

Diagnostic Use of Circulating Cells and Sub-Cellular Bio-particles

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Abstract

In the bloodstream or other physiological fluids, "circulating cells and sub-cellular bio-particles" include many microscopic biological elements such as circulating tumor cells (CTCs), cell-free DNA (cfDNA), exosomes, microRNAs, platelets, immune cells, and proteins are the most well-known and investigated. These structures are crucial biomarkers in healthcare and medical research for the early detection of cancer and other disorders, enabling treatment to commence before the onset of clinical symptoms and enhancing the efficacy of treatments. As the size of these biomarkers to be detected decreases and their numbers in body fluids diminishes, the detection materials, ranging from visual inspection to advanced microscopy techniques, begin to become smaller, more sensitive, faster, and more effective, thanks to developing nanotechnology. This review first defines the circulating cells and subcellular bio-particles with their biological, physical, and mechanical properties and second focuses on their diagnostic importance, including their most recent applications as biomarkers, the biosensors that are utilized to detect them, the present obstacles that must be surmounted, and prospective developments in the domain. As technology advances and biomolecular pathways are deepens, diagnostic tests will become more sensitive, specific, and thorough. Finally, integrating recent advances in the diagnostic use of circulating cells and bioparticles into clinical practice is promising for precision medicine and patient outcomes.

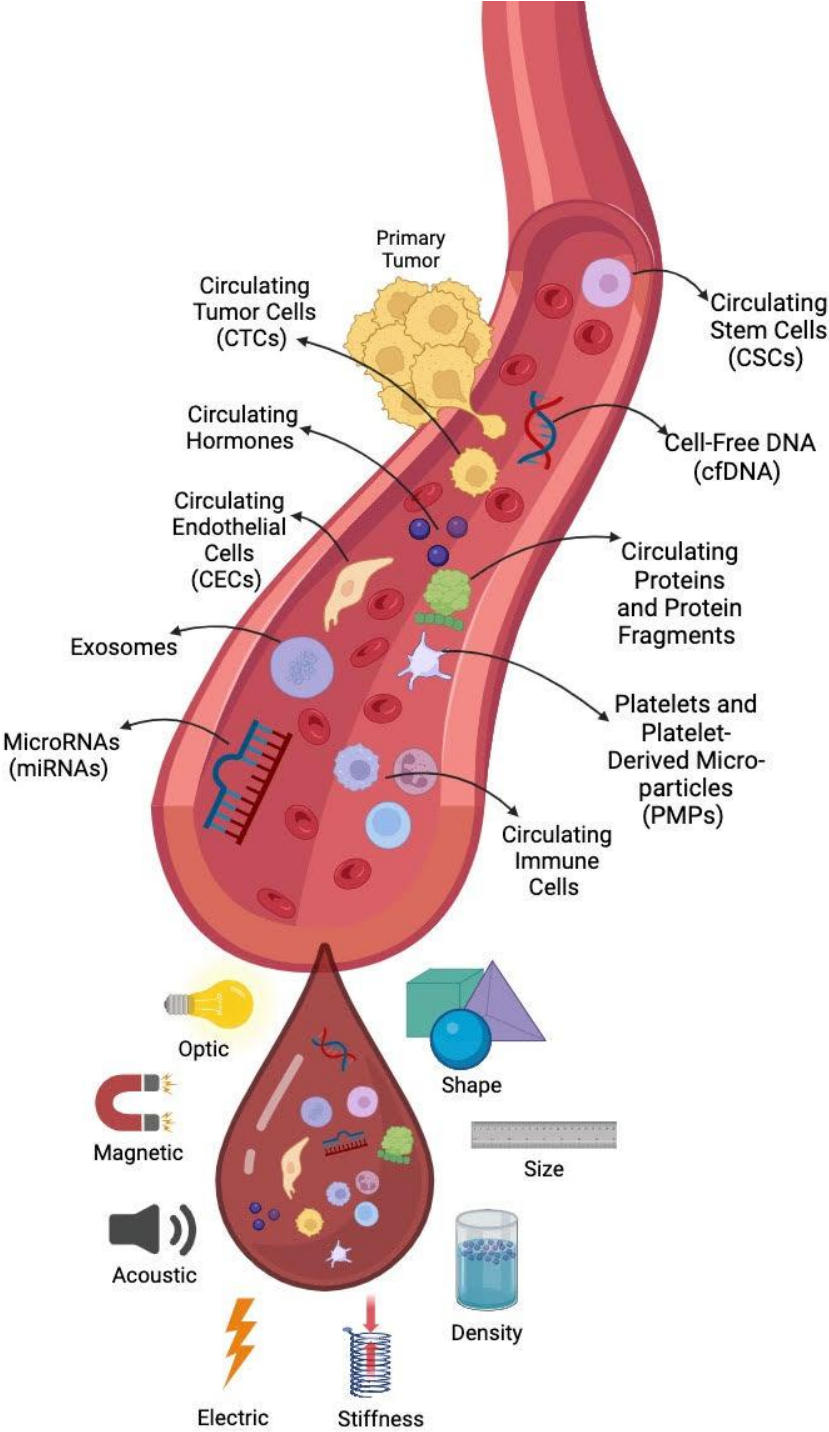
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Graphical Abstract



1. Introduction

Diagnostic tools in the field of medicine cover a diverse range of procedures and technologies that are utilized to diagnose, verify, and monitor various diseases and health situations (Pence and Mahadevan-Jansen, 2016). Diagnostic methods used to date include a clinical examination and physical assessment, a range of imaging techniques including x-ray, Computed Tomography scans (CT), Magnetic Resonance Imaging (MRI) and ultrasound, laboratory analyzes such as blood and genetic testing, molecular diagnostics such as Polymerase Chain Reaction (PCR) and Next-Generation Sequencing (NGS), endoscopy, electrocardiography, spirometry, functional tests, biopsy as tissue sampling, point-of-care testing (Nichols and Geddes, 2021). These technologies contribute to the wide range of diagnostic options available to healthcare professionals and are of great importance in facilitating healthcare practitioners in making precise and prompt diagnoses, customizing treatment strategies, and enhancing patient care outcomes by utilizing condition-specific information (Ho et al., 2020; Li and Diamandis, 2016).

Liquid biopsies have emerged as a promising diagnostic tool in the field of healthcare and diagnostics due to their numerous advantages (Lianidou and Pantel, 2019). These include the ability to detect diseases at early stages, offering a non-invasive and minimally discomforting diagnostic approach (Borros Arneht, 2018). Liquid biopsies also enable real-time monitoring of disease progression, which is particularly beneficial in the context of cancer diagnosis and classification. Moreover, they support the practice of personalized medicine, provide prognostic information, and facilitate research and drug development. By enabling timely and accurate diagnoses, liquid biopsies have the potential to reduce healthcare costs (Alix-Panabières and Pantel, 2021). Additionally, their non-invasive nature enhances patient acceptance and improves disease management. Liquid biopsies exhibit broad applicability across various diseases and demonstrate high sensitivity and specificity in detecting disease-related biomarkers (Martins et al., 2021). The combined attributes of liquid biopsies establish them as potent instruments that can revolutionize the process of illness diagnosis and enhance patient care (Martins et al., 2021). Although liquid biopsies have numerous benefits, they are not devoid of obstacles. The factors encompassed in this category consist of the establishment of uniform procedures, appropriate management of samples, and the requirement for diagnostic assays that possess high sensitivity and specificity. Furthermore, the clinical applicability of liquid biopsies may exhibit variability contingent upon the specific sort and stage of the ailment (Heitzer et al., 2019; J. Wu et al., 2020). Despite the aforementioned obstacles, liquid biopsies are continuously advancing and have significant potential to improve disease identification and treatment (Beije et al., 2019). Therefore, it is of utmost importance

to define precise definitions for circulating cells and subcellular bio-particles in the realm of liquid biopsy (De Rubis et al., 2019). These entities play a significant role in providing useful disease-related information, both indirectly and directly, which can be efficiently employed in diagnostic applications (J. Wu et al., 2020).

The term "circulating cells and sub-cellular bio-particles" encompasses a wide range of biological constituents that are present in the bloodstream or other body fluids, often at a microscopic level (Natalia et al., 2023). These structures encompass a diverse array of cellular entities, as well as smaller components such as fragments or molecules, and serve significant functions in the realms of diagnostics and research (Das et al., 2023). The most well-known and frequently studied of these structures are CTCs, cfDNA, exosomes, microRNAs, platelets, immune cells, and more, found in bodily fluids like blood (El Mustapha Bahassi, 2013; Heeke et al., 2019). The utilization of these structures as biomarkers in diagnostics is critical in healthcare and medical research since these biomarkers play a significant role in the early detection of cancer and other disorders, allowing for therapy before clinical symptoms appear and, eventually, boosting treatment success (Jayanthi et al., 2017). It is also crucial in monitoring the progression of the disease and tailoring treatment options based on the individual's genetic profile (Srivastava and Creek, 2019). These devices are significant because of their minimally invasive features, providing better therapeutic outcomes with fewer side effects, real-time monitoring capabilities, and role in disease classification and subtyping (Wang, 2015). Furthermore, by facilitating final diagnosis and eliminating unneeded procedures, these biomarkers contribute significantly to current pre-clinical research, the creation of next-generation pharmaceutical products, and the potential for cost-effective healthcare (Gromova et al., 2020).

This review article provides a comprehensive overview of circulating cells and subcellular bioparticles for diagnostic purposes, relevant to researchers in preclinical research, clinical studies, biosensor development, lab-on-a-chip systems, and molecular analysis. It covers both established and emerging methodologies, including Flow Cytometry, Immune-Magnetic Separation, Microfluidic Devices, Size-Based Filtration, EpCAM Enrichment, Quantitative PCR (qPCR), Next-Generation Sequencing (NGS), Fluorescence In Situ Hybridization (FISH), Microscopy and Imaging, Electrical Impedance-Based Sensors, and Mass Spectrometry. Unlike existing reviews, this article offers a holistic perspective by integrating bioengineering, chemical engineering, medical biology, molecular biology, and clinical practice, with a summary of the latest literature. It also addresses practical considerations for clinical

implementation and proposes future research directions, making it a valuable resource for both researchers and clinicians.

2. Types of Circulating Cells and Sub-Cellular Bio-particles

2.1. Circulating Cell Types

Circulating cells, including circulating tumor cells, circulating immune cells, circulating stem cells, and circulating endothelial cells, play crucial roles in disease monitoring, immune response, tissue repair, and vascular health. These cells are continuously present in the bloodstream and are essential for various diagnostic and therapeutic applications. Figure 1 illustrates the circulating cell types found in the bloodstream.

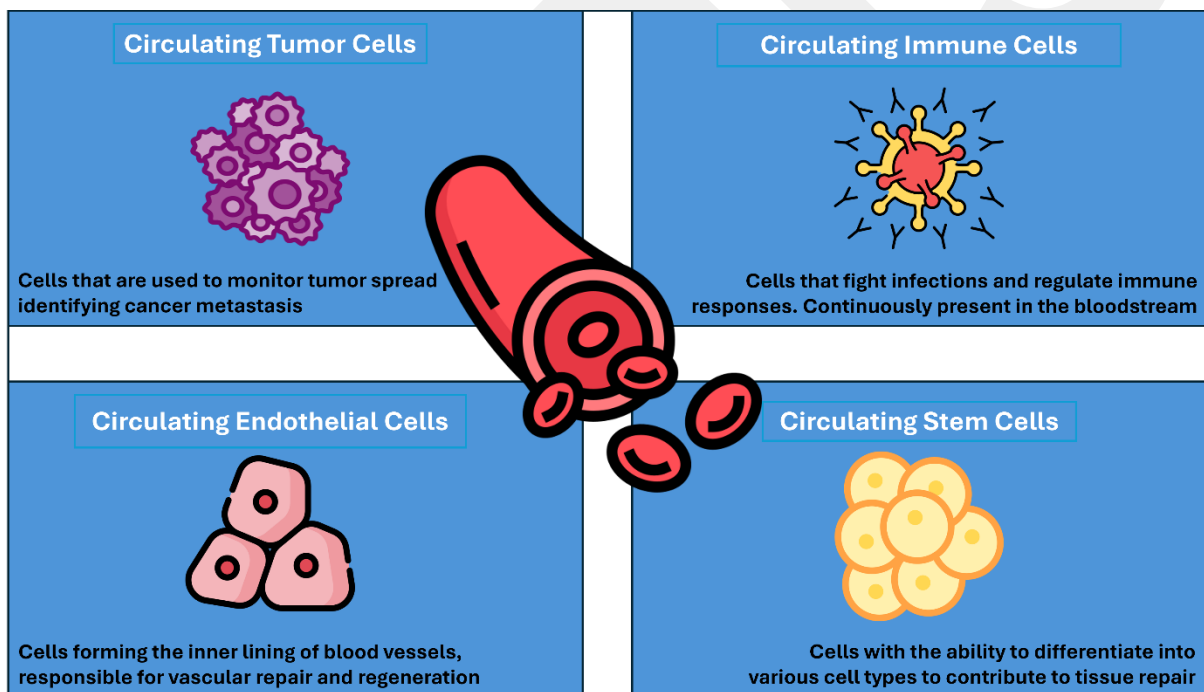


Figure 1. Circulating cell types found in the blood stream.

Circulating Tumor Cells (CTCs)

CTCs refer to a subset of cancer cells that exhibit a low prevalence inside the body (Moon et al., 2018). These cells have undergone detachment from the main tumor and have subsequently infiltrated either the bloodstream or the lymphatic system. This process of dissemination carries the potential for

metastatic colonization within various anatomical sites (Yang et al., 2021). CTCs are derived from primary tumors and gain access to the bloodstream or lymphatic system by the process of invasion, which entails the infiltration of blood or lymphatic vessels (Sharma et al., 2018). The cells have both genetic and behavioral traits that are consistent with those of the original tumor, rendering them highly relevant for investigating the molecular attributes of cancer (Lin et al., 2021).

CTCs demonstrate an exceedingly low incidence rate, with an approximate frequency of one per billion blood cells (Ried et al., 2017). Based on the examination of data collected from individuals who have received a cancer diagnosis, it is generally observed that a minimal risk of malignancy (Stage I) exists when the count of CTCs is below three CTC/ml, with a range of 0.1 to 2.9 CTC/ml (Ried et al., 2017). A moderate risk of malignancy, on the other hand (Stages II and III), is indicative of a CTC count between 3 and 20 CTC/ml (Ried et al., 2017). A significant likelihood of developing malignancy (Stage IV), encompassing metastasis, recurrence, and progression of the disease, is identified when the CTC count surpasses 20 CTC/ml (Ried et al., 2017). There is a positive correlation between the presence of CTCs and the progression of cancer, an increased probability of metastasis, and an unfavorable prognosis (Q. Li et al., 2024). It is evident that tracking CTC counts during cancer treatment may yield important insights into the course of the illness and the efficacy of the treatment (Q. Li et al., 2024).

The detection and isolation of these entities pose significant technical obstacles and several methods and techniques are employed for CTC detection, including immune-magnetic separation, microfluidic devices, size-based filtration, Epithelial Cell Adhesion Molecule (EpCAM) enrichment, nucleic acid-based approaches like quantitative PCR (qPCR) and NGS, fluorescence in situ hybridization (FISH), microscopy and imaging, label-free technologies such as electrical impedance-based sensors, functional assays, and in vivo imaging (Deng et al., 2022). Each method possesses distinct merits and drawbacks (Goldstein et al., 2023; Q. Li et al., 2024; Yeo et al., 2023). CTCs are highly significant instruments in the realm of cancer biology since they provide crucial insights into the mechanisms underlying medication resistance and the variety of diseases. In both preclinical and clinical research settings, they are employed to facilitate the advancement of novel therapeutic approaches and evaluate the effectiveness of treatments (Kahounová et al., 2023). CTCs play a vital role in liquid biopsies, a type of non-invasive diagnostic technique that examines biomarkers present in blood or other physiological fluids for cancer detection and monitoring (Lim et al., 2019). The utilization of CTC analysis has demonstrated its potential in various aspects of cancer management, including but not limited to the diagnosis of cancer, evaluation of individual risk, selection of appropriate treatment strategies, and monitoring of disease progression (Pesta et al., 2015).

The identification of CTCs holds significant relevance in the context of metastatic cancer, as it can provide valuable guidance for making informed decisions on treatment strategies although the study of CTCs encounters significant obstacles attributed to their infrequency and heterogeneity (Lim et al., 2019). The continuous improvement of standardized methodologies and procedures for the isolation and analysis of CTCs is a subject of continuous investigation in the scientific community (Guo et al., 2023).

In conclusion, CTCs hold great significance in the realm of cancer research and diagnostics due to their ability to offer valuable insights into the metastatic capabilities of cancer and present potential avenues for treatment techniques.

Circulating Immune Cells

The immune system is a complex network of cells and molecules that work together to protect the body from harmful pathogens (Kaur and Fang, 2020). Key players in this defense system include circulating immune cells, which include various types of white blood cells, which are crucial components circulating in the bloodstream, constantly searching for any signs of infection or disease (Trowsdale and Parham, 2004). White blood cells, also known as leukocytes, are responsible for detecting and eliminating foreign invaders, such as bacteria, viruses, and fungi (Kohok, 2022). They are produced in the bone marrow and can be broadly classified into two main categories: granulocytes and agranulocytes (Kohok, 2022). Granulocytes contain granules within their cytoplasm that aid in the destruction of pathogens. The three main types of granulocytes are neutrophils, eosinophils, and basophils. Neutrophils are the most abundant type of white blood cell and are known for their rapid response to infections (Sinning and Berliner, 2020). Lymphocytes, including T cells, B cells, and NK cells, are responsible for the body's adaptive immune response (Rich and Chaplin, 2019). Immune response coordination and direct cell-to-cell attack capabilities are possessed by T cells, whereas particular pathogen recognition and neutralization are facilitated by antibodies produced by B cells (Moss, 2022). When it comes to eliminating cancer cells and cells contaminated with viruses, NK cells are masters (Qi et al., 2021). The maturation of monocytes into dendritic cells and macrophages aids in the eradication of infectious diseases (Chaplin, 2010). Dendritic cells distribute antigens to other immune cells, which activates the immune system, and macrophages absorb and break down foreign particles (Chaplin, 2010). In the fight against allergies and parasites, eosinophils and basophils are crucial (Chaplin, 2010). Eosinophils hunt for and kill parasites, whereas basophils secrete allergens (Metcalfe et al., 2016). Blood tests and flow cytometry are valuable tools for evaluating immune cell subsets and studying immune system health (Kaur and Fang, 2020).

The usage of circulating immune cells in diagnosis spans a range of medical applications such as infection and inflammation detection, immune system profiling, autoimmune diseases, cancer immunodiagnosis, hematological disorders, allergy diagnosis, monitoring immunotherapy, and vaccine response (Marchetti and Engelhardt, 2020). Changes in the composition and activation of these cells serve as crucial indicators for various conditions. In infection and inflammation detection, alterations in circulating immune cells can signal the presence of infections or inflammatory conditions, with elevated white blood cell counts being a common marker of infection (Zeming et al., 2021). Immune system profiling involves analyzing the subsets and proportions of different immune cell types, offering insights into overall immune system health, and identifying abnormalities that may suggest immune system disorders (Marshall et al., 2018). In autoimmune diseases, where the immune system targets the body's tissues, examining the activity of circulating immune cells and the presence of autoantibodies aids in diagnosing conditions like rheumatoid arthritis, lupus, and multiple sclerosis (Giancetti et al., 2021; Iwata and Tanaka, 2021). Cancer immunodiagnosics uses immune cells, particularly lymphocytes, that play a role in recognizing and destroying cancer cells, and techniques to assess the immune response to cancer are extremely helpful in early diagnosis and monitoring treatment responses (Rochigneux et al., 2020). Abnormalities in immune cell counts or function may indicate hematological disorders, including leukemias and lymphomas, and advanced techniques like flow cytometry assist in characterizing these disorders (Ma and Tangye, 2019). Allergy diagnosis involves the role of circulating immune cells, such as mast cells and eosinophils, in allergic reactions, with blood tests measuring specific IgE antibodies and eosinophil counts contributing to diagnosis (Stone et al., 2010). Monitoring changes in circulating immune cell populations is vital in immunotherapy, providing insights into the effectiveness of treatment, especially in conditions like cancer (Zhang and Zhang, 2020). Studying the response of circulating immune cells to vaccines provides information about the effectiveness of immunization and the development of protective immunity, and the detection of circulating immune cells in clinical diagnosis highlights their importance in understanding and managing a wide range of medical conditions (Chen et al., 2021; Marcos Rubio et al., 2023; G. Yu et al., 2022).

Circulating immune cells have been routinely assessed in the clinic for years through blood tests that measure the total white blood cell count and distinguish various cell types, and changes in their composition and activation state contribute greatly to the identification of infections, immune system disorders, allergic reactions, and certain types of cancer (King et al., 2018; Pollard and Bijker, 2021). Advanced techniques such as flow cytometry and molecular analyzes allow for a more detailed

characterization of immune cell subsets and their functions, contributing to a better understanding of immune system health and dysfunction (Bruss et al., 2022; van Wolfswinkel et al., 2023).

Circulating Stem Cells (CSCs): CSCs are undifferentiated cells found in the bloodstream and can differentiate into various cell types (Abbaszadegan et al., 2017). Their role in diagnosis is notable for their impact on regenerative medicine and disease detection, and the identification of different types of CSCs circulating in the bloodstream provides valuable diagnostic information (Yao, 2016). These cells are of high importance as potential biomarkers for certain diseases, and changes in their levels are known to be indicative of pathological conditions (Bahmad et al., 2020).

The differentiation potential of CSCs makes them relevant to regenerative medicine, where their therapeutic application aims to repair and replace damaged tissues (Barbon et al., 2021). The diagnostic utility of CSCs extends to the identification and monitoring of hematological disorders and cancers, providing valuable information for early diagnosis and treatment planning (Roufosse et al., 2004). Understanding the roles of CSCs in disease contexts contributes to the advancement of personalized medicine, where specific diagnostic and therapeutic strategies can be developed based on specific properties of circulating stem cells (Yang et al., 2015). In summary, CSCs play a crucial role in diagnosis by providing insights into regenerative potential, disease detection, and development of targeted therapeutic approaches.

Circulating Endothelial Cells (CECs): CECs are cells shed from the inner lining of blood vessels entering the bloodstream, and their role in diagnosis is multifaceted and provides important information regarding vascular health and a variety of diseases (Blann et al., 2005). Elevated CEC levels may serve as indicators of endothelial damage or dysfunction, especially in cardiovascular disorders such as atherosclerosis and hypertension, and thus CECs serve as biomarkers that provide diagnostic information about cardiovascular diseases through changes in their levels (Fortini et al., 2021; Heinisch et al., 2022). Techniques like diagnostic imaging, including ultrasound and angiography, enable the visualization of CEC-related abnormalities, assisting in the diagnosis of vascular disorders.

In the context of inflammatory conditions, monitoring the presence and activation status of CECs aids in diagnosing disorders like vasculitis (Farrah et al., 2019). In cancer diagnostics, assessing CEC behavior is crucial for understanding angiogenesis—the formation of new blood vessels to support tumor growth (Pydi and Rentala, 2018). Additionally, CEC analysis contributes to the evaluation of systemic diseases, such as diabetes, by providing diagnostic information about endothelial dysfunction (Foret et al., 2022). Monitoring changes in CEC levels over time can also serve as an indicator of the effectiveness

of therapeutic interventions, helping healthcare professionals assess responses to treatments aimed at improving vascular health (Řádek et al., 2019). In summary, CECs play a vital role in diagnosis, offering valuable information across cardiovascular health, inflammatory conditions, cancer, and systemic diseases.

2.2. Circulating Sub-Cellular Bio-particles

Circulating sub-cellular bio-particles, such as circulating cell-free DNA (cfDNA), microRNAs (miRNAs), circulating platelets and platelet-derived micro-particles (PMPs), exosomes, hormones, and proteins and/or protein fragments, are vital for disease detection, genetic analysis, and intercellular communication. These particles provide valuable diagnostic information and are essential for personalized medicine and therapeutic monitoring. Figure 2 illustrates the circulating sub-cellular bio-particles found in the bloodstream.

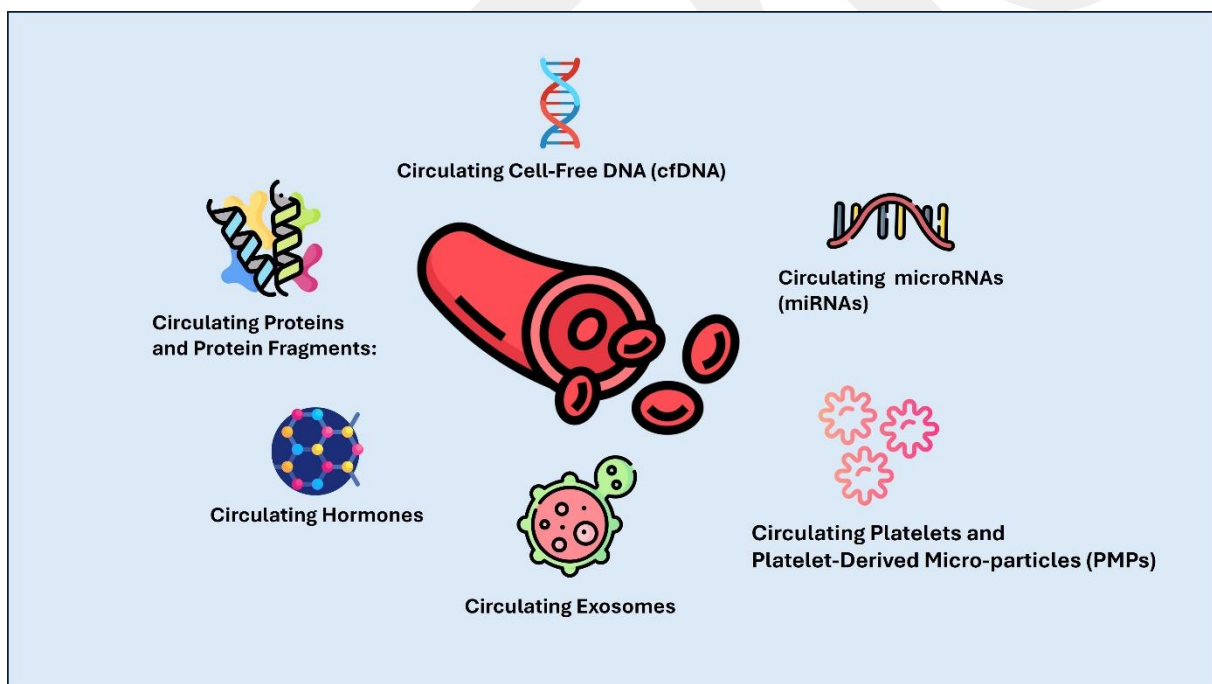


Figure 2. Circulating sub-cellular bio-particles found in the blood stream.

Cell-Free DNA (cfDNA)

cfDNA is a remarkable component of our biological landscape and these small DNA fragments, released into the bloodstream from various cells, provide a unique opportunity to non-invasively study our

genetic makeup, health, and diseases (Bronkhorst et al., 2022). cfDNA is composed of short DNA fragments (usually 150-200 base pairs) released by cell apoptosis, necrosis, or active secretion thence it reflects the genetic profile of the cells from which it originates, offering a dynamic snapshot of the body's genetic information (Su, 2023). It is also found in various bodily fluids, with blood being the most commonly studied (Y. Liu, 2021).

Detection methods of cfDNA play a crucial role in enabling scientists to identify and measure them and thence, several indispensable methods have been identified up to date (Y. Liu, 2021). Quantitative polymerase chain reaction (qPCR), a widely employed molecular technique, serves as a valuable tool for the precise quantification of targeted DNA sequences within cfDNA (Neuberger et al., 2021). This method finds extensive application in the investigation of genetic mutations and gene expression patterns. By leveraging the principles of PCR amplification and fluorescence detection, qPCR enables researchers to accurately measure the abundance of specific DNA sequences present in cfDNA samples (Id et al., 2020). The quantitative nature of this technique allows for the determination of absolute or relative DNA quantities, thereby facilitating the assessment of genetic alterations and gene expression levels in various biological contexts. Consequently, qPCR has emerged as a prominent method in molecular biology research, offering insights into the intricate mechanisms underlying genetic variations and gene regulation (Koval et al., 2023). Besides, NGS has emerged as a powerful tool in the field of genomics, enabling researchers to obtain comprehensive genomic profiles of cfDNA (Jackson et al., 2021). This cutting-edge technology has revolutionized the detection and analysis of various genetic alterations, including mutations, structural variations, and epigenetic modifications. By leveraging NGS, scientists can now delve into the intricate landscape of cfDNA and unravel its hidden secrets (Jackson et al., 2021). The ability to detect mutations at a high resolution has greatly enhanced our understanding of the genetic basis of diseases, such as cancer (Gao et al., 2022). Structural variations, including insertions, deletions, inversions, and translocations, can also be accurately identified, shedding light on the complex genomic rearrangements that underlie various pathological conditions (Shu et al., 2017). Moreover, NGS has opened up new avenues for investigating epigenetic changes in cfDNA (F. Wang et al., 2022). On the other hand, Digital PCR, a cutting-edge molecular technique, has emerged as a powerful tool for the precise quantification of specific DNA sequences (Huerta et al., 2021). This innovative method holds great promise, particularly in the realm of rare mutation detection. By leveraging the principles of partitioning and amplification, digital PCR offers enhanced sensitivity and accuracy compared to traditional PCR methods (Huerta et al., 2021). Lastly, one promising approach to uncovering these alterations is the analysis of cfDNA methylation patterns (Zhao et al., 2023). Methylation, a chemical modification of DNA, plays a crucial role in gene regulation and can be influenced by various environmental factors and disease processes (Kwon et al., 2023). By

examining the methylation patterns of cfDNA, researchers can gain insights into the epigenetic changes associated with different diseases (Moss et al., 2018).

Exosomes

Exosomes, which are diminutive extracellular vesicles (EVs) originating from cells, have emerged as crucial participants in the process of intercellular communication (Kalluri and LeBleu, 2020). Exosomes are lipid bilayer vesicles with a nanoscale size range of around 30-150 nanometers in diameter that are derived from the endosomal compartment within cells (Boriachek et al., 2018). Micro-vesicles are capable of enclosing a diverse range of bioactive molecules, such as proteins, lipids, and nucleic acids, which mirrors the makeup of the cells from which they originate (van Niel et al., 2018). Exosomes are produced by two distinct mechanisms: the ESCRT pathway, which is an endosomal sorting complex required for transport, and ESCRT-independent pathways (Gurung et al., 2021). Once exosomes are released into the extracellular space, they possess the capability of being internalized by adjacent or distant cells. This internalization process enables the cargo to be transferred to the recipient cells, which subsequently affects their function and behavior (Gurung et al., 2021).

Classification of EV subtypes, as outlined in the MISEV2018 recommendations, is based on their size, density, biochemical content, and host cell characteristics, and this approach avoids the use of specific names such as exosomes, microvesicles (MVs), and apoptotic bodies (Jia et al., 2022). EVs can be classed based on their size into two categories: small EVs (sEVs) and medium/large EVs (m/IEVs). The former refers to EVs with a diameter of less than 200 nm, while the latter encompasses EVs with a diameter above 200 nm. The categorization criterion of EV density is generally not considered to be effective due to its limited range of 1.1-1.2 g/ml (Di Bella, 2022). EVs exhibit a wide range of integral membrane tetraspanins such as CD81, CD9, and CD63, as well as scaffold proteins including ERM proteins, syntenin, and Alix (Pegtel and Gould, 2019). Additionally, proteins from the ESCRT machinery are also present in EVs, highlighting their complex biochemical composition (Pegtel and Gould, 2019). The state of the parental cells is also regarded as a crucial determinant in the composition of extracellular vesicles. As an illustration, apoptotic bodies or diminutive apoptotic vesicles, typically ranging in size from approximately 100 to 5,000 nanometers, are discharged during the process of cellular apoptosis (Battistelli and Falcieri, 2020). However, the study of extracellular vesicles (EVs) is complicated by the requirement to purify, measure, and isolate them, and these issues must be adequately addressed before EV-focused approaches can be extensively used (Konoshenko et al., 2018).

Exosomes have emerged as promising tools in the field of diagnostics, with various applications being explored across different medical domains (Konoshenko et al., 2018). Notably, exosomes have shown great potential in the diagnosis of cancer, neurological disorders, infectious diseases, cardiovascular diseases, and liquid biopsies (Konoshenko et al., 2018). In the realm of cancer diagnostics, exosomes have garnered significant attention due to their ability to carry tumor-specific molecules, such as proteins, nucleic acids, and microRNAs (Zhou et al., 2021). In contrast to traditional methods of cancer diagnosis, which often rely on invasive procedures or the detection of tumor-specific biomarkers in the bloodstream, these tiny vesicles can be isolated from bodily fluids such as blood, urine, or saliva, and their cargo can provide valuable insights into the presence, progression, and even the specific type of cancer (Zhou et al., 2021). Thus, by analyzing the molecular composition of exosomes, clinicians can detect cancer at an early stage, monitor treatment response, and even predict patient outcomes (X. Wang et al., 2022). As a result, exosomes provide a comprehensive snapshot of the tumor's molecular landscape, making them valuable tools for cancer diagnosis, prognosis, and treatment.

The role of exosomes in the central nervous system (CNS) has garnered significant attention in the field of neurodegenerative diseases (Liu et al., 2019). These small extracellular vesicles have emerged as potential biomarker carriers that can provide valuable insight into the pathogenesis and progression of debilitating conditions such as Alzheimer's disease, Parkinson's disease, and amyotrophic lateral sclerosis, showing great promise for both patients and healthcare providers (Thompson et al., 2016).

Early detection and accurate monitoring of these diseases are crucial for effective management and the development of targeted therapies. However, current diagnostic methods have attracted increasing interest in the potential of exosomal content analysis as a valuable tool for understanding and diagnosing neurodegenerative conditions such as Alzheimer's and Parkinson's disease (Gao et al., 2021; Vandendriessche et al., 2020). One of the key advantages of analyzing exosomal content is the non-invasive nature of sample collection, and in this context, cerebrospinal fluid (CSF) and blood, both easily accessible biofluids, have been extensively studied for their exosomal cargo (Whitehead et al., 2017; Zanganeh et al., 2023). By isolating and characterizing exosomes from these sources, researchers were able to obtain valuable information about the molecular signatures associated with neurodegenerative diseases (Hornung et al., 2020; Sjoqvist et al., 2020).

An intriguing phenomenon has surfaced within the domain of infectious diseases: the liberation of exosomes during the course of infections (Chaudhari et al., 2022). It has been discovered that these tiny vesicles, which are secreted by numerous cells throughout the body, may contain molecules originating from pathogens. Exosomes comprise origin-specific proteins, lipids, microRNAs, and mRNAs and these components facilitate cell-to-cell communication whether they limit or propagate an infection depends on the pathogen type and the cells that it targets (Ghosh and Ghosh, 2022). Exosomes are involved in the transmission of information between cells, modulation of immune responses, and intercellular transportation during microbial infections (Elsayed et al., 2023). Although they possess the ability to enhance innate and specific immune reactions against invading microorganisms, they also exhibit immunosuppressive properties (Domenis et al., 2019). Potential applications for exosomes include drug and therapy delivery, diagnostic markers, and vaccine therapy. It is imperative to fathom the function of exosomes in viral infections to effectively respond to emerging viral diseases and to grasp viral mechanisms (Fu et al., 2023; Sun and Zhang, 2022).

In recent years, there has been increasing interest in the potential of exosomes derived from cardiac cells or endothelial cells as a valuable source of biomarkers for assessing cardiovascular health (Bei et al., 2017). These small extracellular vesicles, due to their ability to transport a variety of molecules, including proteins, nucleic acids, and lipids, have emerged as promising candidates that can provide valuable insight into the physiological and pathological processes occurring within the cardiovascular system (Sahoo et al., 2021). Exosomes derived from cardiac cells, such as cardiomyocytes, have been found to harbor specific biomarkers that reflect the functional status of the heart (Chistiakov et al., 2016). These biomarkers may include proteins involved in cardiac contractility, ion channel regulation, and cellular stress (R. Zhou et al., 2020). Analysis of exosomal content has emerged as a promising approach in the field of cardiovascular medicine, offering potential information for diagnosing and monitoring critical conditions such as myocardial infarction or heart failure (Emanueli et al., 2016; Tian et al., 2021). By examining the composition of exosomes, researchers can gain valuable information about the underlying mechanisms and molecular signatures associated with cardiovascular diseases (Ma et al., 2021). This noninvasive and easily accessible method holds great promise in improving patient care in the cardiovascular field and advancing our understanding of complex conditions (Chen and Luo, 2022).

The emergence of exosomes as a potential liquid biopsy has revolutionized the field of biomarker research (Boukouris and Mathivanan, 2015). These tiny extracellular vesicles hold immense promise as a non-invasive source of valuable biomarkers (Yu et al., 2021). In other words, one of the key

advantages of exosomal cargo analysis is its non-invasive nature. Traditionally, the diagnosis and monitoring of diseases often require invasive procedures such as biopsies or blood draws. These procedures can be uncomfortable, time-consuming, and carry certain risks (D. Yu et al., 2022). However, with the isolation and analysis of exosomal cargo, clinicians can obtain valuable information about a patient. Exosomes offer diagnostic potential in liquid biopsy for different diseases, including kidney cancer, bladder cancer, prostate cancer, and other types of cancer (Valencia and Montuenga, 2021). Liquid biopsies based on exosomes may change the way many diseases, including cancer, are diagnosed and treated (B. Zhou et al., 2020).

MicroRNAs (miRNAs)

miRNAs, which are small non-coding RNA molecules, have been identified as crucial modulators of gene expression and the deregulation of miRNAs has been implicated in a range of disorders, hence establishing miRNAs as potential candidates for diagnostic applications (Zaheer et al., 2019).

miRNAs are transcribed from the genomic DNA, resulting in the formation of primary miRNAs. These primary miRNAs then undergo a series of processing steps, leading to the production of precursor miRNAs. Finally, the precursor miRNAs are further processed to generate mature miRNAs. The functionality of mature miRNAs is achieved through their interaction with messenger RNA (mRNA), resulting in the inhibition of translation or the facilitation of degradation. This regulatory process effectively controls gene expression (Komatsu et al., 2023). The association between abnormal miRNA expression and various diseases, such as cancer, cardiovascular problems, and neurodegenerative ailments, can be attributed to the participation of miRNAs in essential cellular activities (Ha, 2011).

miRNAs have been identified as significant diagnostic markers in diverse medical fields. Altered expression profiles of miRNAs have a crucial role in facilitating the early diagnosis, classification, and tracking of therapy responses in cancer (Smolarz et al., 2022). They can act as both oncogenes and tumor suppressors, making them potential biomarkers for the diagnosis and prognosis of brain tumors (Balachandran et al., 2020). In prostate cancer (PCa), aberrant miRNA expression has been observed, suggesting their potential as diagnostic and prognostic biomarkers, as well as therapeutic tools (Kanwal et al., 2017). The diagnosis of cardiovascular disorders, such as myocardial infarction and heart failure, can be aided by the identification of particular miRNAs in blood samples (Mir et al., 2021). On the other hand, the analysis of miRNAs in physiological fluids presents valuable insights into the early detection and progression of neurodegenerative disorders (D. You et al., 2022). miRNAs are also involved in the

pathogenesis of infectious disorders, wherein their identification offers valuable diagnostic insights into viral or bacterial infections (Haidar and Langley, 2020). Furthermore, the detection of stable urine miRNAs provides a non-invasive approach to the diagnosis of kidney disorders, urinary tract infections, and colon cancer (Crudele et al., 2021; Lin and Tsai, 2021; Ye et al., 2022).

The aforementioned literature examples demonstrate the significance of miRNAs in gene regulation and disease etiology, rendering them excellent diagnostic instruments in various medical diseases (Galvão-Lima et al., 2021). The utilization of the distinct characteristics exhibited by miRNAs allows for a non-intrusive and highly perceptive method for identifying, categorizing, and tracking diseases at an early stage. This advancement lays the foundation for the implementation of tailored and accurate medical treatments in various clinical environments (Galvão-Lima et al., 2021). Nevertheless, despite the considerable diagnostic potential of miRNAs, certain problems need to be addressed. These challenges encompass the standardization of detection methods, the management of miRNA expression heterogeneity, and the establishment of their specificity towards particular diseases (Jet et al., 2021). The utilization of emerging technology, such as next-generation sequencing and quantitative PCR, holds promise in addressing these obstacles and enhancing the clinical applicability of miRNA-based diagnostic methods (Y. Wu et al., 2020).

Platelets and Platelet-Derived Micro-particles (PMPs)

Platelets, which are diminutive, disc-shaped, non-nucleated cellular entities that derive from megakaryocytes have conventionally been acknowledged for their involvement in the processes of hemostasis and blood coagulation (Aggarwal et al., 2023). Blood clots are formed at the site of vascular injury, playing a vital function in the prevention of bleeding (Moffett and Carroll, 2021). In addition to their primary tasks, platelets can produce micro-particles (PMPs), which contain a cargo of bioactive chemicals (Li and Wang, 2023). PMPs are diminutive vesicles that are released from the active surface of platelets. A diverse array of bioactive substances, such as proteins, lipids, and nucleic acids, are transported by them (Chen et al., 2019).

Platelets and PMPs, demonstrate diagnostic importance in various medical domains. In the context of cancer, there exists a positive correlation between increased levels of PMPs and the advancement of the disease as well as its spread to other parts of the body (Haghbin et al., 2021). The examination of the contents of PMPs, which include both RNA molecules and proteins, holds the potential to diagnose cancer and monitor the response to treatment (Q. Zhang et al., 2023). Platelet activation and levels of

PMPs are crucial factors in the development of thrombosis and atherosclerosis, both of which are significant contributors to cardiovascular illnesses. The assessment of platelet activation and PMP levels serves as a valuable tool in evaluating the likelihood of experiencing cardiovascular events (Baidildinova et al., 2021). Platelets and PMPs play a significant role in the immune response against infectious illnesses (Portier and Campbell, 2021). Consequently, deviations in their numbers or activation patterns have the potential to serve as diagnostic and monitoring indicators. On the other hand, these blood components have been found to play a role in neuro-inflammation and neurodegenerative states associated with neurological disorders, hence providing valuable insights into the pathogenesis of diseases such as Alzheimer's and Parkinson's (Angiulli et al., 2021). Moreover, the identification of aberrant platelet activity and the release of PMPs in coagulation disorders serves to underscore their significance in the diagnosis and management of these pathological illnesses (Di et al., 2023). The significance of platelets and PMPs in comprehending and resolving intricate medical concerns is shown by their diagnostic adaptability (Zahran et al., 2021).

The above-mentioned literature examples demonstrate that platelets and their derivatives, known as PMPs, possess a broader significance beyond their traditional function in hemostasis (Chowdhury and Deb, 2021). They as a diagnostic marker offer useful insights into other disorders such as cancer, cardiovascular illnesses, infectious diseases, neurological disorders, and coagulation disorders presents novel opportunities for comprehending and addressing intricate medical problems (Chowdhury and Deb, 2021). However, despite their potential for diagnosis, some hurdles need to be addressed to fully utilize platelets and PMPs in a clinical setting including the need for standardization of measuring methodologies, a comprehensive understanding of the diverse platelet and PMP populations, and the identification of their specific features concerning different diseases (Hamad et al., 2022; K Sekar et al., 2023; Kopeikina and Ponomarev, 2021). Continuing research endeavors seek to address these obstacles and harness the complete diagnostic potential of platelets and PMPs (C. Zhang et al., 2023).

Circulating Hormones: Circulating hormones are signaling molecules produced by endocrine glands and released into the bloodstream to regulate various physiological processes, and these molecules play important roles in diagnosis and serve as key biomarkers to identify and understand hormonal imbalances and endocrine disorders (Neumann et al., 2019). Hormones play an important role in maintaining homeostasis, and deviations from normal levels often indicate underlying health problems (Myers et al., 2021). Diagnostic tests that measure hormone concentrations, such as blood tests and hormonal stimulation tests, are crucial in evaluating and monitoring conditions such as diabetes, thyroid disorders, and adrenal dysfunction (Winter et al., 2020). Analysis of circulating hormones is also

particularly important in reproductive health by providing crucial information about fertility problems, menstrual irregularities and pregnancy-related conditions (Kowal, 2023). A comprehensive understanding of circulating hormones and their role in diagnosis is essential for tailoring effective treatment plans and optimizing patient care in various endocrine-related disorders, and hormones have an important place in both conventional diagnostics and next-generation diagnostic studies (Bidlemaier et al., 2022).

Circulating Proteins and Protein Fragments: Circulating proteins and protein fragments play crucial roles in diagnosis, exemplifying their significance across various medical contexts (Moaddel et al., 2021). Various circulating proteins and protein fragments, such as cardiac troponins, C-reactive protein (CRP), and brain natriuretic peptide (BNP), are used as biomarkers for cardiovascular disease, inflammation, and organ damage (Vuillaume et al., 2022). Cardiac troponins, specific protein fragments released during heart muscle damage, serve as pivotal markers for diagnosing myocardial infarction (Lackner, 2022). Cancer diagnostics leverage specific circulating proteins like prostate-specific antigen (PSA) for prostate cancer and CA-125 for ovarian cancer, aiding in early detection and treatment monitoring (Sturgeon et al., 2008). In infectious diseases, antibodies or proteins produced in response to pathogens are indispensable for diagnosis; for example, the detection of HIV-specific proteins helps diagnose HIV infection (Silverman and Green, 2021). As another example, protein biomarkers such as anti-nuclear antibodies (ANA) are used to diagnose the autoimmune disorder lupus (Duraisamy, 2021). Another example involves assessing proteins such as hemoglobin A1c to monitor long-term glucose control in metabolic conditions such as diabetes (Suastika, 2021). The application of immunoassays, a common technique for protein analysis, allows for precise and sensitive detection of these circulating biomolecules (Birhanu, 2023). The evolving field of proteomics continues to unveil novel protein markers, contributing to the advancement of personalized medicine by tailoring diagnostic and treatment approaches based on individual protein profiles (Su et al., 2021). In essence, circulating proteins and protein fragments serve as invaluable diagnostic tools, offering insights into diverse health conditions and shaping the landscape of modern medical diagnostics.

3. Applications in Disease Diagnosis

Circulating cells and bioparticles present an exciting opportunity for non-invasive diagnosis of diseases and monitoring. These biomarkers, which are released into physiological fluids such as blood, urine, and cerebrospinal fluid, provide important information on the underlying pathophysiology of a variety of illnesses, including cancer, infectious diseases, autoimmune disorders, and cardiovascular ailments. Leveraging the diagnostic capability of circulating cells and bioparticles offers significant promise for

enhancing personalized medicine by allowing for early identification, precise prognosis, and targeted treatment methods. A summary of research on circulating objects has been attempted to provide a brief overview of the bioparticle identified, the detection method used, the performance of the diagnostic device developed, and the type of detection used. Table 1 presents a current overview of research on different types of cells found in the bloodstream, whereas Table 2 presents a current overview of research on sub-cellular bioparticles.

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Table 1. Recent literature examples highlighting the use of circulating cells in the bloodstream for diagnostic purposes.

Types of Circulating Cells	Reference	Detected Cells	Type of Detection	Performance	Diagnostic Tool
CTCs	(Zhu et al., 2022)	MCF-7 human breast cancer cells	intravenous Surface Plasmon Resonance (SPR) fiber probe functionalized with an EpCAM antibody	Limit of Detection: ~1.4 cells/ μ L Detection Time: 15 m Recovery Rate: NS	a sensor for real-time detection of CTCs in bloodstreams
	(Lv et al., 2024)	A549 human non-small cell lung cancer cells	a surface-enhanced Raman spectroscopy (SERS) method based on aptamer-SERS bio-probe recognition coupled with micropore membrane filtration	Limit of Detection: 100 cells/mL Detection Time: 10 s Recovery Rate: 92% – 96%	a sensor for the detection of CTCs at single cell level
	(Jiang et al., 2023)	SMMC-7721 human liver cancer cell	a microfluidic microarray technology by utilizing dielectrophoretic force	Limit of Detection: 600 cells/mL Detection Time: 1 h Recovery Rate: 94.3%	a sensor separating and enriching CTCs

<p>(Ye et al., 2023)</p>	<p>SMMC-7721 human liver cancer cell, HepG2 human liver carcinoma cell, HT-29 human colorectal carcinoma cells, MCF-7 human breast cancer cell, MDA-MB-231 human breast cancer cell, HeLa human cervical carcinoma cells</p>	<p>a BSA-coated adhesive microplate coupled with a low-speed centrifuge</p>	<p>Limit of Detection: 600 cells/mL Detection Time: 20 m Recovery Rate: 70.7–86.6%</p>	<p>a rapid and low-cost method for CTC isolation</p>
<p>(Peng et al., 2022)</p>	<p>MCF-7 human breast cancer cell</p>	<p>a dual-recognition-controlled electrochemical biosensor</p>	<p>Limit of Detection: 3 cells/mL Detection Time: ~2h Recovery Rate: NS</p>	<p>a selective, strongly stable, and anti-interference electrochemical biosensor for cancer diagnosis and personalized medicine</p>

CICs	(Diks et al., 2021)	antigen-specific serum immunoglobulins (Ag-specific Ig) in blood samples of vaccinated humans	high-throughput multicolor flow cytometry	NS	a flow cytometry-based analysis of multiple circulating immune subsets in blood samples simultaneously
	(Jeong et al., 2023)	Egg Ara h 2 protein in egg white	a biosensing platform was constructed by immobilizing nanovesicles containing anti-immunoglobulin E (anti-IgE) antibody receptors derived from RBL-2H3 immune cells onto a carbon nanotube-based transistor	Limit of Detection: 0.1 fM Response Time: tens of seconds	a biosensing platform containing nanovesicles fixed on a carbon nanotube field-effect transistor (CNT-FET) for the detection of specific matters in the field of food and pharmacology
	(Park et al., 2020)	citrullinated histone H3 (CitH3) released by neutrophils in a sepsis mouse model	a label-free detection with a plasmophotonic nanostructure device	Limit of Detection: 56 fM in 2.5 μ L Response Time: 20 min	a sensitive biosensor for detecting CitH3 can avert life-threatening septic shock in infections

CSCs	(Chenaghlou et al., 2021)	CD 133 antibody as cancer stem cell biomarker in human serum	gold nanostars (AuNSs)-enhanced electrochemiluminescence (ECL) emission from graphitic carbon nitride nanosheets (g-CN nanosheets)	Limit of Detection: 0.257 ng/mL Recovery Rate: 92.16% to 109.64%	an easy-to-use, label-free ECL immunosensor that can detect and quantify various antibodies with pinpoint accuracy
	(Cho et al., 2018)	Both CCSCs and several major breast cancer CTC subtypes using five different surface markers: CD133, EpCAM, EGFR, HER2, and MUC1	nanoparticle-mediated Raman-Active Nanoprobe (RAN)-based chip platform	Efficiency: 90% Accuracy: 93%	a RAN-based Raman imaging sensor for monitoring cancer metastasis and relapse via CCSC detection
	(Gening et al., 2021)	Stem like antibodies stem-like CD44, CD133 and ALDH	Flow cytometry	NS	CD133+ALDH+ circulating stem-like tumor cells have valuable prognostic potential in ovarian cancer among the examined phenotypes

CECs	(Debrece ni et al., 2020)	plasma serine proteases MASP-2, C1r, and kallikrein	a label-free resonant waveguide grating (RWG) based optical sensing	NS	a powerful instrument for identifying new biological pathways and efficiently evaluating different chemicals on endothelial cells
	(Guervilly et al., 2020)	circulating endothelial cells in blood samples of human	immunomagnetic separation with beads coated with CD146 and counting by fluorescence microscopy after labeling	Limit of Detection: 6 CEC/mL	a diagnostic tool providing evidence that endothelial damage is an important marker of COVID-19

Table 2. Recent literature examples highlighting the use of circulating sub-cellular bio-particles in the bloodstream for diagnostic purposes.

Types of Circulating Bio-particles	Reference	Detected Bio-particles	Type of Detection	Performance	Diagnostic Tool
cfDNA	(Lee et al., 2023)	Human urine sample-derived DNA	DNA Extraction and Targeted Deep Sequencing of DNA	Specificity: 100 % Sensitivity: 83.7 %	a biosensor for early and precise detection of bladder cancer tumor DNA in the urine of hematuria patients
	(Faria and Zucolotto, 2019)	Primers and capture probe DNA sequence of the Zika virus	an impedimetric electrochemical DNA biosensor	Limit of Detection: 25.0 ± 1.7 nM Response Time: 1.5h	a bio sensor for the label-free detection of Zika virus
	(Jafari-Kashi et al., 2022)	Cytokeratin 19 fragment 21–1 (CYFRA21-1) DNA	an electrochemical DNA-biosensor related to the signal of guanine oxidation	Limit of Detection: 2.14 fM Response Time: immobilization 3h hybridization 5h	a label-free, fast, low-cost, and efficient electrochemical biosensor for the detection of lung cancer
	(Bonini et al., 2021)	a CRISPR/Cas12a system	an electrochemical impedance spectroscopy (EIS) based label-free DNA-biosensor	Limit of Detection: 3 nM Response Time: 1.5h	a biosensing assay for the detection of <i>Escherichia coli</i> and <i>Staphylococcus aureus</i> , two bacterial species commonly associated with blood stream infections

Exosomes	(Su et al., 2022)	PC-3 human prostate cancer cell line-derived exosomes	an electrochemically improved sandwich-type immunoassay	Limit of Detection: 7.23 ng in 5 μ L Response Time: Less than 2h	a rapid, sensitive, and portable biosensor in combination with smartphones and screen-printed electrodes for the quantification of exosomes in serum samples and the cell culture supernatant
	(M. Zhang et al., 2023)	SK-BR-3 breast cancer cell line-derived exosomes	a one-step multiplex analysis electrochemical aptasensor based on a multi-probe recognition	Limit of Detection: 3.4×10^3 particles/mL Response Time: NS	a sensor that is simple to operate, has good stability, strong specificity and works with high sensitivity, and can distinguish breast cancer exosomes from other exosomes
	(Song et al., 2023)	exomiRNAs in clinical serum samples of patients diagnosed with Alzheimer's Disease	a novel plasmonic nanoarchitecture based on the DNA-directed crystallization technique and develop a highly sensitive plasmonic biosensor	Limit of Detection: attomolar-level Response Time: NS	a biosensor classifies individuals into Alzheimer's disease, mild cognitive impairment patients, and healthy controls through profiling and quantifying their serum exosomal miRNAs
	(Q. You et al., 2022)	exosomes extracted from the A549 human pulmonary epithelial cell line	a novel electrochemical biosensor based on hierarchical Au nanoarray-modified MXene membranes for sensitive detection of exosomes	Limit of Detection: 58 particles/ μ L Response Time: 10-30 min	a biosensor implementing 2D membrane platforms for reliable biomarker diagnosis and monitoring applications

	(Deng et al., 2024)	exosomes extracted A2780 human ovarian carcinoma cell line and simian virus 40-transformed human ovarian surface epithelial cells (IOSE-80)	an enzyme-free electrochemical biosensor by constructing an entropy-driven autocatalytic DNA circuit (EADC)	Limit of Detection: 30 particles/ μ L Response Time: 90 min	a biosensor with exceptional performance in distinguishing the clinical diagnosis of ovarian cancer patients exosomes from healthy samples
miRNAs	(Yu et al., 2024)	neutrophil-based exosome (Neu-Exo) derived miRNA in the clinical blood sample	droplet digital PCR (ddPCR) based miRNA detection	Limit of Detection: 7.8×10^5 particles/mL Recovery Rate: 81 %	a biosensor based on the novel and accurate Neu-Exo miRNAs biomarkers for the detection of gastric cancer diagnosis by ddPCR technology
	(Yan et al., 2023)	miRNA-126 bladder cancer marker in clinical urine samples	enzyme-free and nucleic acid-based catalytic hairpin assembly and cascade recognition reactions strategy with fluorescence signal	Limit of Detection: 0.25 aM Specificity: 100 % Sensitivity: 90 %	a biosensor for clinical cancer diagnosis based on urinary miRNA detection and for achieving tumor point-of-care testing
	(Wu et al., 2021)	miR-1, miR-133a, miR-208a, and miR-499 heart-associated markers in clinical serum samples of AMI patients	the polymerization-induced fluorescence-off (PIFO) signal amplification technique on a microfluidic chip equipped with a fluorescence sensor	Limit of Detection: 3 miRNAs in 1 μ L serum sample Response Time: 45 min	a rapid and multiparametric microfluidic biosensor capable of discovering miRNA signatures with high specificity

	(Lim et al., 2022)	miR-574-5p in blood samples of Alzheimer's Disease model mice	hydrogel-based sensor containing catalytic hairpin assembly (CHA) reaction-based probes, leading to fluorescence signal amplification without enzymes and temperature changes	Limit of Detection: 1.29 pM Response Time: 2 h	a liquid biopsy platform capable of early diagnosis of Alzheimer's Disease as POCT
Platelets and PMPs	(Rahil et al., 2022)	PDGF from human platelets	a colorimetric biosensor to diagnose PDGF by using gold nanoparticles (AuNPs) and PDGF binding aptamers	Limit of Detection: 0.01 µg/ml Response Time: within few minutes	a naked-eye PDGF detection sensor for conceivable ovarian cancer screening
	(W. Wang et al., 2022)	megakaryocyte-(MKMPs) and platelet-derived MPs (PMPs) in the serum of immune thrombocytopenia patients (ITP)	flow cytometry	Sensitivity: 73.1% Specificity: 77.3%	flow cytometry-based analysis of MKMPs and PMPs as potential biomarkers for ITP diagnosis
	(Rejeeth et al., 2022)	platelet-derived growth factor receptor (PDGFR)	electrochemical carbon nanotube (MWCNT)-coated indium tin oxide (ITO) based sensor	Limit of Detection: 1.5 pg/mL Repeatability: 2.51 % Stability: 30 days	a novel, cost-effective biosensor for detecting PDGFR based on a charge-based affinity bait molecule

Circulating Hormones	(Sankiewicz et al., 2023)	follicle-stimulating hormone (FSH) in human plasma	a specific array surface plasmon resonance imaging (SPRi)	Limit of Detection: 0.905 ng/mL Recovery Ratio: 94% to 108%	a biosensor using the promising array SPRi technique for the detection of FSH by liquid biopsy in body fluids such as blood plasma without any signal amplification
	(Baluta et al., 2023)	free triiodothyronine (fT3) in synthetic human serum	biorecognition element integrated electrochemical biosensor	Limit of Detection: 27 nM Recovery Ratio: 96.5% Stability: over 30 days	a biosensor with high potential for the early detection of diseases related to fT3 level disorders such as hyperthyroidism, working with a simple technique (DPV) that can also be potentially used in mobile sensor devices
	(Kumari and Nayak, 2021)	human progesterone hormone	label-free electrochemical immunosensor	Limit of Detection: 0.15 pM Recovery Rate: 88%-104%	an immunosensor detecting PGN hormone proposed for environmental and clinical research, and disease diagnostics
Circulating Proteins	(J. Chen et al., 2023)	cardiac Troponin I (cTnI)	Recycling Walker-cTnI-Glucometer (RWCG)	Limit of Detection: 0.01 ng/mL Recovery Ratio: 97.55 ~104.3% Response Time: 8 sec	an innovative, portable, and real-time biosensor that combines a personal glucometer and a DNA walker for the prompt and efficient detection of acute myocardial infarction

	(Ghedir et al., 2023)	N-terminal Natriuretic Peptide (NT-proBNP) in human artificial saliva	electrochemical based immunobiosensor	Limit of Detection: 0.2 pg/mL Sensitivity: 0.03-0.9 pg/mL Response Time: simultaneously	a highly sensitive multiplexed immunosensor for simultaneous NT-proBNP and cortisol detection in human artificial saliva for cardiovascular disease detection
	(Liu et al., 2021)	C-reactive protein	A capacitive biosensor based on mass-producible three-dimensional (3D) interdigital electrode arrays	Limit of Detection: 0.01 ng/mL Sensitivity: 1 pg/mL	a biosensor offers the prospect of clinical use for cardiovascular and periodontal disease-associated biomarker identification

4. Methodologies used to Identify Circulating Cells and Sub-Cellular Bio-particles

Identification of circulating cells and subcellular bioparticles relies on the use of advanced methodologies, each individually or in combination, designed to detect specific entities in body fluids (Soda et al., 2022). Flow cytometry is widely used due to its ability to analyze the physical and chemical properties of cells or particles in a liquid as they pass through at least one laser; this makes it particularly useful in identifying CTCs and immune cells (Muchlińska et al., 2022). Immuno-magnetic separation, on the other hand, uses antibodies conjugated to magnetic beads to selectively capture and isolate specific cell types or bioparticles, increasing purity and yield (Gheorghiu, 2020). In recent years, microfluidic devices enable precise manipulation and sorting of cells and particles in microscale channels, enabling high-throughput analysis and isolation based on size, shape, and other physical properties (Cha et al., 2022). Size-based filtration is another technique where cells and particles are separated through membranes with specific pore sizes, allowing enrichment of target populations (Chen et al., 2022). On the other hand, the Epithelial Cell Adhesion Molecule (EpCAM) enrichment technique amplifies EpCAM expression on epithelial cells, including many CTCs, to isolate these cells using antibody-coated surfaces (Jiang et al., 2024). Nucleic acid-based approaches such as quantitative PCR (qPCR) and next-generation sequencing (NGS) enable high-throughput analysis and quantification of cell-free DNA (cfDNA) and RNA (cfRNA), enabling the identification of genetic mutations, expression profiles, and other molecular alterations (Choudhury et al., 2022). Fluorescent in situ hybridization technique (FISH) is used to detect and localize specific DNA sequences on chromosomes and is often applied to identify genetic abnormalities in CTCs (She, 2023). On the other hand, microscopy and imaging techniques, including confocal and fluorescence microscopy, allow visualization and characterization of cells and subcellular bioparticles at high resolution (Hickey et al., 2021). Label-free technologies, such as recent sensors based on electrical impedance, measure changes in electrical properties as cells or particles pass through a sensing region and offer a non-invasive method to identify and quantify bioparticles (Yang et al., 2023). Lastly, the mass spectrometry identifies and quantifies proteins and metabolites, providing detailed molecular information without the need for labels (Stincone et al., 2024). Each technique mentioned here has advantages and disadvantages compared to each other, and these are presented in Table 3. Therefore, by employing these approaches together, the ability to identify and analyze circulating cells and subcellular bioparticles is enhanced, resulting in improved accuracy and precision. This, in turn, leads to advancements in diagnostic and therapeutic applications within the fields of healthcare and medical research.

Table 3. Advantages and disadvantages of identification techniques for circulating cells and sub-cellular bio-particles.

Identification Technique	Advantages	Disadvantages	Reference
Flow Cytometry	High-throughput, multi-parameter analysis, rapid results, single-cell analysis	Requires fluorescent labeling, may miss rare cells, potential for data complexity	(Muchlińska et al., 2022)
Immune-Magnetic Separation	High specificity and purity, easy to use, suitable for various sample types	Can be expensive, limited to known markers, potential loss of rare cells	(Gheorghiu, 2020)
Microfluidic Devices	Precise control, high-throughput, minimal sample requirements, low reagent use	Complex fabrication, potential for clogging, requires specialized equipment	(Cha et al., 2022)
Size Based Filtration	Simple, cost-effective, rapid processing, scalable	Limited specificity, potential loss of smaller particles, may require multiple filters	(Chen et al., 2022)
EpCAM Enrichment	Effective for epithelial cells, widely used in CTC detection, high purity	Not applicable to non-epithelial cells, potential false negatives, depends on EpCAM expression	(Jiang et al., 2024)
Quantitative PCR (qPCR)	High sensitivity, quantification of nucleic acids, rapid, cost-effective	Limited to known sequences, requires optimization, potential for contamination	(Peng et al., 2023)

Next-Generation Sequencing (NGS)	Comprehensive genetic analysis, high throughput, detects novel mutations	Expensive, requires extensive data analysis, long turnaround time	(Tébar-Martinez et al., 2023)
Fluorescence In Situ Hybridization (FISH)	High specificity, detects genetic abnormalities, direct visualization	Time-consuming, requires fluorescent probes, limited to known sequences	(W. Li et al., 2024)
Microscopy and Imaging	High resolution, visual characterization, spatial context, real-time monitoring	Labor-intensive, may require extensive sample preparation, operator skill required	(Hickey et al., 2021)
Electrical Impedance-Based Sensors	Label-free, non-invasive, real-time monitoring, minimal sample preparation	Limited sensitivity, may require complex instrumentation, potential for low throughput	(Yang et al., 2023)
Mass Spectrometry	Label-free, high sensitivity, detailed molecular information, high specificity	Expensive equipment, requires skilled operation, complex data analysis	(Van Acker et al., 2023)

5. Discussion

The concept of biomarkers can be traced back to ancient civilizations, where physicians observed changes in bodily fluids and tissues as indicators of disease. For example, the presence of blood in urine (hematuria) was recognized as a sign of kidney or bladder disease in ancient Egypt and Greece (Bax et al., 2019). The field of clinical chemistry emerged in the 19th century, with the advent of chemical tests to analyze bodily fluids. In 1848, the German chemist Friedrich Julius Otto introduced the concept of urinalysis for diagnosing diseases based on changes in urine composition (Wilkinson, 2002). In the late 19th and early 20th centuries, scientists began isolating and characterizing hormones, signaling molecules that regulate physiological processes. The discovery of insulin by Frederick Banting and Charles Best in 1921 revolutionized the diagnosis and treatment of diabetes mellitus (Vecchio et al., 2018). The mid-20th century saw significant advances in immunology, leading to the identification of antibodies and antigens as biomarkers of immune responses. The development of serological tests, such as enzyme-linked immunosorbent assays (ELISA), enabled the detection of specific biomolecules in blood and other bodily fluids (Bagut et al., 2013). The advent of molecular biology techniques in the latter half of the 20th century revolutionized biomarker discovery and analysis. Polymerase chain reaction (PCR), introduced by Kary Mullis in 1983, allowed for the amplification and detection of nucleic acids, facilitating the identification of genetic biomarkers (Deepak et al., 2007). The completion of the Human Genome Project in 2003 and subsequent advancements in genomics and proteomics accelerated biomarker research. High-throughput sequencing technologies and mass spectrometry enabled comprehensive profiling of genetic and protein biomarkers associated with various diseases (Hood and Rowen, 2013). In the 21st century, the concept of personalized medicine emerged, emphasizing the use of biomarkers to tailor medical treatment to individual patients. Biomarker-driven approaches have transformed cancer diagnosis and treatment, guiding targeted therapies and immunotherapies based on molecular profiling of tumors (Wang and Wang, 2023). Recent years have witnessed the integration of multiple omics technologies, including genomics, transcriptomics, proteomics, metabolomics, and microbiomics, to comprehensively study biomolecular signatures associated with health and disease. This systems biology approach holds promise for identifying multi-dimensional biomarker panels for precision medicine (C. Chen et al., 2023).

Overall, the history of biomarkers reflects a continuum of scientific discoveries and technological advances that have driven progress in disease diagnosis, prognosis, and therapeutic intervention. As our understanding of biomolecular pathways expands, the identification and validation of biomarkers will continue to play an important role in the development of personalized healthcare. Looking at the path from past to present, it can be seen that biomarkers used for diagnostic purposes have shifted

from cellular levels to subcellular particles over time. Undoubtedly, as the structure to be detected becomes smaller in size and its number decreases, detection materials have started to be forced to be smaller in size, more sensitive, and more effective, thanks to the developing nanotechnology.

Circulating cells, such as CTCs and CICs, are often easier to detect due to their relatively larger size compared to subcellular particles like cfDNA and miRNAs (Słomka et al., 2022). CTCs are pivotal for cancer detection and monitoring metastasis but are rare in the bloodstream, making them challenging to capture and analyze (Bailey and Martin, 2019). In contrast, cfDNA and miRNAs, although present in smaller quantities, are more frequently found and can provide a broader snapshot of the genetic landscape of a disease (Cai et al., 2015). The specificity of these biomarkers varies. For example, CTCs offer high specificity for detecting and monitoring cancer, while cfDNA and miRNAs can indicate a wide range of diseases, including cancer, cardiovascular conditions, and infectious diseases (Adhit et al., 2023). This versatility makes cfDNA and miRNAs valuable for early diagnosis and personalized treatment strategies. Recently, the use of exosomes, which carry proteins, lipids, and nucleic acids, has become a popular approach as they are emerging as potent biomarkers due to their ability to reflect the molecular state of their cells of origin (Cheng et al., 2022). Combining the use of circulating cells and subcellular bio-particles can enhance diagnostic accuracy (Keup et al., 2022). For example, integrating the detection of CTCs with cfDNA analysis can provide comprehensive insights into tumor heterogeneity and resistance mechanisms. Such a multi-faceted approach leverages the strengths of both types of biomarkers, offering a more robust and detailed understanding of disease states (Bamodu et al., 2023).

On the other hand, from visual inspection to advanced microscopy techniques, the detection of cells and sub-cellular bioparticles progresses from macroscopic to microscopic spectrum, allowing researchers to explore biological systems with increasing resolution and precision (Y. Wang et al., 2022). This spectrum of detection methods includes visual inspection for color changes or precipitate formation (Chandran et al., 2022), chemical assays such as spectrophotometric and enzymatic assays for quantitative measurements (Hoekstra and Smith, 2023), electrophoresis and chromatography for separation based on charge and affinity (Coskun, 2016), mass spectrometry for precise identification and quantification (Chu et al., 2023), fluorescence and luminescence techniques for light emission upon excitation (Deshpande and Kanungo, 2023), advanced imaging methods like confocal and super-resolution microscopy for visualizing biomolecules at cellular and subcellular levels (Bond et al., 2022), atomic force microscopy for nanoscale imaging with atomic precision (Canepa et al., 2024), and cryo-

electron microscopy for high-resolution structural analysis of biomolecules (Chari and Stark, 2023). Each technique offers unique advantages in terms of sensitivity, resolution, and applicability, providing researchers with a comprehensive toolkit for studying cellular and biomolecular interactions in biological systems.

6. Available Technologies, Current Challenges and Future Perspectives

In recent years, the clinical application of circulating cells and sub-cellular bio-particles has seen significant advancements, driven by the development of commercial tools and methodologies. One of the most widely used clinical tools is the CellSearch® system, which is FDA-approved for detecting and enumerating circulating tumor cells (CTCs) in patients with metastatic breast, prostate, and colorectal cancers (Vasseur et al., 2021). This system utilizes immuno-magnetic separation and fluorescence-based detection, providing a reliable method for monitoring disease progression and therapeutic response. Additionally, liquid biopsy platforms such as Guardant360® and FoundationOne® Liquid offer comprehensive genomic profiling of cell-free DNA (cfDNA), enabling the detection of actionable mutations and guiding personalized treatment strategies in oncology (Venetis et al., 2023).

Microfluidic devices have also gained traction in clinical settings due to their ability to isolate and analyze rare cells and bio-particles with high precision. Companies like BioFluidica and Vortex Biosciences have developed commercial microfluidic platforms that facilitate the isolation of CTCs and other circulating biomarkers, providing critical insights into tumor heterogeneity and metastatic potential (Sung et al., 2022; Witek et al., 2023). Furthermore, advanced molecular techniques such as quantitative PCR (qPCR) and next-generation sequencing (NGS) are now routinely employed in clinical laboratories for the analysis of cfDNA and circulating microRNAs (miRNAs), offering high sensitivity and specificity in detecting genetic alterations associated with various diseases. Commercially available assays from companies like Qiagen and Thermo Fisher Scientific have streamlined these processes, making them accessible for widespread clinical use (Sorbini et al., 2024).

These commercially available tools and techniques have revolutionized the clinical landscape by enabling minimally invasive, real-time monitoring of disease states, thus improving patient management and outcomes. Their integration into clinical practice underscores the importance of continued innovation and validation to further enhance their diagnostic utility (Yu et al., 2023). However, despite significant advancements, several challenges persist, necessitating ongoing research

and innovation to overcome current limitations and harness the full potential of these biomarkers. Identifying current challenges can be categorized into four main areas: standardization and reproducibility, sensitivity and specificity, sample heterogeneity, and clinical validation and translation (G. Liu, 2021). The lack of standardized protocols for sample collection, processing, and analysis hinders the reproducibility and comparability of results across different laboratories and platforms. Variability in methodologies and quality control measures poses challenges for establishing robust diagnostic assays (Brancato et al., 2024). Achieving adequate sensitivity and specificity is crucial for the accurate detection and characterization of cells and sub-cellular bioparticles. Current techniques may lack the sensitivity required to detect rare or low-abundance biomarkers, leading to false-negative results or inadequate sensitivity for early disease detection (Gao et al., 2023). Biological variability within and between patients introduces challenges in interpreting biomarker data. Tumor heterogeneity, cellular plasticity, and the dynamic nature of circulating bioparticles pose challenges to capturing the full spectrum of disease characteristics and monitoring treatment response accurately (Gilson et al., 2022). Despite promising preclinical data, the clinical utility of biomarkers derived from cells and sub-cellular bioparticles requires rigorous validation in large, diverse patient cohorts. Demonstrating the clinical relevance, prognostic value, and predictive power of these biomarkers is essential for their successful translation into routine clinical practice (Rusling and Forster, 2021).

Future possibilities may be classified into four categories: technological advancements, multi-omics approaches, liquid biopsy in clinical practice, biomarker discovery and validation (Armakolas et al., 2023). Continued innovation in technology, including improvements in sequencing technologies, microfluidics, and imaging modalities, will enhance the sensitivity, specificity, and throughput of diagnostic assays (Galan et al., 2020). Integration of artificial intelligence and machine learning algorithms for data analysis and interpretation will further enhance the accuracy and efficiency of biomarker detection (Wang et al., 2024). Integration of multi-omics data, including genomics, transcriptomics, proteomics, and metabolomics, will provide comprehensive insights into disease biology and facilitate the identification of robust biomarker signatures. Combining multiple biomarkers from different omics layers can enhance diagnostic accuracy and enable personalized treatment strategies (Wekesa and Kimwele, 2023). Liquid biopsy-based approaches, including the analysis of circulating cells and sub-cellular bioparticles, are poised to become routine clinical tools for cancer diagnosis, monitoring treatment response, and detecting minimal residual disease (Armakolas et al., 2023). Implementation of liquid biopsy-based assays in clinical trials and real-world settings will validate their clinical utility and pave the way for widespread adoption in routine patient care (Sisodiya et al., 2023). Collaborative efforts among researchers, clinicians, and industry partners are essential for

accelerating biomarker discovery and validation (Ou et al., 2021). Large-scale prospective studies, biomarker discovery consortia, and data-sharing initiatives will facilitate the identification of novel biomarkers and validation of existing candidates across diverse patient populations and disease contexts (Bose et al., 2022).

The diagnostic use of cells and sub-cellular bioparticles holds great promise for transforming healthcare by providing non-invasive, real-time insights into disease biology and treatment response. Addressing current challenges through technological innovation, multi-omics approaches, and collaborative research efforts will facilitate the successful translation of these biomarkers into routine clinical practice, ultimately improving patient outcomes and advancing precision medicine.

7. Conclusion

This review provides a structured approach to covering various aspects of the use of circulating cellular and sub-cellular bioparticles for diagnostic purposes, from their types and applications to the techniques involved, current challenges, and future directions in the field. In conclusion, the diagnostic application of circulating cells and bioparticles represents a paradigm shift in healthcare, offering non-invasive and real-time insights into disease biology. By harnessing the diagnostic potential of these biomarkers, clinicians can improve the accuracy and timeliness of disease diagnosis, monitor treatment response, and tailor therapeutic interventions to individual patients. The versatility of circulating cells and bioparticles extends across a wide range of medical specialties, from oncology to infectious diseases, autoimmune disorders, and cardiovascular conditions. As technology continues to advance and our understanding of biomolecular pathways deepens, the diagnostic landscape will evolve, enabling the development of more sensitive, specific, and comprehensive assays. Ultimately, the integration of circulating cells and bioparticles into routine clinical practice holds tremendous promise for advancing precision medicine and improving patient outcomes.

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Declaration of Competing Interest

The corresponding author declares that this manuscript is original, has not been published before, and is not currently being considered for publication elsewhere. There is no conflict of interest.

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