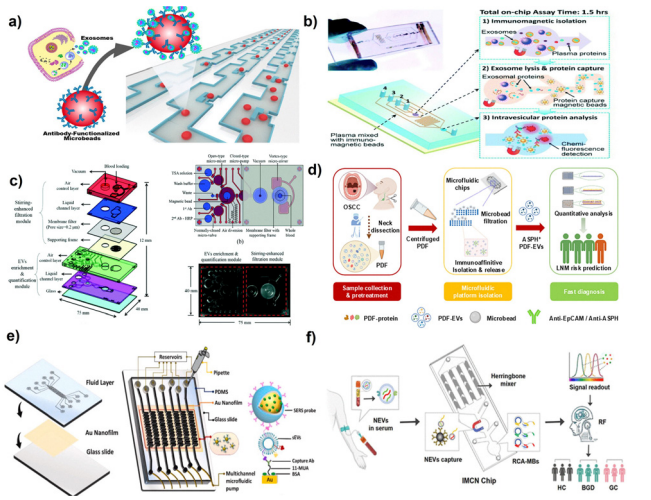

Fig. 3 Labelling-based microfluidic separation and detection technology for EVs. (a) High-specificity microfluidic EV extraction system utilizing streptavidin–biotin affinity pairing for selective vesicle isolation. Adapted with permission from ref. 54. Copyright 2020 American Chemical Society. (b) Immunomagnetic bead-based microfluidic platform for specific capture and isolation of extracellular vesicles through antibody–antigen interactions. Adapted with permission from ref. 55. Copyright 2014 Royal Society of Chemistry. (c) Precision microfluidic system leveraging fluid mechanical design and antibody-conjugated magnetic beads for targeted EV separation in plasma samples. Adapted with permission from ref. 57. Copyright 2019 Royal Society of Chemistry. (d) A microfluidic-based platform for extracellular vesicle enrichment and molecular profiling in postoperative PDF of OSCC patients. Adapted with permission from ref. 60. Copyright 2024 Springer Nature. (e) SERS-integrated microfluidic platform for label-free detection and molecular profiling of extracellular vesicles in ovarian cancer patient serum. Adapted with permission from ref. 61. Copyright 2025 Elsevier. (f) High-specificity microfluidic system for neutrophil EV subpopulation analysis in pre-clinical gastric cancer screening. Adapted with permission from ref. 64. Copyright 2025 American Chemical Society.

overcome the challenges of blood matrix complexity (Fig. 3(c)).[57] Compared to label-free methods, labelling-based separation offers superior specificity, enabling the development of fully integrated microfluidic devices that combine isolation with downstream detection. This approach represents a major advancement in EV research and a key direction for next-generation microfluidic technologies in EV isolation.

3.3 Microfluidic technology for the specific detection of EVs

Specific detection of EVs primarily involves two complementary approaches: (i) profiling of surface protein signatures through immunoaffinity techniques, and (ii) analysis of intravehicular nucleic acids (miRNAs, mRNAs, cfDNA). The traditional methods for the detection of surface proteins (Western Blot and enzyme-linked immunosorbent assay, ELISA) and nucleic acids (polymerase chain reaction, PCR) face challenges in long processing time and are labour-intensive, which limit their application in EV analysis.[46] Therefore, a variety of microfluidic-based techniques have been developed. Leveraging its high-throughput capability, microfluidic technology enables rapid multiplexed analysis of EV protein and nucleic acid biomarkers. Researchers have developed an integrated 3D microfluidic platform incorporating quantum dot labelling and vesicle fusion technology, allowing simultaneous *in situ* detection of multiple EV markers (CD81, EphA2, CA19-9, miR-451a, miR-21, and miR-10b) for highly accurate cancer diagnosis.[58] Bu *et al.* conducted comprehensive proteomic profiling of EVs isolated from postoperative drainage fluid, identifying aspartate β-hydroxylase-enriched EV subpopulations as promising diagnostic biomarkers for lymph node metastasis in oral squamous cell carcinoma patients. Their work established a novel liquid biopsy approach leveraging surgical byproducts for metastatic risk assessment.[59] Based on this, a microfluidic platform was designed for enrichment and detection of EVs to assess the risk of postoperative lymph node metastasis in patients with oral squamous cell carcinoma, and its effectiveness was demonstrated by double-blind verification (Fig. 3(d)).[60] To overcome challenges in ovarian cancer early detection, Xing *et al.* engineered a SERS-based multichannel microfluidic platform (S-MMEV) for small extracellular vesicle (sEV) phenotyping. This innovative assay quantitatively profiles sEV surface biomarkers with single-vesicle resolution, demonstrating clinical validity through blinded serum testing with 92.3% sensitivity for Stage I/II disease (Fig. 3(e)).[61] Additionally, Liu *et al.* developed a microfluidic platform capable of isolating plasma EVs directly from whole blood and performing specific protein profiling (CD81, CD24, EpCAM). This approach offers a novel strategy for blood-based EV analysis in diagnostic applications.[62]

Apart from the detection of surface proteins, increasing interest has been put on the detection of nucleic acids inside EVs for the diagnosis of specific diseases. For instance, Zhao *et al.* proposed a microfluidic-SERS sensor based on rolling ring amplification and tyramine signal amplification strategies to detect exosomal miRNA which was further applied to detecting breast cancer cell-derived EVs.[63] Zhang *et al.* proposed an integrated microfluidic chip for the analysis of neutrophil EVs, which used 10 μL serum samples to achieve multiple detection of surface proteins and miRNA of NEVs, thereby enabling early diagnosis of gastric cancer (Fig. 3(f)).[64]

The continuous development of these studies has put forward new detection strategies for the specific detection of EVs. These next-generation platforms enable highly specific EV detection with significant advantages over traditional techniques, including: (i) rapid processing times (min *versus* hours), (ii) exceptional specificity, and (iii) high-level system integration (combining isolation, enrichment, and analysis into a single miniaturized device). This technological synergy not only enhances detection accuracy but also facilitates clinical translation by enabling high-throughput, standardized workflows with minimal sample requirements.

3.4 Digital microfluidic chip detection

The integration of advanced digital analysis and artificial intelligence (AI) with microfluidic systems has propelled significant advancements in digital microfluidic technology. Digital microfluidics has evolved into high-throughput, intelligent systems for automated multi-step assays. In EV analysis, researchers have developed advanced protein detection methods, particularly digital ELISA platforms. Chang *et al.* developed a digital ELISA platform which achieved 80% EV capture efficiency using immunomagnetic beads and dual-enzyme colorimetry, demonstrating utility in ovarian cancer chemotherapy resistance assessment.[65] Similarly, Zeng *et al.* introduced a microfluidic-enhanced, partition-free dELISA (μTIP-dELISA) for EV detection in Ewing's sarcoma diagnosis. Their approach utilized a streamlined microfluidic device incorporating single-molecule signal amplification technology to generate a morphological nano-gap array, which substantially enhanced enzymatic reactions on surface-bound targets. Compared to conventional ELISA, this method exhibited over 300-fold improvement in sensitivity for detecting four Ewing's sarcoma protein biomarkers; while achieving diagnostic accuracy of 97% (Fig. 4(a)).[66] These advancements highlight the potential of digital microfluidic-based digital ELISA platforms in enabling highly sensitive and accurate EV biomarker detection for clinical applications.

For the detection of EV-derived nucleic acids, researchers have primarily focused on advancing digital PCR (dPCR) technologies to enable high-throughput and rapid nucleic acid analysis with enhanced sensitivity. Yao *et al.* designed a 4-plex droplet digital PCR (ddPCR) assay capable of simultaneously quantifying four EV-derived mRNA biomarkers (PGR, ESR1, ERBB2, and GAPDH). By integrating machine learning algorithms, this approach significantly improved the diagnostic accuracy for ovarian cancer, demonstrating high clinical efficacy (Fig. 4(b)).[67] In another study, Zheng *et al.* developed a multifunctional droplet digital immuno-PCR (dd-iPCR) platform that combines the high specificity of immuno-PCR with the exceptional sensitivity of ddPCR. This method enabled single-EV nucleic acid profiling by simultaneously detecting breast cancer-associated mRNAs (CD9/CD63/CD81, HER2, EpCAM) and hepatocellular carcinoma-specific transcripts (CD9/CD63/CD81, GPC-3, EpCAM). The assay exhibited robust

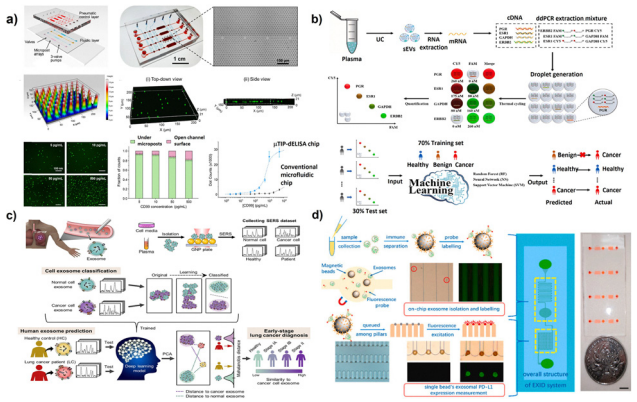

Fig. 4 Digital microfluidic chips combined with AI model learning for detecting single EVs. (a) The μTIP-dELISA microfluidic platform for detecting the surface protein of individual EVs is used for the diagnosis of Ewing's sarcoma. Adapted with permission from ref. 66. Copyright 2025 American Chemical Society. (b) The 4-plex droplet digital PCR detection platform combined with machine learning algorithms, for detecting EVs and achieving efficient diagnosis of ovarian cancer. Adapted with permission from ref. 67. Copyright 2021 Elsevier. (c) The SERS combined with deep learning algorithm detection platform is used to detect EVs in the blood of lung cancer patients and achieve early diagnosis of lung cancer. Adapted with permission from ref. 70. Copyright 2020 American Chemical Society. (d) A rapid and highly sensitive detection and analysis platform for PD-L1 protein in single EV can be used for the diagnosis and detection of various cancers. Adapted with permission from ref. 75. Copyright 2022 Elsevier.

discrimination between healthy donors and cancer patients, underscoring its potential for clinical diagnostics.[68] These advancements in digital nucleic acid detection highlight the growing role of multiplexed, high-sensitivity dPCR platforms in EV-based liquid biopsy applications.

In recent years, with the burst in use of AI, the integration of this newly emerging technology with microfluidics has exhibited significant potential in EV detection.[69] A notable example is the study by Choi *et al.*, which combined deep learning with SERS to profile EVs, establishing a novel approach for the early diagnosis of lung cancer. In this work, 43 plasma-derived EV samples from lung cancer patients were analysed by the developed deep learning model (Fig. 4(c)).[70] Moreover, this AI model predicted the higher similarity of detected EVs to lung cancer cell-derived EVs and the positive correlation with disease progression, which suggested the potential of AI in assisting EV analysis and disease diagnosis. On the basis of this work, the same group utilized a similar approach to analyse plasma exosomes in other malignancies, including ovarian, breast, colon, and pancreatic cancers. Their AI-assisted detection system demonstrated robust diagnostic performance, achieving a sensitivity of 90.2% and a specificity of 94.4%.[71] These findings underscore the potential of AI to enhance EV-based diagnostics, paving the way for more precise and non-invasive disease detection across multiple cancer types.

3.5 Single EV analysis

Single EVs demonstrate both cell-type specific signatures and population heterogeneity derived from their parental cells. During disease progression, pathological alterations in tissues induce distinct modifications in EV cargos and surface markers, making these biomolecules valuable targets for diagnostic applications.[72,73] While single-EV analysis has tremendous potential for precision diagnostics, its clinical translation faces substantial challenges, primarily due to difficulties in achieving both high-throughput processing and target-specific detection.[74] To meet these requirements, microdroplet systems have been employed to generate microreactors of single EVs, facilitating further detection using various readout techniques. For instance, Mao *et al.* developed an integrated microfluidic platform for single-EV detection, which realized accurate quantification of PD-L1 protein in a reduced total analysis time of less than 2 h (Fig. 4(d)).[75] Researchers have also dedicated efforts to developing new methods for multiplexed analysis. Ko *et al.* reported an iddPCR approach enabling simultaneous detection of multiple protein markers on individual EVs, demonstrating the feasibility of integrated protein–nucleic acid analysis at the single-EV level.[76] Similarly, David *et al.* developed a microfluidic-based dual digital assay for ultrasensitive single-molecule detection in individual EVs, enabling PD-L1 quantification in melanoma-derived EVs. This breakthrough demonstrates significant potential for evaluating cancer biomarkers related to treatment response and disease progression, advancing EV-based liquid biopsy for precision oncology.[77] Current trends in microfluidics emphasize the development of fully integrated systems for multiparametric single-EV analysis. These technological advances are establishing new paradigms for disease diagnosis through exosome-based liquid biopsies. The ability to perform comprehensive molecular profiling at the single-EV level represents a transformative approach in the diagnostic medicine field.

4. Advanced biomedical application of microfluidic-assisted analysis of EVs

Analysis of EVs offers distinct advantages for disease diagnosis and prognostic evaluation. As research on EV biogenesis and function advances, disease-associated alterations in EV biomarkers are emerging as a novel class of indicators for monitoring pathological progression (Fig. 5).[78] In the field of tumour diagnostics, EV-based detection technologies have demonstrated significant advancements, progressively evolving toward high-throughput, high-sensitivity, and high-specificity detection platforms.[79] Beyond their well-established role in oncology, EVs demonstrate unique diagnostic value across multiple disease domains, including cardiovascular disorders, neurological conditions, infectious diseases (encompassing both microbial infections and tropical parasitic diseases), and autoimmune pathologies (Table 2).

4.1 Cancer

Cancer remains a severe threat to human health.[80] Through continuous efforts in tumour research, a variety of diagnostic methods have been applied in clinical practice, including imaging diagnostics and needle biopsy. However, due to the

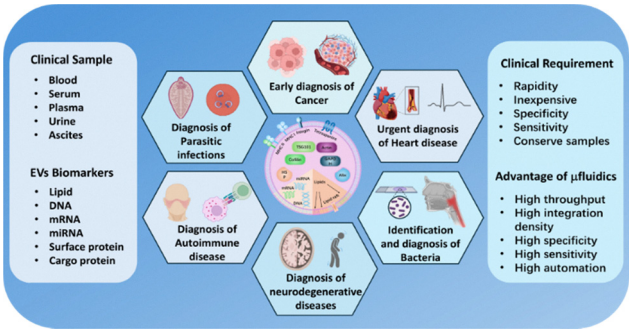

Fig. 5 EV-based detection demonstrates significant diagnostic potential for multiple clinical disease categories (cancer, cardiovascular diseases, neurological diseases, bacterial infection, parasite infection and autoimmune diseases) and clinical requirements and advantages of microfluidic detection.

inherent limitations of these methods, a significant number of cancer patients are still diagnosed at an advanced stage. Notably, the stage of a tumour is closely correlated with patients' long-term survival rates. With the continuous advancement of clinical diagnosis and treatment, the demand for precise tumour diagnosis and treatment has emerged,[81] and this precision has become indispensable in clinical practice.

Liquid biopsy technology enables accurate disease diagnosis under non-invasive or minimally invasive conditions.[82] Nevertheless, the development of liquid biopsy techniques first requires the discovery of disease markers with high specificity and sensitivity. Tumour-derived EVs can serve as such tumour markers.[83] Currently, an increasing number of researchers are utilizing microfluidic technology as a detection platform for early cancer diagnosis through EV analysis. For instance, Yang's research group developed a novel microfluidic chip filled with transparent microbeads, which forms a non-uniform electric field. This design ultimately achieves rapid, sensitive, and adjustable EV capture and detection, and by analysing specific proteins, it enables high-accuracy and high-sensitivity diagnosis of breast cancer.[84] Matt and his colleagues developed an innovative multiplexed microfluidic platform that enabled highly specific capture and sensitive detection of multiple EV biomarkers through tuneable alternating current electrophoresis. This advanced approach facilitates high-accuracy breast cancer diagnosis with exceptional sensitivity.[85] Based on these findings, they developed a surface plasmon resonance platform for quantifying the surface protein composition of blood-derived EVs from breast cancer patients. The results revealed that approximately 14–35% of patients exhibited significant expression of human epidermal growth factor receptor 2 (HER2) on their circulating EVs. This discovery not only confirms the potential of EV-based biomarkers but also offers a new approach for improving breast cancer diagnosis.[86] Ding and colleagues have developed a cascade-triggered individual EV nanoencapsulation strategy, enabling ultrasensitive and specific detection of EV subsets in a one-pot manner. This approach has demonstrated excellent performance in the diagnosis of hepatocellular carcinoma (Fig. 6(a)).[87]

Small nucleic acid molecules carried by tumour-derived EVs (tEVs) also hold promise as novel tumour diagnostic markers. Wu *et al.* focused on miRNAs encapsulated in EVs and proposed a coding fusion strategy to analyse miRNA characteristics in tEVs for pancreatic cancer diagnosis. Their method allows the analysis of six pancreatic cancer-associated miRNAs from a mere 2 μL of plasma, eliminating the need for EV isolation or lysis, thereby achieving accurate diagnosis of pancreatic cancer.[88] Beyond the analysis of single types of diagnostic markers, researchers can leverage microfluidic devices to simultaneously detect multiple markers. For example, Jung's team designed a new microfluidic device capable of selectively isolating EVs derived from epithelial and mesenchymal cells. This enables efficient and accurate analysis of both EV populations, facilitating the diagnosis of breast cancer (Fig. 6(b)).[89] Additionally, James and his team achieved specific diagnosis of pancreatic cancer through the dual analysis of Glypican 1 protein and mRNA within EVs using a microfluidic reaction system.[90]

In the ongoing battle against tumours, accurate early diagnosis has gradually become an essential requirement in clinical practice. Research into these technologies not only provides new insights into the practical applications of EVs but also holds promise for translation into clinical practice, offering new solutions to improve the diagnostic accuracy of tumours and further enhance patient survival rates.

4.2 Cardiovascular diseases

In recent years, as human lifestyles have changed and living standards have improved, cardiovascular diseases have gradually emerged as a major threat to human health. No longer confined to older populations, they are increasingly affecting younger individuals, with their impact expanding across age groups.[91] Cardiovascular diseases encompass both chronic conditions such as hypertension, hyperlipidaemia, atherosclerosis, and acute critical conditions including coronary heart disease, myocardial infarction, heart failure, and aortic dissection.[92,93] These acute conditions can often endanger patients' lives in a very short time. Traditional diagnosis of chronic cardiovascular diseases typically relies on enzymatic assays and conventional imaging tests. While the diagnosis of acute cardiovascular diseases has become relatively mature, challenges such as delayed detection and ambiguous diagnosis persist, and emergency clinicians are required to possess a certain level of clinical expertise. Against this backdrop, the integration of EV detection with microfluidic technology is expected to provide new insights for advancing diagnostic techniques for cardiovascular diseases.

An increasing number of mechanistic studies have proved that EVs play an indispensable role in the pathophysiological process of cardiovascular diseases.[94] Multi-omics analyses have also demonstrated that EVs can be used as biomarkers for atherosclerosis, coronary heart disease, ischemic heart disease and cardiac fibrosis.[95] For practical applications, Lee's group developed a highly integrated microfluidic system equipped with highly sensitive field-effect transistors that can perform EV

Table 2 Summary of various clinical applications based on EV analysis for disease diagnosis

Type of disease	Disease	EVs biomarkers	Time	Advantages	Disadvantages	Ref
Cancer	Ovarian cancer	CD24, EGFR, EpCAM	2 h	Multiple markers, high sensitivity and specificity	Expensive and time consuming	61
	Breast cancer	EpCAM, MUC1	13 min	Rapid, high throughput	Low marker specificity	63
	Hepatocellular carcinoma	EpCAM, PDL-1, MUC1	2 h	Machine analysis, high throughput	Complicated equipment and time consuming	87
	Pancreatic cancer	miR-21	2 h	High throughput, digital	Low diagnostic specificity	88
	Breast cancer	EpCAM, CD49f	6.7 min	Rapid, high throughput	Low marker specificity	89
	Pancreatic cancer	GCP1 protein and GCP1 mRNA	2–3 h	Dual maker, high specificity and high throughput	Time consuming	90
Cardiovascular diseases	Early myocardial infarction	miR-21 and miR-126	5 h	High sensitivity and high specificity	Time consuming	96
	Atherosclerosis of the arteries	Urine EV miR-155	5 h	Non-invasive, high throughput	Time consuming	97
	Classification of hypertension	Urine EV mRNA	1–2 h	Non-invasive, high specificity	Complicated equipment and expensive	100
Neurological diseases	Alzheimer's disease	Glycerol phosphoserine	5 h	High sensitivity, BBB penetration	Complicated equipment and time consuming	106
	Brain injuries	EVs miRNA	2 h	BBB penetration, machine analysis, high efficiency	Expensive	108
	Glioma	EGFR	10–30 min	BBB penetration, high sensitivity	Low marker specificity	109
Infectious diseases	Tuberculosis in children	Glucolipids arabinomannan	2 h	High specificity	Low marker specificity and time consuming	126
	Helicobacter pylori infection	HIF-1α	10–30 min	High efficiency and specificity	Complicated equipment and expensive	128
	Chronic American trypanosomiasis	IgG-2, IgG-4	10–30 min	High specificity	Low marker specificity	132
	Plasmodium vivax infection	hsa-miR-150-5p, has-miR-15b-5p	2 h	High sensitivity and specificity	Low efficiency and time consuming	135
Autoimmune diseases	Evaluate the effect of rheumatic treatment	miR-212-3p, miR-338-5p, miR-410-3p	2 h	Dual maker, high specificity	Complex procedure and time consuming	139
	Systemic lupus erythematosus	tiRNA5-Lys-CTT-1	5 h	High sensitivity	Complex procedure and time consuming	141

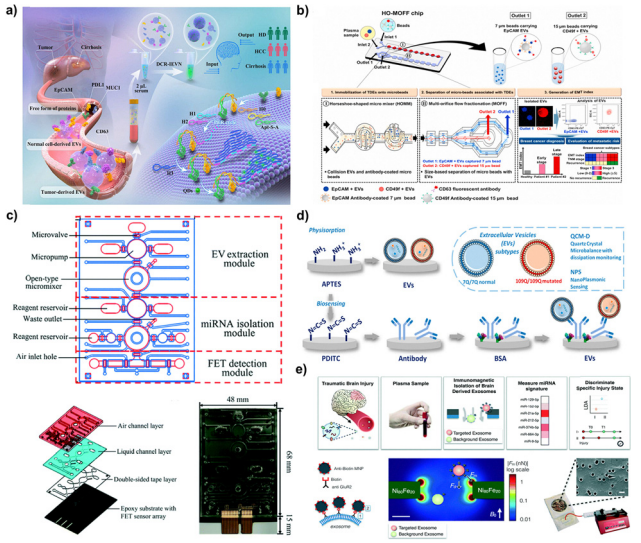

Fig. 6 Microfluidic platform for targeted EV detection in cancer, cardiovascular diseases, and neurological diseases diagnostics. (a) One-step micro-nano platform for early hepatocellular carcinoma diagnosis *via* specific EV capture and on-chip analysis. Adapted with permission from ref. 87. Copyright 2024 American Chemical Society. (b) A dual-targeting microfluidic platform for selective isolation and phenotypic detection of epithelial and mesenchymal derived EVs in early-stage breast cancer. Adapted with permission from ref. 89. Copyright 2021 Elsevier. (c) Integrated microfluidic platform for on-chip EV extraction, lysis, and cardiac-specific microRNA detection for early cardiovascular disease diagnosis. Adapted with permission from ref. 96. Copyright 2018 Royal Society of Chemistry. (d) A microfluidic immunoassay platform for high-specificity detection of Huntington's disease-associated EVs proteins. Adapted with permission from ref. 105. Copyright 2022 American Chemical Society. (e) A microchip-based platform for brain-specific EV miRNA profiling and quantitative assessment of traumatic brain injury severity. Adapted with permission from ref. 108. Copyright 2018 Royal Society of Chemistry.

extraction, EV cleavage, target miRNA isolation, and miRNA detection within 5 hours. Thus, early diagnosis of cardiovascular disease can be achieved (Fig. 6(c)).[96] For the diagnosis of atherosclerosis, Belton's group found that microRNA-155 was increased in urinary EVs during the progression of atherosclerosis,[97] providing a new rationale for EV-based diagnosis of atherosclerosis. Binder *et al.* found that in the pathology of myocardial infarction, the level of EVs carrying oxidation-specific epitopes (OSE) derived from lipid peroxidation was markedly increased at the infarct site. Importantly, natural IgM antibodies specific for OSE, such as malondialdehyde, have been shown to modulate the functional impact of EVs and been associated with reduced cardiovascular risk.[98] For myocardial infarction disease, EVs are not only of diagnostic significance, but also of high utilization value in the treatment of myocardial infarction.[99] In terms of the classification of hypertension, EVs also provide new diagnostic potential. Pizzolo *et al.* found that by analysing the mRNA carried by EVs derived from urine, they can be used to reflect the pathological changes of the kidney, to further analyze the type of hypertension.[100]

4.3 Neurological diseases

The spectrum encompasses neurodegenerative disorders[101] (such as Parkinson's disease, Huntington's disease, Alzheimer's disease, and cerebral atrophy), acute brain injury[102] and tumour lesions[109] (*e.g.* glioma, other tumours, brain metastases). Due to the non-regenerative nature of nerve cells, patients with nervous system diseases often experience a severe decline in the quality of life in the late stage, imposing substantial psychological and economic burdens. The clinical diagnosis of neurodegenerative diseases relies more on behavioural changes and a limited number of biomarkers to cross the BBB,[103] all of which suffer from low specificity and diagnostic accuracy. Therefore, researchers turn to using EVs as a potential solution for the diagnosis of neurological diseases.[104] For instance, Boujday *et al.* designed a sensor platform that combines a quartz crystal microbalance with dissipative and nano plasma sensing for EV tracking analysis, and application of this multi-sensing strategy to analyze small EVs isolated from striatal cells of knock-in mice expressing the mutant allele or the wild-type allele of the huntingtin gene suggests that EVs can serve as effective biomarkers to reflect cell-to-cell changes associated with neurodegenerative diseases (Fig. 6(d)).[105]

In another study, Vella and colleagues verified that EVs can cross the BBB and reflect the lipid homeostasis in the brain. They also developed an approach to isolate EVs from plasma while minimizing the co-segregation of lipoproteins. The lipid composition of EVs in the human frontal cortex is described for the first time, and these lipids have been shown to play an important role in AD, thus successfully demonstrating the unique significance of EVs in AD diagnosis and treatment.[106] EVs hold unique value in the diagnosis and treatment of brain injury. The Leppik team isolated EVs from the plasma of brain injury patients and analysed the expression of surface epitopes on these EVs *via* neural-specific multi-flow cytometry, with healthy controls as a reference. Their findings revealed that EVs were enriched with specific proteins in response to brain injury, highlighting the potential of EVs in diagnosis.[107] Ko *et al.* developed a microchip-based diagnostic technique that evaluates brain injury status by measuring a panel of brain-derived EV miRNAs and processing the data through machine learning algorithms. This approach demonstrated that RNA contained in brain-derived EVs enabled a more comprehensive characterization of brain injury (Fig. 6(e)).[108] In the context of nervous system tumours, Yang *et al.* established and optimized a bead-based auxiliary detection method using flow cytometry to assess EGFR protein expression in serum EVs from glioma patients. Their results confirmed that EGFR expression in EVs serves as an effective diagnostic marker for glioma, with a strong correlation between EGFR levels and glioma malignancy.[109,110]

EVs exhibit distinct advantages in the diagnosis of nervous system diseases. Owing to their capacity to cross the BBB, EVs can more accurately reflect the pathophysiological changes within the nervous system, thereby holding promise as novel diagnostic markers and therapeutic platforms for such diseases.

4.4 Infectious diseases and tropical diseases

Infectious diseases, among the most prevalent ailments in daily life, are caused by the invasion of pathogenic microorganisms (bacteria, fungi, parasites, *etc.*) into the human body. These

pathogens can infect various bodily systems, particularly those in contact with the external environment, such as the respiratory system,[111] digestive system,[112] and urinary system.[113] Meanwhile, in individuals with severely compromised immune systems (*e.g.* tumour patients with long-term chemotherapy, HIV infected patients, ICU patients, *etc.*), infections can also affect other systems, leading to severe complications like sepsis,[114] and nervous system infections.[115] The onset of such infectious diseases is often accompanied by systemic pathological reactions, including acid–base imbalance, dyspnoea, and persistent high fever, which may threaten patients' lives.[116] Due to unique climatic, environmental, and healthcare conditions, infectious diseases in tropical regions frequently exhibit more distinct features and graver consequences.[117] Moreover, the tropics harbour a great diversity of endemic pathogens responsible for infectious diseases, such as *Plasmodium*,[118] *Burkholderia pseudomallei*,[119] dengue virus,[120] and various fungi and parasitic worms.[121]

Bacterial infection. In the diagnosis and treatment of infectious diseases, bacterial infection represents the most common focus of clinical diagnosis. In practical clinical settings, the primary task is to identify the type of infectious microorganisms and the drug resistance traits of pathogens, enabling clinicians to deliver targeted, precision treatment tailored to the specific pathogen. However, the most widely used clinical method for identifying pathogenic microorganisms suffers from long incubation time. While emerging technologies have been developed, such as mass spectrometric analysis of pathogens[122] and high-throughput drug resistance profiling,[123] the time-consuming nature of pathogenic bacterial culture remains a significant challenge. Critically, in cases of acute infectious disease onset, clinicians require rapid and accurate pathogen analysis. Due to limitations in detection methods, they often rely on empirical treatment or broad-spectrum antibiotics to manage acute infections.

In addressing these challenges, advances in EV detection technology offer a novel solution. Studies have shown that when pathogens infect the human body, infected host cells produce specific EVs,[124] while pathogen cells themselves also secrete distinct EVs that exert various physiological and pathological effects.[125] These pathogen-specific or infection-associated EVs hold promise as new diagnostic markers for infectious diseases. Hu's group developed a nanoparticle-enhanced immunoassay to detect two *Mycobacterium tuberculosis* virulence factors (glucolipid arabinomannan and its carrier protein) on the surface of circulating EVs in bodily fluids, achieving accurate diagnosis of pediatric tuberculosis (Fig. 7(a)).[126] Joseph *et al.* analyzed the entire proteome of EVs derived from human retinal cells infected with *Staphylococcus aureus* (*S. aureus*) and *Pseudomonas aeruginosa*, identifying 38 infection-specific proteins that could serve as diagnostic markers for bacterial infection-induced retinal diseases.[127] Additionally, Quest *et al.* isolated and analyzed EVs from infected or uninfected immortalized normal gastric GES-1 cells, revealing that EVs secreted by *Helicobacter pylori*-infected cells induce higher IL-23 secretion, thereby increasing the carcinogenic potential of normal gastric cells and identified hypoxia-inducible factor-1α (HIF-1α) as a specific detection target.[128]

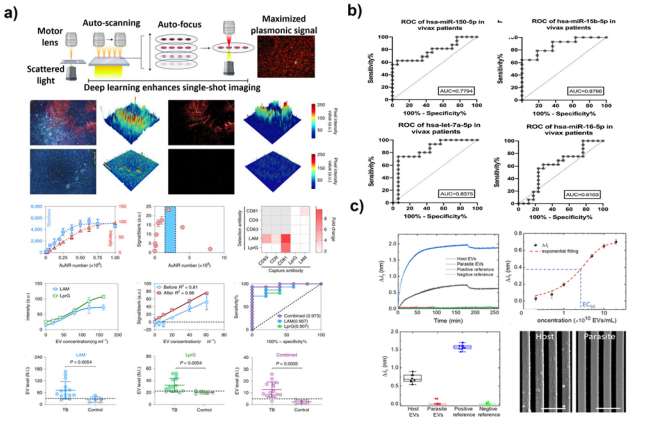

Fig. 7 The diagnostic efficacy of EV detection for bacterial infections and parasitic infections. (a) A nanoparticle-enhanced diagnostic platform enables childhood tuberculosis detection through simultaneous quantification of two *Mycobacterium tuberculosis* virulence factors in circulating EVs. Adapted with permission from ref. 126. Copyright 2022 Nature. (b) ROC analysis demonstrates high diagnostic accuracy for *Plasmodium vivax* infection using a four-miRNA signature (hsa-miR-150-5p, hsa-miR-15b-5p, hsa-let-7a-5p, has-miR-16-5p) in plasma-derived EVs (with an area under the curve (AUC) of 0.7794, 0.8766, 0.8375,0.6103). Adapted with permission from ref. 132. Copyright 2020 Springer Nature. (c) A microfluidic platform capable of rapidly differentiating EVs produced by host cells from those produced by parasites and its detection efficacy. Adapted with permission from ref. 135. Copyright 2018 American Chemical Society.

Parasite infection and tropical disease. In the context of parasite infection detection, studies have shown that multifunctional EVs facilitate intercellular communication, contributing to the persistence of parasites within the host. These secreted vesicles exhibit heterogeneous sizes ranging from 30 to 500 nm, cell of origin, target destination, and contents.[129] In practical detection research, Osuna *et al.* investigated circulating EVs in the serum of patients with chronic Chagas disease and the types of IgG antibodies elicited by these EVs in the host. They identified specific Chagas infection-associated proteins (such as GP63) in these EVs, offering new insights for the diagnosis of Chagas infection.[130] Schwartz *et al.* isolated EVs from the serum of schistosomiasis patients and analysed the miRNAs carried by EVs. They discovered that two highly expressed miRNAs (bantam and miR-2c-3p) of EVs exhibited 86% sensitivity and 84% specificity, respectively, suggesting their potential as novel markers for schistosomiasis detection.[131] For the detection of tropical-specific parasites, Palasuwan *et al.* extracted EVs from the serum of 18 malaria-infected patients and analysed the miRNA content, with 20 healthy donors as controls. In patients infected with *Plasmodium vivax*, the relative expression levels of hsa-miR-150-5p and hsa-miR-15b-5p were significantly higher, providing a new approach to malaria diagnosis (Fig. 7(b)).[132]

Meanwhile, when the parasite-specific antigen stimulates the body's immune cells, these cells secrete increased numbers of EVs, which in turn enhance the activation of the host immune response. Peng *et al.* investigated miRNAs carried by EVs derived from *Toxoplasma gondii*-infected dendritic cells, revealing that these EVs further promote the body's immune processes.[133] Nejsum *et al.* demonstrated that parasite-derived

specific antigens can also activate immune cells, inducing them to produce more EVs that amplify the immune response. Their research involved stimulating bone marrow-derived macrophages with soluble products of *Trichuris suis* and collecting the EVs secreted by these BMDMs. They found that TSP indirectly regulates immune responses by triggering EV release from BMDMs, thereby exerting anti-inflammatory effects on recipient cells.[134]

To date, extensive research has explored the physiological and pathological changes in EVs induced by infectious diseases. However, further efforts are still required to translate these findings into clinical biomarkers and practical applications. The first critical issue to address is the validation of the sensitivity and accuracy of biomarkers. Additionally, the development of novel research methods, such as integration with microfluidic technology can enable effective detection of infection-induced EVs. Dong and colleagues developed a microfluidic platform capable of rapidly distinguishing host cell-derived EVs from parasite-derived EVs (Fig. 7(c)).[135] It is anticipated that with ongoing research, microfluidic-based EV detection will find broader applications in the diagnosis of infectious diseases.

4.5 Autoimmune diseases

Autoimmune diseases, including rheumatoid disease, systemic lupus erythematosus (SLE), and Sjogren's syndrome, are systemic disorders characterized by the erroneous recognition and attack of the body's own tissues by its immune cells.[136] Clinical diagnosis of autoimmune diseases primarily relies on symptomatic assessment and laboratory detection of specific antigens and antibodies. EVs, which play a crucial role in intercellular communication, also mediate various immune responses.[137] In the treatment of autoimmune diseases, real-time monitoring of therapeutic efficacy is essential. Compared with traditional antigens and antibodies, EVs exhibit greater uniformity and higher sensitivity in evaluating treatment outcomes.[138] Pratt *et al.* isolated serum EVs from 46 rheumatoid arthritis patients and analysed their miRNA content. They identified 798 miRNAs and compared them with those in serum EVs from rheumatoid arthritis patients who responded effectively to methotrexate treatment. Significant differences were observed in the expression of miR-212-3p, miR-338-5p, miR-410-3p, and miR-537, confirming that EVs have promise as markers for assessing methotrexate efficacy in rheumatoid arthritis patients.[139] Rheumatoid arthritis patients often exhibit abnormal coagulation during treatment, with pathological activation of the procoagulant system increasing the risk of cardiovascular disease. Antovic *et al.* enrolled 20 female rheumatoid arthritis patients and 20 healthy donors, extracted and analysed their serum EVs, and measured fibrinogen levels across all participants. They found elevated levels of circulating EVs from various cell types in rheumatoid arthritis patients, accompanied by abnormal fibrinogen production, supporting the potential of EVs as markers for detecting coagulation abnormalities in these patients.[140] Lupus nephritis represents one of the most severe organ lesions in SLE, with critical implications for SLE treatment. Liang and colleagues compared 20 SLE patients with lupus nephritis and 20 SLE patients without lupus nephritis, revealing upregulated levels of tiRNA5-Lys-CTT-1 in EVs from the former group. This finding validated urinary EVs as a specific marker for lupus nephritis.[141]

In the detection of autoimmune diseases, clinical research has continuously identified numerous diagnostic markers.[142] However, for systemic diseases, a definitive diagnosis alone is insufficient to guide clinical management. Unlike traditional antigen–antibody markers, EVs possess more complex compositions, enabling multi-dimensional, multi-marker monitoring of treatment responses and disease progression in autoimmune disease patients. Microfluidic technology offers a promising solution for high-throughput, multi-marker detection needs. While such detection technologies are still in the early stages of development, advances in this field are expected to enable high-throughput, multi-marker EV detection, providing improved diagnostic tools for patients with autoimmune diseases.

5. Summary and future perspectives

To date, the use of EVs as novel liquid biopsy markers for diagnosing clinical diseases and monitoring treatment responses has garnered increasing attention. EVs hold great promise as liquid-biopsy markers for early diagnosis of tumours,[143] traditional common diseases such as infectious diseases,[144] and rare diseases which are difficult to diagnose, like autoimmune diseases.[145] However, there are still many significant challenges in microfluidic-based EV analysis when it comes to clinical application, which can be categorized as technical, biological, clinical, *etc.*[146]

First, regarding clinical detection methods for EVs, traditional detection methods are unsuitable for clinical use due to their inherent limitations.[147] Microfluidic technology, with its advantages of high efficiency, low reagent consumption, rapid response and high integration, is expected to emerge as a new platform for the EV detection. While ongoing research efforts have led to the development of various microfluidic-based EV detection platforms, these methods still fall short of clinical application requirements. Further improvements are needed to enhance detection sensitivity and specificity, as well as to shorten detection time. Therefore, in the development of new microfluidic-based detection platforms, emphasis should be placed on high-throughput analysis with increased sensitivity and accuracy. Microfluidic technology can integrate multiple chemical detection methods for EV analysis. For surface protein profiling of EVs, it can be combined with optical techniques such as SERS,[148] fluorescence detection,[149] and other techniques like electrochemical detection.[150]

Second, due to the inherent heterogeneity of EVs in size and contents, the application potential of these vesicles is constrained by their complex biological characteristics.[151] To solve this challenge, EV analysis at the single vesicle level is critical to reveal the biological characteristics for further detection. Research on single EV analysis has advanced rapidly in recent years.[73] For instance,

fluorescence imaging-based techniques such as super-resolution microscopy and total internal reflection fluorescence microscopy enable single-vesicle based imaging on microfluidics for further quantification *via* fluorescence response. Droplet-based single-EV counting systems including ddELISA and ddPCR represent advanced microfluidic platforms for analysing the protein and nucleic acid contents in individual vesicles. Moreover, other optical technologies such as Raman tweezers microspectroscopy, interferometric plasmonic imaging also provide solutions for single EV detection, enabling researchers to gain deep insights into EVs' biology.

Third, despite significant advancements in developing microfluidic platforms for EV detection across various diseases in preclinical studies, there is still a lack of technologies that have been successfully translated into routine clinical laboratory diagnostics. This translational gap stems primarily from several key challenges, including complex sample preparation workflows, prolonged detection timelines, insufficient sensitivity and specificity, and high operational costs. To bridge this gap, next generation EV microfluidic platforms must prioritize clinically relevant design goals, focusing on achieving highly specific and sensitive diagnostic methods. Additionally, critical considerations such as detection limits, quality control in real-world applications, and the establishment of reliable detection thresholds must be addressed. Beyond optimizing sensitivity and specificity, future platforms should aim to enhance batch-to-batch consistency and reproducibility while simultaneously reducing detection time and cost to facilitate widespread clinical adoption.

Fourth, in terms of data analysis, emerging digital microfluidic technologies and deep learning algorithms can be incorporated to generate detection and analysis results in the shortest possible time.[152] In recent years, the integration of AI and microfluidic technology has provided revolutionary solutions for clinical diagnostic technologies. AI-driven machine learning and deep learning algorithms can optimize microfluidic system design, enable real-time data analysis, and facilitate automated decision-making, thereby enhancing the precision and throughput of clinical testing. This transformative integration is expected to fundamentally reshape the traditional clinical laboratory model by enabling the use of mobile health platforms including microfluidic chips, imaging modalities, auxiliary components, and intelligent software algorithms. Such platforms have already made it possible to detect molecules, viruses, cells, and parasites in non-laboratory settings.[153] Currently, the application of AI-microfluidic integration in EV analysis remains very limited. However, as advanced analytical technologies continue to evolve, AI is expected to play an increasingly significant role in EV detection for biomedical applications.

In the future, the development of microfluidic technology to detect EVs can be developed in combination with different clinical disease types. In the field of tumour diseases, great efforts are focused on the development of new high-sensitivity and high-specificity tumour biomarkers for early diagnosis and staging. It is necessary to improve the detection sensitivity and detection speed in cardiovascular diseases and neurological diseases. In the field of infectious diseases, more efforts are focused on the differential detection of pathogenic microbial-derived EVs and host cell EVs. Meanwhile, new detection techniques should be developed for special infectious diseases in tropical areas. For autoimmune diseases, attention should be paid to the detection of biomarkers caused by other lesions during the development of systemic diseases.

In summary, the development of EV analysis platforms using microfluidic technology for disease diagnosis and monitoring exhibits substantial promise. However, to fully align with the requirements of clinical practice, significant advancements are still needed in key areas such as detection sensitivity, specificity, and the portability of devices. We anticipate that microfluidics-based EV analysis will play an increasingly pivotal role in clinical detection, ultimately transforming the landscape of clinical diagnostics.

Conflicts of interest

The authors declare no competing interests.

Data availability

No primary research results, software, or code have been included, and no new data were generated or analysed as part of this feature article.

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