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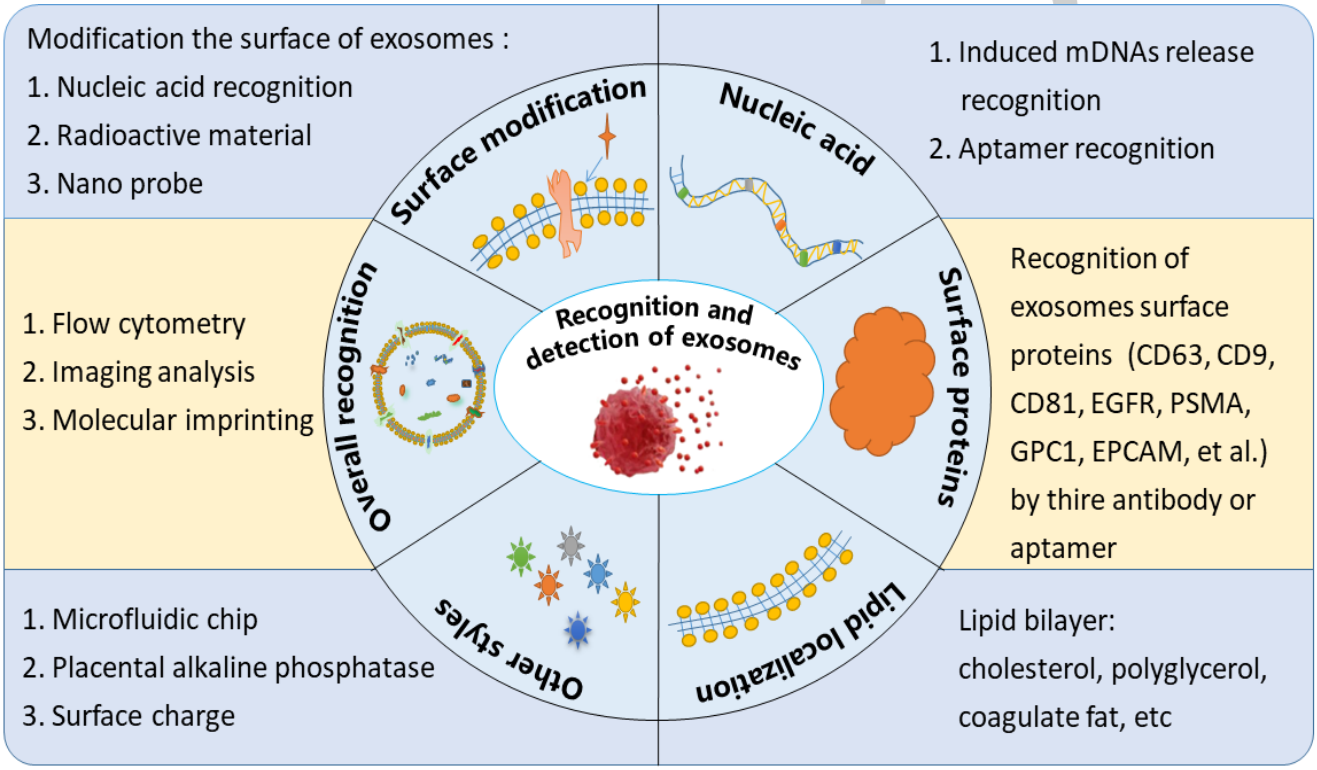
Recent Progress on the Isolation and Detection Methods of Exosomes

Zejun Jiang, [a] Guangyan Liu, [a, b] Jianping Li*[a, b]



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Abstract: Exosomes are known as one of extracellular vesicles, which are found in various body fluids and released by cells. As transport carrier, exosomes participate actively in intercellular communication and reflect its characteristics uniquely to the origin cells. Due to its unique biological physical properties and physiological functions, exosomes are considered to be one of best biomarkers of cancer diagnosis. At the same time, exosomes are potential therapeutic targets and drug delivery carrier. Therefore, the characteristics, functions and their analytical methods of exosomes have increasingly attracted wide attention among scientists. In this review, the recent research progress on the basic characteristics and functional applications of exosomes are summarized. Furthermore and importantly, this review focuses on the recent advance in the purification and test methods of exosomes in recent years. Finally, the issues existed in exosomes detection are presented. Based on newly discovered characteristic of exosomes, the opportunity and challenge for the future research of the purification and quantitative detection methods are prospected.

1. Introduction

Exosomes are found in various body fluids, such as ascites, blood, cerebrospinal fluid, tear, urine as well as saliva, etc. Exosomes which can be used for communication between cells[1] and as a medium for the regeneration of different organs[2] display a wealth of intracellular and extracellular biological information. Exosomes also can be used as diagnostic biomarkers, because they carry physiological change imformation in the source cells and tissues[3]. With the deepening of scientific research on exosomes, exosomes show important research value in clinical diagnosis[4], disease treatment[5], tissue regeneration and other aspects[6]. However, the characteristic, separation and quantitative detection of exosomes are very critical in the researches.

In previous reviews[7] of exosomes, they concentrated mainly on biosensing technologies, microfluidics-based detection and the difference of analytical methods. Based on the exosomes analytical methods established in recent years, this review focus on analyzing and summarizing the difference of recognition sites of exosomes analysis, providing some enlightenment and references for the establishment of simple, efficient and reliable standardized procedures for exosomes isolation and analysis in the future.

1.1. Characteristics of exosomes

As carriers of cargo transport, exosomes play an essential role in intercellular communication and reflect the state and characteristics of the source cells. Exosomes are extracellular vesicles of endocytosis origin that are approximately 30 to 150nm in diameter. It has been found that surface proteins and other related receptors in the endosomal pathways exist in exosomes. The common proteins are CD9, CD63 and CD81, which can reflect specific biological sources. These proteins have important effect for the identification and determination of exosomes. Due to the lipid bilayer of exosomes, the inclusion including nucleic acids, proteins and amino acid keep wrapped in stability and activity[8]. With the further exploration of exosomes by researchers, more and more functional proteins have been discovered, such as Rab GTPases, annexin, Flotillin, ESCRT complex, integrins and tetraspanins[9].

Exosomes have membrane structure and lipid bilayer. The lipids involved include polyglycerin, phospholipid, ceramide, sphingomyeline, cholesterol and phosphatidylinositol. Exosomes also show another biological feature that can distinguish them from other vesicles, that is, exosomes contain nucleic acid substances such as messenger RNA (mRNA) non-coding nucleic acid (miRNA)[10] and genomic DNA (gDNA)[11]. It's also reported that vesicles are not carriers for the release of active DNA[12]. These genetic materials which are subsumed in the structure of lipid bilayer may be transferred between the source cell and the recipient cell through the endocytic fusion of the cell.

The biogenesis of exosomes begins with the endocytosis of cell membranes, including receptor-mediated endocytosis, nuclear endocytosis of inclusion proteins and pinocytosis[13]. As shown in the formation process of exosomes in figure 1, the early endosomes are formed by the endocytosis firstly. While the early endosomes are developing into late endosomes, it exists continuous intracellular exchange of substances by invagination. This is the main reason that exosomes carry information from origin cells. The invagination in the late endosomes leads to the formation of intraluminal vesicles (ILVs, future exosomes) . Multivesicular bodies (MVBs) can be transported to cell membranes through cytoskeletal and microtubule networks. Exocytosis allows exosomes to be released and have lipid bilayer structure. A variety of marker proteins have been found on the surface of exosomes, including CD63, CD81, CD9, GPC1, etc. In addition, nucleic acid, specific protein, metabolites and amino acids, etc, are surrounded by the lipid bilayer of exosomes.

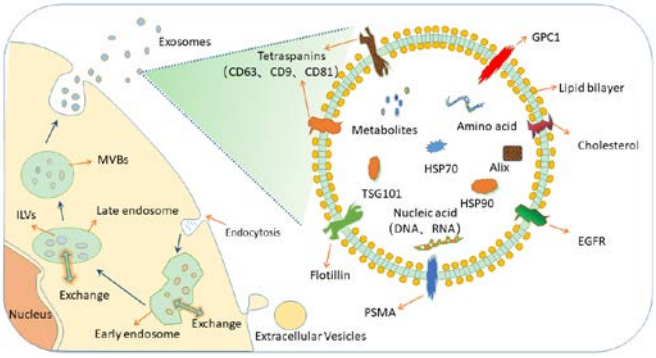

Figure 1 The source, structure and main components of exosomes

1.2 The functions and applications of exosomes

The contents of proteins and nucleic acids in exosomes from origin cells are in a stable state and can be ideal biomarkers for many diseases[14]. Several researches show that exosomes are common in different cancer subtypes，which will be used as potential biomarkers[15]. Some scholars found several miRNAs in exosomes separated from peripheral blood of patients with pancreatic cancer, such as Mir-17-5p, Mir-10b, Mir-550, etc., then the research results show that they can be considered as markers of early cancer[16]. The study on the mechanism of exosomes show an important positive effect on diabetes. Exosomes from patients with Type 1 diabetes mellitus (T1D) may become new diagnostic markers as well as potential therapeutic targets[17].

Nowadays, researchers worked hard to use exosomes as drug carriers to target the delivery of data drugs. Exosomes can be used to carry RNA, proteins and small-molecule drugs. Perets and his colleagues found that exosomes released from mesenchymal stem cells were used to treat autism spectrum disorders through intranasal treatments, showing significant therapeutic effect[18]. Exosomes showed excellent performance in tolerability, which made the therapeutic application of exosomes had a broad prospect[19]. After repeated experiments, the exosomes of mesenchymal cells and epithelial cells were injected in mice without adverse reactions[20]. In order to realize the therapeutic application of exosomes, scientists made a lot of efforts[21] to establish an exosomes platform that could target regions of tumor hypoxia. There are also studies on the functionalization of exosomes to improve their application value in therapeutic[22].Through the efforts of scientists, the research of exosomes in promoting the operation and regulation mechanism of cell transport system has made great progress in recent years[23]. It also has a positive effect on the immune mechanism of malignant tumors[24].

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2. The isolation and purification of exosomes

The extraction and purification of exosomes are the premise of exosomes research. The reliability and stability of exosomes extraction technology are important prerequisites for exosomes research. Because the exosomes in live samples are very small in size and few in number, the isolation and purification of exosomes is a great challenge. Due to exosomes have shown great potential in targeted drug therapy carriers and disease diagnostic markers, the development of exosome separation technology has attracted more and more attention. Due to thire differences in size, protein, nucleic acid, etc, exosomes from different sources can be extracted from cell cultures or body fluids by different separation methods[25].

2.1 Centrifugation

The centrifugation method can be divided into ultracentrifugation method and density gradient ultracentrifugation method. According to the size difference of exosomes, exosomes are separated by the ultracentrifugation method which is an unlabeled, high-throughput method[26]. Nowadays this method is most common for exosomes extraction. It is also considered as the "gold standard" for exosomes isolation. This method has the

advantages of extracting a large number of samples at one time and low cost. However, the shortcomings are the long treatment time, the expensive equipment, the low recovery and the low purity for this method. In addition, the damage of exosomes may be caused by repeated centrifugation, which may lead to the degradation of exosomes.

As a continuation of the ultracentrifugation, density gradient ultracentrifugation method can be used for exosomes purification, when additional purity is required. The exosomes can maintain their activity and morphology without extrusion or deformation. However, this method is characterized by heavy workload, complicated steps, time-consuming and low recovery[27]. The centrifugation effect is related to the density of exosomes in samples. The higher the density difference is, the more significant the separation effect will be. However, there is no relation with the particle size and shape of exosomes. The control of centrifugation time will also directly affect the purification effect.

2.2 Precipitation

Polyethylene glycol (PEG) precipitation is a separation method which hydrophilic PEG is added to the sample at a certain salt concentration. The combination of water molecules reduces the solubility of the solute, thus resulting in the condensation and precipitation of exosomes under the condition of low-speed centrifugation. Mass separation of exosomes can be achieved without the need of high-end and expensive equipment and without any impact on biological activity by this method. However, the exosomes isolated by this method may contain impurities, such as protein polymers. For example, Weng et al.[28] isolated exosomes from the supernatant of cell culture successfully by the PEG-based precipitation and revealed the basic mechanism. They observed the size and shape of exosomes clearly by a high resolution electron microscope. The PEG separation method is characterized by low cost, simplicity and high efficiency.

2.3 Immunoaffinity

Exosomes are rich in specific membrane proteins, including CD9, EPCAM, CD63, RAB5, CD81, ALIX, ANNEXIN, etc. The Immunoaffinity method is achieved isolation by labeling specific proteins of exosomes. Specific proteins can achieve specific binding with corresponding antibodies on different carrier substrates, thus achieving the separation and enrichment of exosomes[29]. Based on the difference of coated antibodies substrates, the methods of immune separation can be divided into magnetic bead immune separation, chromatographic stationary phase separation, enzyme-linked immunosorbent separation, etc. The method is characterized by high specificity, unaffected exosomes morphology and simple operation. However, the exosomes isolated are subclasses of exosomes with positive markers, but not all types of exosomes. The extraction efficiency is low. In addition, the activity of exosomes is susceptible to the pH and salt concentration[30].

2.4 Size-based isolation method

Ultrafiltration and size exclusion chromatography are two dominating separation modes based on exosomes particle size. The separation efficiency of ultrafiltration depends on the size and molecular weight of the suspended substance in the sample. At present, some scholars have isolated exosomes successfully by this method[31]. The sample pretreatment process is simple and time-consuming. But in fact, separated exosomes by this method may be deformed or broken by pressure. It is also prone to hole blocking, which affects the separation efficiency. The purity of exosomes is not high enough.

Size exclusion chromatography is a chromatographic separation technique that takes advantage of particle size differences of analytes. The exosomes isolated can be separated efficiently from impurities (proteins, lipids, etc.) and maintain their integrity and biological activity by this method. In addition, the operation process is simple, while the purity of exosomes is high[32]. However, it's required the relatively high-quality chromatographic column, which is contaminated easily by proteins or lipids during exosomes separation. Due to the low reused rate of chromatographic column, this method is costly and time-consuming[33]. Meanwhile, some researchers[25] combined ultrafiltration and size exclusion chromatography to the separation and purification of vesicles or exosomes, achieving a satisfactory separation effect[34].

2.5 Microfluidic separation

Microfluidic separation is a microscale rapid separation method developed in recent years, which shows good application prospects in the improvement of recovery, reducing sample volume and shortening treatment time[35]. Microfluidic platform which characterized by good flow control and rapid mixing, is a universal tool for the study of small size and fine structure lipid nanocapsules[36]. The separation and purification of exosomes using microfluidic technology is attributed to the differences in physical and biochemical characteristics of exosomes[37]. Based on the conjugate effect of phosatidylserine-specific proteins on exosomes, Kang et al.[38] achieved the separation of exosomes from plasma, all of which are associated with cancer, using microfluidic exosomes separation devices. Its working principle is shown in figure 2. In addition, Ye's and Sun's group used microfluidic technology to bind protein chips to achieve the isolation and detection of exosomes simultaneously [39].

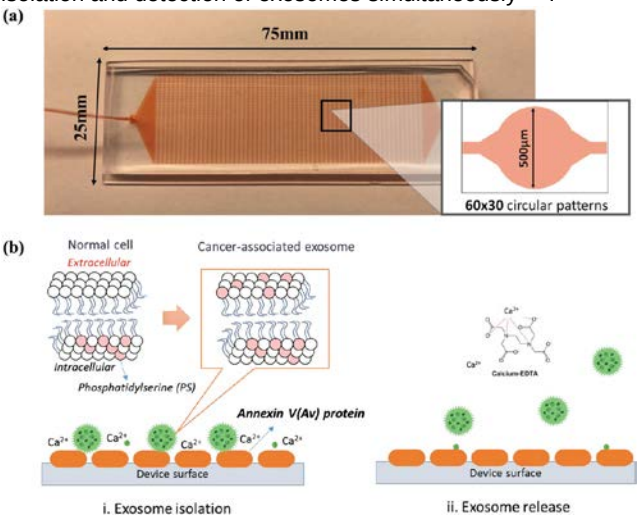

Figure 2 The microfluidic device design and working principle[38] .

3 Methods of exosomes detection

Previous researches found that exosomes had an important impact on tumorigenesis, tumor metastasis and targeted therapy. The recognition and analysis of exosomes are the premise and guarantee in clinical research[40]. Therefor, the quantitative analysis of exosomes is of great significance. In order to improve the efficiency, sensitivity and reliability of exosomes analysis, many researchers proposed new analysis methods by specific recognition of exosomes, such as nucleic acid information, specific proteins, lipid recognition, surface modification recognition and overall recognition, etc., as shown in figure 3.

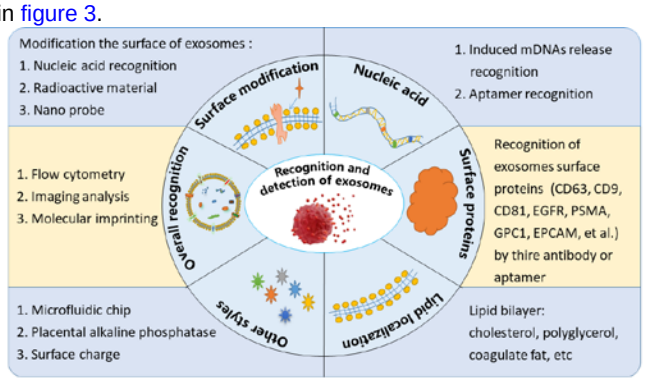

Figure 3 The recognition and detection of exosomes

3.1 Nucleic acid information dependent identification

As one of the main inclusion of exosomes, nucleic acid materials carry important genetic information from origin cells, mainly including RNA, DNA and microRNA[41]. Nucleic acid information can transmit and communicate freely between cells in the body, which makes it possible for more RNA-based personalized cancer drug therapy[42]. Nucleic acid substances carried by exosomes may be associated with diseases, especially in the diagnosis of cancer. what's more, it provide important information in reflecting physiological changes. The nucleic acid substances are mainly distributed in the inner layer of exosomes. In other words, they are surrounded by lipid bilayer, which increases the difficulty of nucleic acid recognition in the analytical process. There are few methods to detect exosomes with this component as the identification object. Based on the auxiliary detection of DNA nanostructure and nanotetrahedron, Tan's group constructed an aptamer electrochemical sensor that could detect exosomes in hepatocytes[43].

Exosomes usually carry nucleic acid substances concentrated in the inner layer. Therefore, nucleic acid substances need to be released in a certain way to realize the recognition of exosomes. Based on the adaptor magnetic bead bio-conjugate capturing exosomes and inducing the release of multiple mDNAs, Zhang and his colleagues[44] established a method for tumor exosomes analysis in LNCaP cells. The method also involves Exo III-assisted cyclic amplification of mDNA to improve sensitivity. Thus, the detection limit can be up to 70 particles/μL.

3.2 Protein recognition

Due to some specific proteins often play an important role in the diagnosis of cancer as tumor markers, many efficient detection methods were established[45]. Previous researches found that the surface of exosomes was expressed with a variety of specific proteins, which distinguished exosomes from other vesicles. These proteins include tetraspanins (CD9, CD82, CD81 and CD63), heat shock proteins (Hsp 60, Hsp 70, Hsp 90), biosynthetic protein (TSG, Alix) and surface growth factor receptor (EGFR), etc. In addition, tetraspanins are highly enriched on the surface of exosomes, which make them be ideal markers for exosomes identification and quantitative analysis.

3.2.1 Protein recognition based on immunoreaction

At present, it is a very mature technology to identify and detect proteins by using the immune response of antigens and antibodies. Exosomes are enriched with specific proteins on the surface. Therefore, detection of exosomes using immune recognition of proteins is one of the most common methods in identification. Researchers have established a variety of exosomes analytical methods by combining protein immunorecognition technology with electrochemical analysis[46], chromatographic analysis[47], surface-enhanced Raman scattering (SERS)[48] and microfluidic detection platform[49].

In recent years, protein immunorecognition technology has been used in variety of exosomes analytical methods[50]. Yu and colleagues[51] developed a field-effect transistor biosensor for quantitative analysis of exosomes using an antibody-modified reduced graphene oxide that specifically identifies the CD63 protein on exosomes.This method shew good specificity and low detection limit（33 particles/μL）. Wang and his colleagues[52] reported a surface acoustic wave sensor amplified by gold nanoparticle for high sensitivity detection of exosomes. The carboxyl group was formed by Au-S bond through self-assembly of thioglycolic acid. Anti-CD63 was fixed on the chip for specific recognition of exosomes, and EpCAM antibodies were used as secondary antibodies for amplification and recognition. A surface acoustic wave (SAW) sensor was established to detect the content of exosomes in the blood samples of cancer patients combined with gold nanoparticles. The decoration process is shown in figure 4. The sensor can detect at least 1.1×10^3 exosomes/mL

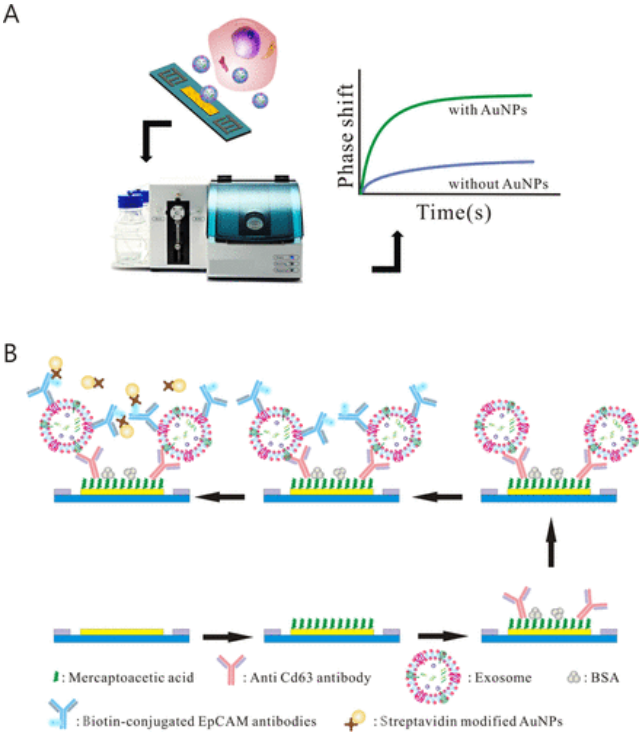

Figure 4 SAW chip was modified by double antibody sandwich method and AuNPs amplification[52]

In addition, some researchers also used CD63 protein on the surface of exosomes for specific recognition with corresponding antibodies to achieve quantitative analysis of exosomes[53].
The surface of exosomes is enriched with a large number of specific proteins. On the one hand, a single protein can be used to achieve the recognition and capture of exosomes; on the other hand, multiple specific proteins can also be used for multiple recognition and determination. Fan et al.[54] established a SPRi biosensors through the different recognition sites and biological affinity of antibodies to multiple identify exosomes derived from non-small cell lung cancer(NSCLC) with high sensitivity. Thus, accurate identification of exosomes protein patterns can differentiate exosomes effectively from normal lung and NSCLC cells. Moura's group[55] achieved immunomagnetic separation of exosomes using magnetic particles modified by CD81 antibody, labeled exosomes with enzyme-bound second antibodies (CD24 or CD340), then performed optical reading detection by standard microplate reader.

3.2.2 Protein recognition based on aptamer affinity

Aptamer are oligonucleotides selected through the systematic evolutionary process of exponential enrichment of ligands (SELEX) and have the basic characteristics of nucleic acid chain flexibility, spatial conformational diversity and so on. Proteins achieve a binding in high affinity and specificity with aptamers in the form of superposition, complementary shape, electrostatic and hydrogen bond[56]. Compared with antibodies, nucleic acid aptamers have the advantages of easy chemical modification, low cost, good long-term stability and easy synthesis. In recent years, there have been many researches using aptamers of surface marker proteins of exosomes to achieve specific recognition of exosomes detection[57].
Based on the aptamer of exosomes surface proteins, the quantitative analysis of exosomes was realized by the recognition of surface proteins combined with electrochemical detection. CD63 is the most common specific protein on the surface of exosomes. Many scholars[58] have realized the detection of exosomes by CD63 aptamer with good detection results. Zhang and his colleagues[59] used gold nanoparticle modified by Ti3C2 and CD63 aptamer to construct a hypersensitive electrochemiluminescence sensor for exosomes and their surface proteins. In this strategy, not only exosomes were specifically and efficiently recognized, but also bare catalytic surfaces of gold nanoparticles with high electrocatalytic activity were provided. The schematic diagram of detection is shown in figure 5. In addition, some analytical methods[44, 60] used other protein aptamers to detect exosomes. At the same time, based on the labeling of surface proteins of exosomes in serum with seven fluorescent aptamers, Sun et. al constructed a thermophoretic aptasensor to realize the detection and classification of prostate cancer by linear discriminant analysis, which showed high sensitivity and low cost[61].

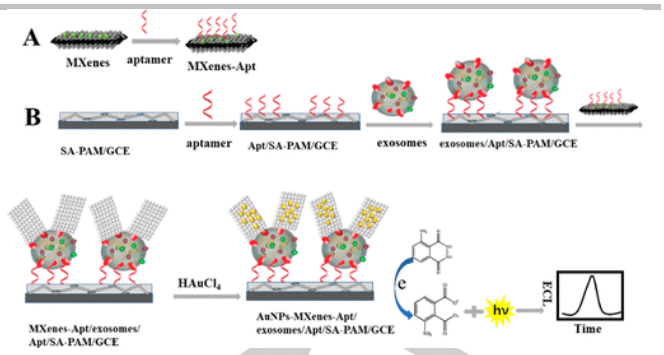

Figure 5 The principle of detecting exosomes based on Ti3C2 MXenes biosensor ECL modified by in situ formation of gold nanoparticles[59]

The detection of exosomes by using surface specific protein aptamers recognition combined with fluorescence analysis is also an important aspect in exosomes analysis. Several research groups[62] have established exosomes detection methods by this way. Zhang et al.[63] developed a fluorescence polarization method based on aptamer for exosomes quantification, which can directly quantify exosomes in human plasma. In this study, the intrinsic affinity between the aptamers and the membrane proteins of exosomes played a key role in exosomes capture. In addition, some scholars have established some detection methods of exosomes by combining surface proteins and aptamers recognition with other analysis methods, including colorimetric analysis[64], surface plasma resonance (SPR)[65], giant magnetoresistance (GMR) biosensors[66], click-chemistry[67], and luminescent resonant energy transfer (LRET)[68]. Tan's group[69] used the specific binding between exosomes surface proteins and aptamers to cause color changes. The surface proteins of exosomes could be analyzed by naked eye within a few minutes. Based on the specific recognition and capture of EpCAM proteins on the surface of exosomes by aptamers, Fang et al. constructed a dual-mode photothermal and electrochemiluminescence biosensor, which could detect exosomes reliably and efficiently in serum samples[70]. Its schematic diagram of detection is shown in figure 6. In addition, there are also researchers[71] who combine the antibody immunity of proteins with the recognition of aptomers to achieve quantitative analysis of exosomes.

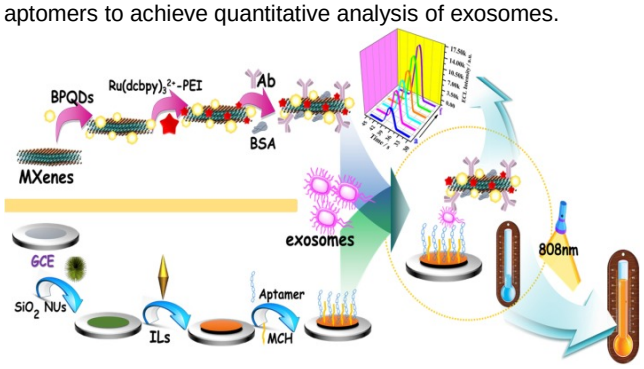

Figure 6. Schematic diagram of two-mode biosensor for exosomes detection[70]

3.3 Lipid recognition

In order to ensure the stability of inclusion in exosomes, the outermost layer of exosomes is a lipid bilayer structure.

According to the lipid components include cholesterol, polyglycerol, phospholipids, etc., the researchers realized specific recognition and detection of exosomes. By anchoring the lipid double layer of exosomes with DNA oligonucleotide binding, Tian et al. [72] constructed a high-sensitivity microchip platform for exosomes detection in combination with integrated nucleic acid amplification microchip digital detection. The surface of exosomes contains rich lipid components. During the detection, the cholesterin nanoprobe was anchored to identify exosomes in urine samples. By amplifying the signal of copper nanoparticles, Ye's group established a fluorescence method to detect exosomes. With the aid of cholesterol-modified magnetic beads, exosomes were magnetically separated due to the hydrophobic interaction between cholesterol molecules and lipid membranes. This method is simple and fast, and can complete the detection within 2 hours[73].

In addition, Lipid recognition can combine with the surface specific proteins of exosomes to achieve common recognition and detection. Double marker recognition was used. These include cholesterol probe targeting lipid layer localization and aptamers recognition of CD63 on exosomes surface, and combined with magnetic bead separation technology. Finally, It's constructed a highly sensitive and specific exosome detection strategy[74]. Based on CD63 aptamer magnetic bead marker capture and cholesterol modified DNA probe for exosomes lipid identification, Zhang et al.[75] used the amplification of the hybrid chain reaction (HCR) signal of alkaline phosphatase to achieve quantitative analysis of exosomes by visual detection or UV-vis spectrophotometer. The detection process is shown in figure 7. This strategy is simple and easy to implement with good specificity. The surface of exosomes has lipid bilayer structure, which is usually combined with other recognition methods in recognition and detection. This method reduces interference signals greatly, obtains high specificity of exosomes detection and ensure high sensitivity. However, the operation and modification process of this method are more complicated with higher cost.

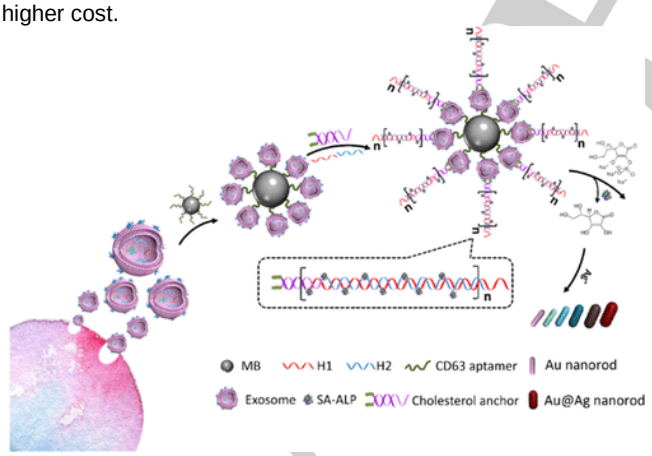

Figure 7. The schematic diagram of the polychromatic visual detection mechanism of exosomes[75]

3.4 Surface modification identification

Exosomes are nanometer particles secreted by cells, so direct analysis and detection are challenging. The researchers try to detect exosomes indirectly by surface modification of exosomes through nucleic acid, radioactive substances and nano-probe, then calculate content of exosomes by determinating decorations. Tan's group realized the directional detection of exosomes from target cells with high specificity. Aptamers were modified on specific exosomes by DNA hybridization chain reaction[76]. Morishita et al. [77] used iodine-125 (125I) based on the streptomycin avidin (SAV) biotin system to label exosomes from specific sources. Afterwards, the exosomes were evaluated by analyzing and testing the 125I content. These results indicate that SAV biotin system for exosome 125I labeling is an effective method for quantitative evaluation. Besides, the surface of exosomes was modified by nanometer probe, which showed good application effect in recognition and detection as well. Tian et al.[78] constructed a biosensor for quantitative detection of exosomes based on nano-probe modified exosomes surface and combined with the detection technology of surfacing enhanced Raman scattering, which has advantages of high sensitivity, convenience and can be applied to the detection of exosomes in biological samples.

3.5 Overall recognition

The overall recognition and analysis of exosomes mainly include flow cytometry[79], imaging analysis and molecular imprinting. Based on the fact that surface plasma wave depth and exosome size are at the same horizontal line, Picciolini et al.[80] proposed a strategy that could directly detect multiple exosomes subgroups. The method quantified neurogenic exosomes in the blood by surface plasmon resonance imaging technology.

By using the intact imprinting or morphological characteristics of exosomes, researchers have realized recognition and capture of exosomes and achieved good test performance. Using molecular surface imprinting technology combined with antibody-modified nanoparticles, Mori et al.[81] established a fluorescence method for quantitative detection of exosomes from prostate cancer. This method does not require pretreatment and shows high sensitivity. By recording the exosomes fingerprint characteristics produced by mass spectrometry, the analysis of exosomes content in melanoma disease is achieved[82]. At the same time, Dong et al.[83] designed ingeniously a platform similar to a beehive to achieve the overall recognition of exosomes by the surface enhanced Raman scattering technology. This strategy was used for separation and detection exosomes in plasma of cancer patients successfully.

3.6 Other recognition

In addition to the above description of recognition, the researchers also achieved the recognition and detection of exosomes by other characteristics of exosomes, such as charge and enzyme. Based on the characteristics of exosomes particle size and charge, Brown and his colleagues analyzed physical and chemical properties by mass spectrometry, which would be beneficial to the classification of exosomes subtypes[84]. Besides, magnetic nanoparticles were used to separate exosomes from placental cells, while it's tested by enzyme-linked immunosorbent assay[85].

With the increasing maturity of microfluidic technology, the identification and detection of exosomes can be applied through the microprocessing of test channels, and the sensitivity shows obvious advantages. Zeng's group[86] proposed a highly sensitive detection strategy for exosomes secreted by tumor cells with the help of microfluidic chip technology. The design that combined with self-assembled 3D herpet-shaped nanoplatform improved the separation efficiency of exosomes

significantly. The schematic diagram and process are shown in figure 8.

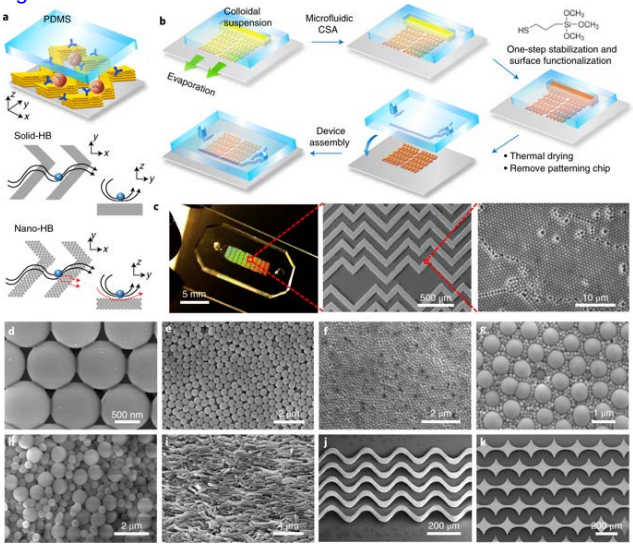

Figure 8. The schematic diagram of the idea strategy of improving biosensors through 3D nanostructures of microfluidics elements[86]

4 Conclusions and outlook

With the development of exosomes research, the structural characteristics, basic components, biogenesis, morphological characteristics and intercellular functions of exosomes have been well understood. In addition, the antibodies or aptamers of CD63 are most commonly used to recognize and capture exosomes. More and more tumor-related biomarkers were found, such as HER2, EpCAM, MUC1, CA125, PTK7 and PSA. Based on the newly discovered exosomes marker proteins, the recognition and detection of exosomes from different source cells were achieved to enrich the efficient separation and analytical methods of exosomes, laying a solid foundation for the application of exosomes in cancer diagnosis and treatment. For example, microfluidic chip technology, owing to the small sample size and high sensitivity, show the potential to be a new tool for exosomes separation and detection.

Nevertheless, the biogenesis, secretion, target cell uptake and function of exosomes have not been fully revealed. Exosomes are produced by cells. Whether exosomes can grow and divide, or participate in signal transduction mechanisms and autonomous biochemical reactions in the appropriate environment, remains to be determined. The existing exosomes separation technology still needs to be further improved. The main challenge is to achieve high-throughput exosomes isolation while achieving high content, low damage and high recovery separation. The challenge of exosomes analysis technology is the selection of exosomes surface target and how to identify the origin of exosomes. In the future, it is not only necessary to determinate their cargo (proteins, lipids and nucleic acids, etc.) in exosomes from various cell sources, but also necessary to analyze the characteristic or origin types and establish a simple, efficient and reliable standardized procedures for exosomes separation and analysis.

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Keywords: exosomes • detection methods • purification • biological characteristics • function

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Entry for the Table of Contents

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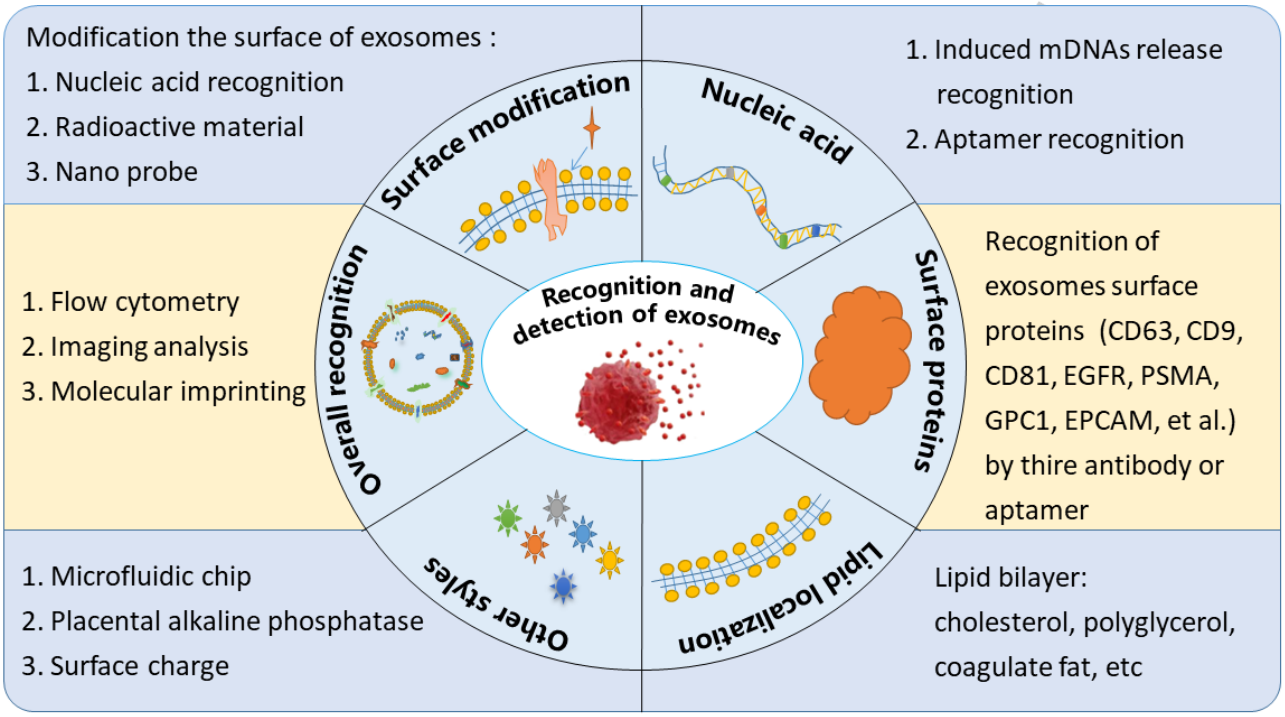
Graphic abstract



This review summarizes the recent progress of exosomes separation, analysis and detection. The future research and development directions of exosomes performance, mass production, purification and quantitative detection were prospected.

Figure captions

Figure 1 The source, structure and main components of exosomes

Figure 2 The microfluidic device design and working principle[38]

Figure 3 The recognition and detection of exosomes

Figure 4 SAW chip was modified by double antibody sandwich method and AuNPs amplification[52]

Figure 5 The principle of detecting exosomes based on Ti3C2 MXenes biosensor ECL modified by in situ formation of gold nanoparticles[59]

Figure 6 Schematic diagram of two-mode biosensor for exosomes detection[70]

Figure 7 The schematic diagram of the polychromatic visual detection mechanism of exosomes[75]

Figure 8 The schematic diagram of the idea strategy of improving biosensors through 3D nanostructures of microfluidics elements[86]

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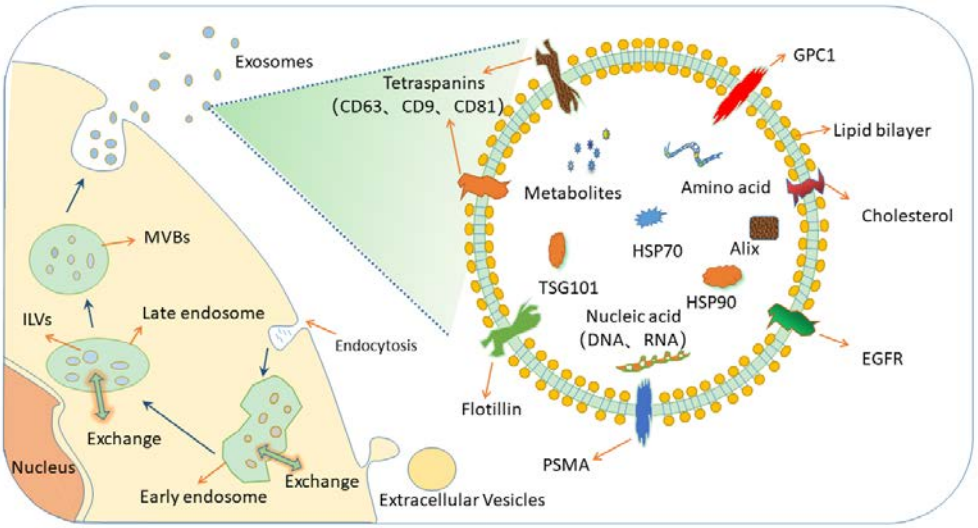

Figure 1 The source, structure and main components of exosomes

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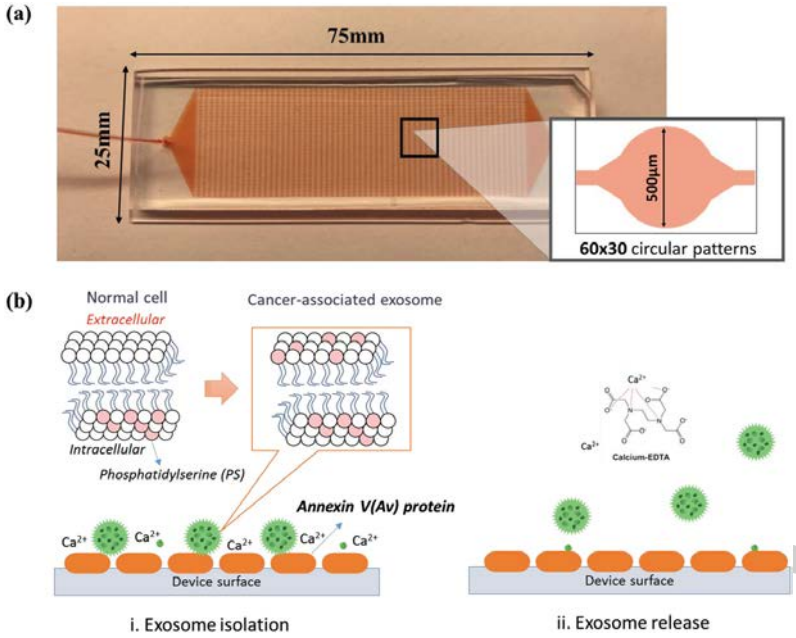

Figure 2 The microfluidic device design and working principle[38]

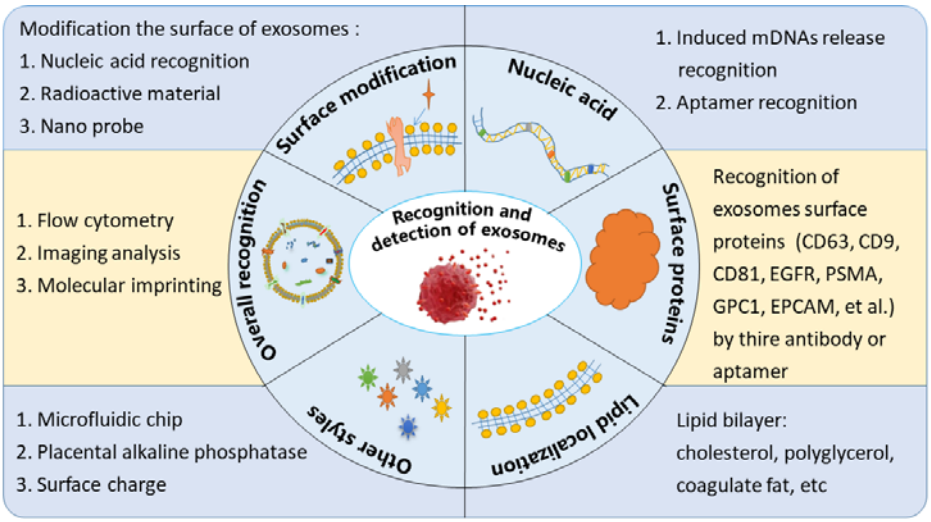

Figure 3 The recognition and detection of exosomes

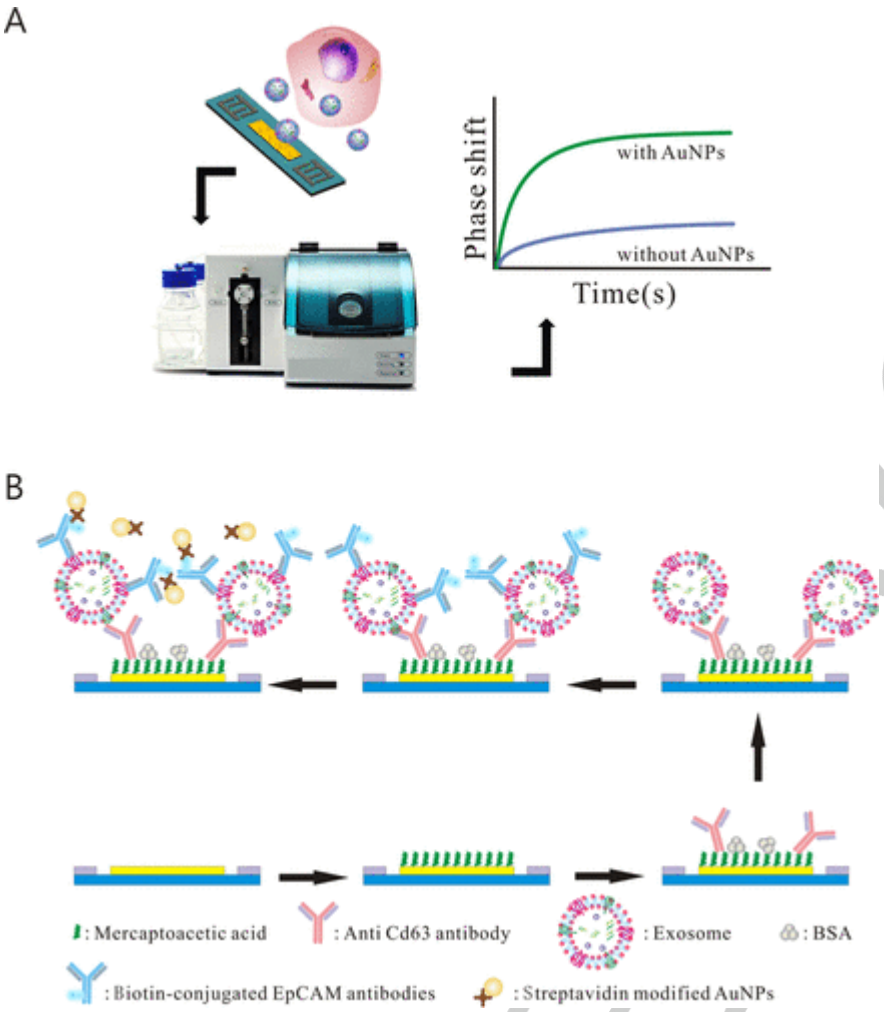

Figure 4 SAW chip was modified by double antibody sandwich method and AuNPs amplification[52]

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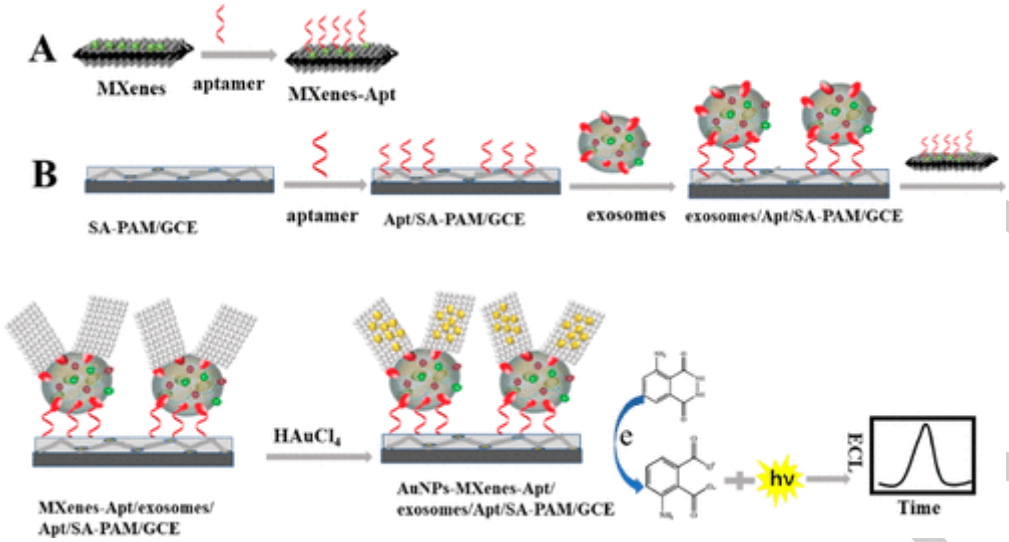

Figure 5 The principle of detecting exosomes based on Ti3C2 MXenes biosensor ECL modified by in situ formation of gold nanoparticles[59]

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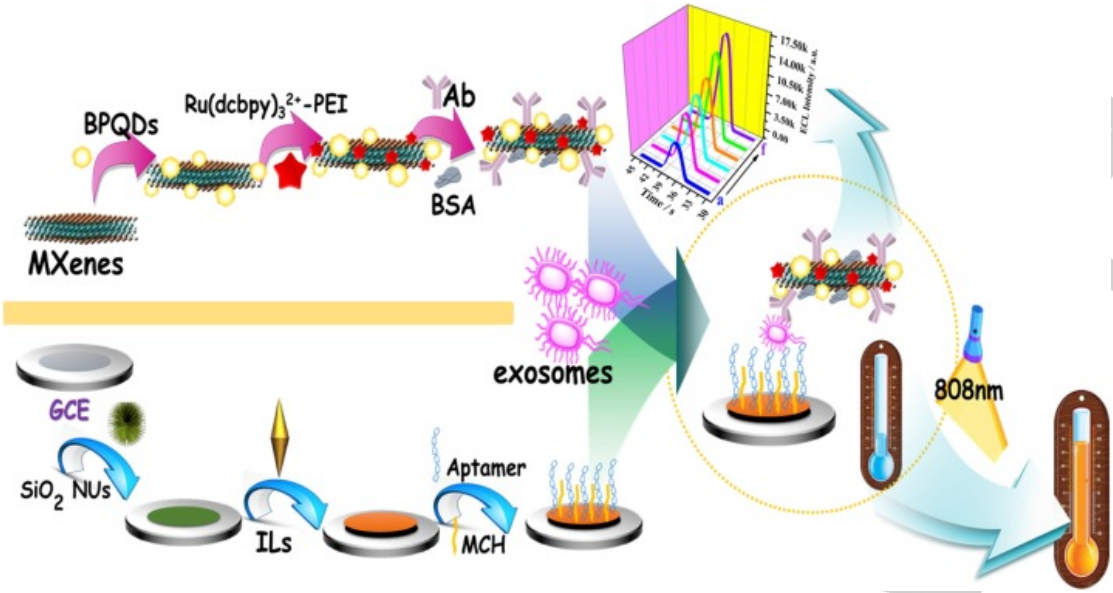

Figure 6. Schematic diagram of two-mode biosensor for exosomes detection[70]

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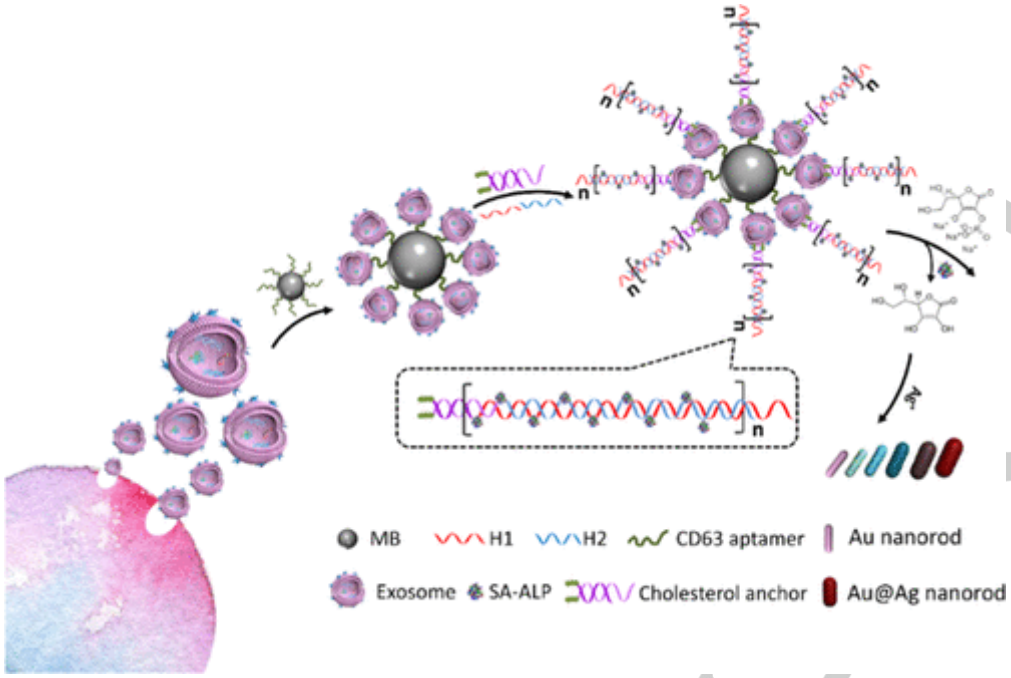

Figure 7. The schematic diagram of the polychromatic visual detection mechanism of exosomes[75]

10.1002/asia.202000873

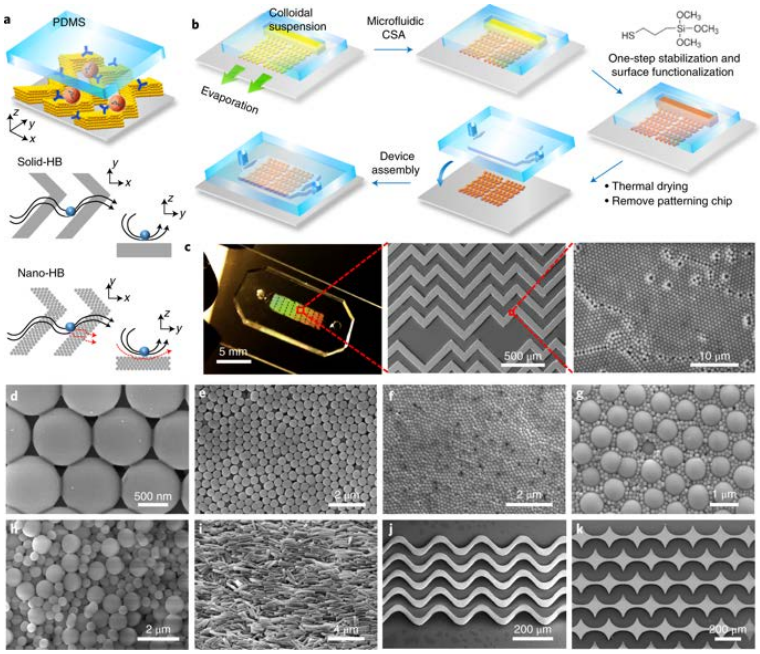

Figure 8. The schematic diagram of the idea strategy of improving biosensors through 3D nanostructures of microfluidics elements[86]