Journal of Medical Sciences 2021, 2(3) 22-31

Derleme / Review

https://doi.org/10.46629/JMS.2021.45

Biosensor Platforms for Cancer Derived Exosomes Detection

Kanser Kaynaklı Eksozom Tespitinde Biyosensör Platformlar

Kübra KELLECİ[1], Sevil ÖZER[2]

KK: 0000-0002-9409-2254 **SÖ:** 0000-0002-0186-763X

[1] Beykoz Üniversitesi, Meslek Yüksekokulu, Tıbbi Hizmetler ve Teknikleri Bölümü, İstanbul, Türkiye
[2] Yeni Yüzyıl Üniversitesi, Mühendislik- Mimarlık Fakültesi, Biyomedikal Mühendisliği, İstanbul, Türkiye

Abstract

Early diagnosis is one of the biggest challenges in the fight against cancer. Traditional cancer diagnosis methods have some disadvantages such as requiring expertise, involving invasive procedures, radiation exposure, high cost and loss of time. For this reason, current research has focused on the development of faster, reliable and cost-effective diagnostic methods.

The use of biomarkers present in body fluids has been promising in this regard. Exosomes secreted by cells into body fluids and responsible for intercellular communication are biological nanoparticles produced by natural pathways. Studies have shown that the change in microenvironmental conditions during tumor development changes exosome secretion. Due to their high cellular activities, tumor cells produce a much higher rate of exosome than healthy cells. Therefore, it is known that the number of exosomes in body fluids is significantly enriched compared to other cells and can act as a diagnostic biomarker alone. Cancer-derived exosomes are very interesting for early detection of cancer and evaluation of therapeutic response. Biosensor is an analytical device used to detect an analyte that combines a biological component with a physicochemical detector. Nowadaysthere is an increasing interest in developing cancer biosensors with superior analytical performance and real-time measurement. The development and dissemination of biosensor applications for exosome detection will be very advantageous for individuals in regions where there is no access to diagnostic devices and resources. Considering the disadvantages of traditional methods, we can say that biosensor studies are much more advantageous because they are cheaper, provide multi-analyte support, provide high sensitivity, and obtain fast and reliable results. In this study, recent developments in biosensor systems for the detection of exosomes secreted from different cancer cells are summarized. In addition, the use of cancer-secreted exosomes for point of care testing (POC) and the challenges in this area are presented and discussed.

Keywords: Biosensor, Biomarker, Exosome, Diagnostic method

Öz

Erken teşhis kansere karşı mücadeledeki en büyük zorluklardan biridir. Geleneksel kanser tanı yöntemlerinin uzmanlık gerektirmesi, invaziv işlem içermesi, radyasyon maruziyeti, yüksek maliyet ve zaman kaybına neden olması gibi birtakım dezavantajlara sahiptir. Bu nedenle mevcut araştırmalar daha hızlı, güvenilir ve uygun maliyetli tanı yöntemlerinin geliştirilmesine odaklanmıştır. Vücut sıvılarında var olan biyobelirteçlerin kullanımı bu anlamda umut verici olmuştur.

Hücreler tarafından vücut sıvılarına salgılanan ve hücreler arası iletişimden sorumlu olan ekzosomlar, doğal yolaklarla üretilen biyolojik nanopartiküllerdir. Tümör gelişimi esnasında mikroçevre koşullarında meydana gelen değişimin ekzosom sekresyonunu değiştirdiği yapılan çalışmalarca ortaya konmuştur. Tümör hücreleri yüksek hücresel aktiviteleri nedeniyle, sağlıklı hücrelere göre çok daha yüksek oranda ekzosom üretimi gerçekleştirmektedir. Dolayısıyla vücut sıvılarında bulunan ekzosomların sayısı, diğer hücrelere göre ciddi oranda zenginleştiği ve tek başına tanısal biyobelirteç olarak görev yapabildiği bilinmektedir. Kanser kaynaklı eksozomlar, kanserin erken teşhisi ve terapötik yanıt değerlendirmesi için oldukça ilgi çekicidir. Biyosensör, biyolojik bir bileşeni fizikokimyasal bir detektörle birleştiren bir analitin saptanması için kullanılan analitik bir cihazdır. Günümüzde, üstün analitik performans ve gerçek zamanlı ölçüm gösterdikleri için kanser biyosensörlerini geliştirmesine yönelik artan bir ilgi vardır. Ekzosom tespiti için biyosensör uygulamaların geliştirilmesi ve yaygınlaştırılması, teşhis cihazlarına ve kaynaklarına erişimi olmayan bölgelerdeki bireyler için oldukça avantaj sağlayacaktır. Geleneksel yöntemlerin dezavantajları göz önünde bulundurulduğunda biyosensör çalışmalarının daha ucuz, çoklu analit desteği sağlaması, yüksek hassasiyet sağlaması ve hızlı, güvenilir sonuçlar elde etmesi nedeniyle çok daha avantajlı olduğu söyleyebiliriz. Bu çalışmada farklı kanser hücrelerinden salgılanan eksozomların tespiti için biyosensör sistemlerdeki son gelişmeler özetlenmiştir. Ayrıca, bakım noktası testleri (POC) için kanserden salgılanan eksozomların kullanımı ve bu alandaki zorluklar sunulmaktadır.

Anahtar sözcükler: Biyosensör, Biyobelirteç, Ekzosom, Tanı yöntemi

* This study was presented as an oral presentation at the 7th science days congress on May 6, 2021.

* Bu çalışma 6 Mayıs 2021 tarihinde İYYÜ 7. Bilim Günlerinde Sözlü Sunum olarak sunulmuştur.

Sorumlu Yazar / Corresponding Author: Kübra KELLECİ
E-posta: kubrakelleci@beykoz.edu.tr

İntroduction

Cancer is one of the most important public health problems in which abnormal cells grow uncontrollably, have the ability to start in almost any organ or tissue of the body, and/or spread to other organs. It is known that cancer, which is the second leading cause of death worldwide, caused an estimated 9.6 million deaths in 2018. The most common types of cancer are lung, breast, prostate, skin cancer and stomach cancer, respectively (1,2).

Current diagnostic techniques for living tissues include dermoscopy, computed tomography (3), X-ray imaging, and magnetic resonance imaging (4), which are non-invasive methods, while at the microscopic level, there are biopsy procedures (5) to learn the type of cancer and cancer stage. These methods, which are used in macroscopic and microscopic cancer imaging, besides their advantages, also contain some disadvantages such as radiation, being unsuitable for claustrophobic people, causing anaphylactic reactions due to the use of contrast agents, time consuming and requiring expertise (6). Biopsy is a conventional method that is often used to remove the skin and determine whether the cells are malignant or benign. However, this method often causes the lesions to spread. It tends to be dangerous, because it may damage healthy cells. Therefore, current research has focused on faster, reliable and cost-effective diagnosis of cancer (7,8).

There is a growing interest in the use of microfluids (circulating tumor-specific molecules, vesicles) to detect tumor presence. For this purpose, the use of cancer cell-specific biomarkers comes to the fore. Biomarkers are molecules of biological origin that contain RNA, DNA or proteinaceous structures such as antibodies, exosomes and hormones found in tissues, blood or other body fluids. Biomarkers are of great importance in the early diagnosis of cancer, in its correct staging, or in determining the response to the treatment and progression of the disease (7). Biomarkers can usually be detected in fluids such as urine, serum, cerebrospinal fluid or blood, or they can be found on or inside cells. Serological tests such as ELISA and PCR, in which biomarkers are used, have advantages such as high sensitivity and specificity, and relatively fast results. However, they have some disadvantages such as being expensive, time- consuming and needing qualified personnel, and the need for a well-equipped laboratory (8,9).

Exosome

Exosomes, which are released by all cells into body fluids and responsible for intercellular communication, are biological nanoparticles produced by natural pathways (10,11). They are extracellular vesicles of endosomal origin with a diameter of 30-150 nm. They can be secreted by all cell types, and are found in body fluids (such as amniotic fluid, blood, CSF,urine). In addition to many metabolites, they have a rich content of biological molecules such as RNA, DNA, protein, lipid (cholesterol, phosphatidylserine and ceramide) (8). Cancer-associated antigens are abundant on the surface of cancer-derived exosomes. Exosomes are cargo carriers that affect neighboring cells and form pre-metastatic niches. The cargoes that they carry differ according to the purpose of secretion and the contents of the cells from which they originate (10). Exosomes contain cargo protein such as integrins, immunoglobulins, many adhesion proteins, cytoskeletal proteins, ESCRT (endosomal separation complex proteins necessary for transport), ceramide and tetraspannin family, heat-shock proteins (HSP70, HSP90), CD63, CD81 and CD9. Due to the the differences in surface contents, simple and rapid identification of exosomes can be easily achieved (11).

It is known that exosomes are responsible for the process of tumorigenesis as well as the induction of distant tissue tumors that metastasize. Due to their high cellular activity, tumor cells produce exosomes at a much higher rate than healthy cells. Therefore, it is known that the number of cancer-derived exosomes in body fluids is significantly enriched compared to healthy cells, and can act as a diagnostic biomarker alone (8,9). Logozzi et al., the release of exosomes from prostate (LNCaP), melanoma (Me30966), osteosarcoma (SaOS2), breast (SKBR3), colon (HCT116) human tumor cell lines was investigated. They have reported a significant exosome release occurred at pH 6.5 compared to the control pH 7.4 (10). Therefore, the results of the study shows us that the exosome release in the acidic environment of the tumor is directly proportional to the tumor metastatic potential.

There are different approaches and methods to detect and analyze exosomes. These methods include traditional detection methods such as Dynamic Light Scattering – DLS (12), Atomic Force Microscopy – AFM (13), Transmission Electron Microscopy -TEM (14)

and Enzyme Linked Immunosorbent Assay- ELISA (15), Western Blot (16). However, these methods have disadvantages such as weak signal, slow detection, manual adjustment of the software, high analyte volume, need for trained personnel and expensive due to the need for advanced laboratories. Considering the disadvantages of traditional exosome diagnostic methods, biosensor technology can play an important role in the reliable and sensitive identification of exosomes.

Biosensor Applications in Exosome Detection

In cancer studies, biosensors can measure the levels of exosomes secreted by tumor cells to determine whether a tumor is present or how effective treatment is in reducing or eliminating cancerous cells.

Biosensors are analytical devices that have been widely used and researched in recent years to detect a biological analyte (antigen, antibody, enzyme, microorganism, exosome biomarker, etc.). The elements in the biosensor platforms are illustrated in Figure 1.

Tetraspanins (CD3, CD9, CD24, CD63, CD82 ets.) or lipid aggregates (cholesterol, phosphatidylserine, and ceramide etc.) on the surface of exosomes can be used as targets for exosome detection (17). In addition to those ones, different proteins can be detected depending on the cell (EpCAM, MIF, HER2, EGFR, LMP1, PTK7, PSMA etc.) in the detection of cancer-derived antigens on the surface of exosomes. The recognition element, one of the most critical elements of the biosensor, specifically recognizes the target analyte. This choice is entirely related to the binding affinity between the recognition element and the target. Antigen, antibody, enzymes or nucleic acids, aptamers can be used for the recognition element.

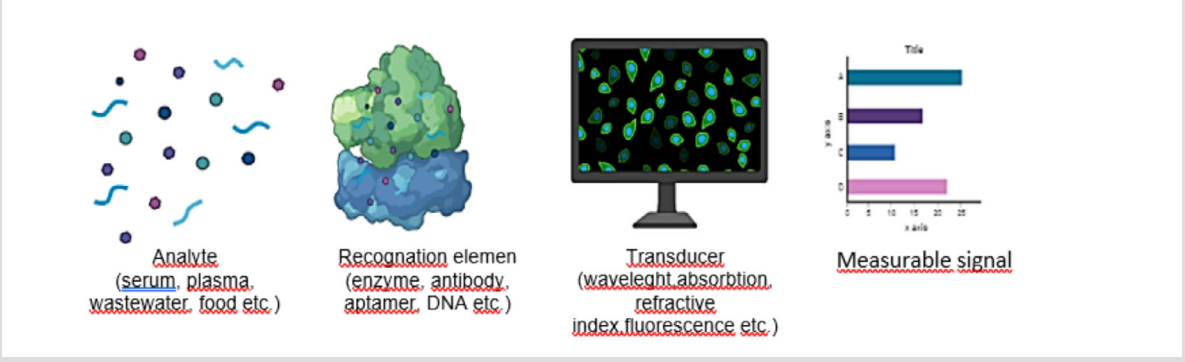
Another important part of the biosensor is the transducer. The transducer converts the molecular signal into electrical or optical signal that can be displayed and analyzed. Transducers can be electrochemical (potentiometry, amperometry) (18,19), optical (fluorescence, colorimetric and interferometric) (20, 21, 22), mass (piezoelectric acoustic waves) (23) or calorimetric (temperature) (24). The transducer and recognition element should be chosen according to the researcher's needs.

Biosensor systems have many advantages such as not needing trained personnel, providing fast and multiple analyte support, low reagent consumption, and not needing safe, portable, aseptic working conditions. The development of highly sensitive, specific and cost-effective biosensors is in great demand as they contribute to the high-precision diagnosis of disease and the realization of personalized medicine. Many studies have reported that various biomarkers have been successfully detected by different biosensors (2,25).

1. Optical Biosensors

Optical biosensors read changes in wavelengths of light. Optic transducers can be fluorescent (20), luminescent (26), SERS (27), SPR (28), colorimetric (21). Optical transducers determinates changes in wavelengths in the identification of the analyte. When the literature is examined, it has been observed that different optical biosen-

Figure 1. Biosensors consist of 3 basic components: recognition element (detector), transducer and output system. The recognition element can be an enzyme, antibody, aptamer, DNA etc. to counter analytes. Transducer is responsible for the conversion of the molecular signal into a mesurable (optical or electrical) signal.



sors (fluorescence, SERS, SRP and colorimetric) have been developed for the detection of exosomal proteins or miRNAs due to low cost, high specificity and sensitivity, and low analyte volume requirement.

In the last few years, fluorescence-based methods have been widely used in the development of biosensors due to their high sensitivity and their fundamental properties. In addition to fluorescent dyes, fluorescent proteins and nanoparticles are also used to obtain a strong fluorescent signal (25,29). Thus, the concentration of the analyte is determined by providing fluorescence. Luminescence biosensors are sensors that work with light emission when the electrons of the molecules return to the ground state after being excited. Examples of these are radioluminescence (29), chemiluminescence (30), photoluminescence (31), and bioluminescence (32).

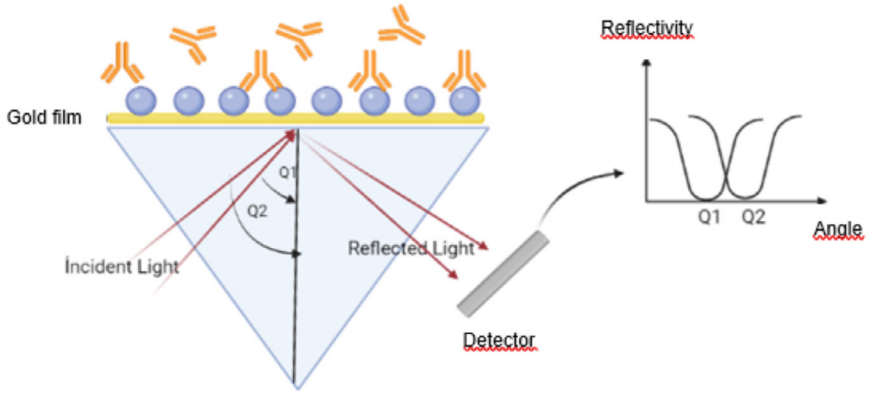
Zhang and coworkers (33) developed a microfluidic platform based on graphene oxide/polydopamine nano-interface for the detection of CD63, CD81, EpCAM exosomes obtained from COLO-1, Ovarian cancer cell line. It has been reported that 50 exosomes / µL are detected by fluorescence methods. They have reported aptamer-modified two-dimensional material Ti3C2 was used to detect CD63 exosome from Melanoma cell line B16, breast cancer cell line MCF-7, human ovarian carcinoma cell line OVACAR-3 and human liver cancer cell line Hep G2. An electrogenerated chemiluminescence (ECL) biosensor was developed using MXenes nanosheets. At the end of the study, the detection limit was found to be 125 particles /µL, which is 100 times lower than the traditional ELISA method (34).

Another work with a fluorescent biodetection platform was developed by Huang and his coworrkers. for the detection of leukemia cell-derived exosomes (35). To bind the exosomes, DNA primer containing AS1411 aptamer, which has a high affinity for the nucleolin on the exosome, was applied. It has been reported that 1×10^2 particles / µL exosome are detected with the fluorescent platform, which is hybridized with gold nanoparticle (GNP)-DNA- fluorescent dye. The types of optical biosensors are mentioned below.

a. SPR Biosensor

Surface plasmon resonance (SPR) biosensors detect changes in the refractive index in real time. SPR results from optical excitation of a surface plasmon that runs along the interface between a highly conductive metal film such as gold, silver etc film and dielectiric material. The excitation conditions are determined by the transmittance of the metal and sample materials, as well as the wavelength and angle of the instantaneous beam. SPR biosensor uses the temporary surface plasmon wave at the surface of a gold layer to probe the optical properties of the contacting region and measuring changes in the number of biomolecules on the sensor surface (36). The resonance angle is sensitive to changes in the refractive index (RI) and dielectric constant at the interface at a distance of up to 800 nm from the real metal surface. As the distance from

Figure 2. Setup of SPR biosensor: SPR biosensors include a light source, detector, and prism attached to the metal-clad surface. The SPR detects the change in the refractive index



the surface increases, the sensitivity drops exponentially, allowing the SPR-based biosensor to work better on small particles. A typical SPR biosensor platform is illustrated in Figure 2. Modification of the sensor surface with capture agents such as antibodies, nucleic acids, aptamers provides information about specific molecular interaction reactions.

Grasso and his coworkers (37) have developed a SPR biosensor for the detection of exosomes which are obtained from breast cancer cell lines (MDA-MB-231, MCF-7, BT-474). In this study, the surface was modified with PEG (which suppresses non-specific binding), neutralavidin and biotinylated antibodies. By using the developed SPR sensor, 6 exosomes, CD63, CD9, CD24, CD44, EpCAM and HER2 were detected in less than 60 minutes with 0.5 mL LOD.

Hosseinkhani et al. (38) has designed an SPR biosensor which is to detect the specific surface markers on the exosome obtained from coronary heart patients. The profile of exosomes in the study was verified by using immunogold labeling, ELISA, and SPR. SPR results indicates that the binding of exosomes from individuals with coronary heart disease to anti-ICAM-1 antibodies increased when compared to exosomes from healthy donors.

Another work conducted by Park et al. in 2018 (39), HSP90, HSP70, TSG101 and CD63, EpCAM, EGFR exosomes from ovarian cancer cell lines (OVCAR 3, OV420, CaOV3) were detected as 104 exosomes per mL in the Nanohole-based SPR (iNPS) biosensor study.

Exosome detection was also performed with the SPR sensor in a research by Sina et al (28). In the study, the gold film was functionalized with biotinylated anti-HER2 using biotin-avidin chemistry, and the analytes were passed through the SPR biosensor to detect 8280 exosomes/μL derived from BT474 breast cancer cell.

b. Colorimetric Biosensor

This technique is a basic and fast method for exosome detection that works by measuring instant color change with the naked eye using enzymes and nanomaterials. For this reason, it is often used in point-of-care testing. Colorimetric biosensors are examined in two different categories as paper and solution-based. Paper usually use a substrate in paper based biosensor. Also this methods are suitable for point of care application due to their ease of use and the fine sample volume required. Solution-based biosensors use signal labels (enzyme, nanomaterials etc.) to obtain color change. These sensors are known to exhibit advantages such as fast and simple detection for large sample volumes.

In a study, Xia et al. (40) reported that the surface of single-walled carbon nanotube (SWCNTs) was functionalized with CD63 aptamer to increase exosome detection from MCF-7 cells. At the end of the study, it was reported that pale blue color visible to the naked eye was formed and exosomes with a detection limit of 5.2×10^5 particles/μl could be detected.

Yang and his partners (41) reported that colorimetric pH sensitive bioanalysis was performed for the diagnosis of CD63 exosome from the MCF-7 breast cancer cell line, and 4.46x 10^3 exosome/μL was detected. By establishing a relationship between the change in pH of the solution and the exosome concentration, it was stated that a low-cost, widely used and commercially available pH test paper could be directly used to quantitatively analyze exosomes.The results indicates that there was a color transition from yellow to red with increasing exosome concentration as shown in Figure 3. The change of colr with increasing concentration is also confirmed by the quantitative absorption data of UV-VIS analysis.

In the 2017, there is an another study by Oliveira-Rodríguez et al (42), which has detected 3.4 x 10^6

Figure 3. The color changes from yellow to red with increasing exosome concentration (41).

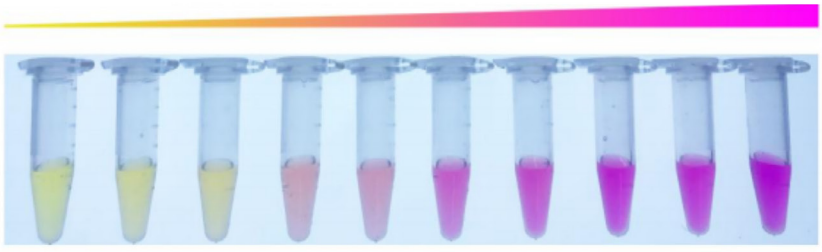
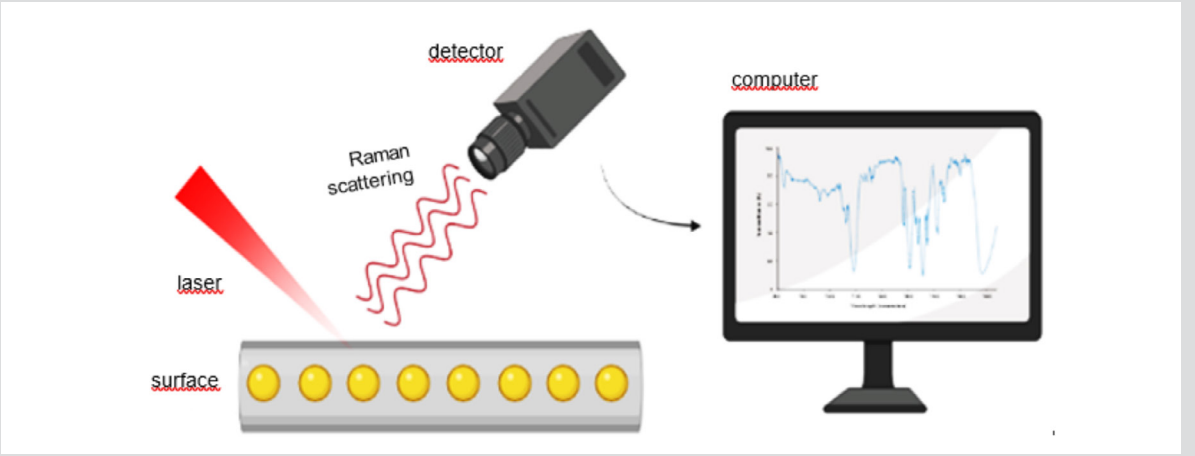


Figure 4. In SERS biosensor, Raman scattering signals are detected using metallic nanoparticles or metallic surfaces at various laser wavelengths.



exosomes in μL with an Au nanoparticle-based colorimetric biosensor for CD63 detection from human serum.

Optical biosensors have come to the fore in biosensor studies for the detection of exosomes due to their remote control and high sensitivity. On the other hand, it is also known that optical biosensors are more costly and more fragile.

c. SERS Biosensor

Surface enhanced Raman spectroscopy (SERS) is a real-time analytical method used in the detection of biomarkers, including exosomes, due to its easy application, label-free use, high sensitivity and specificity, and safety. Analysis is performed with molecules that are absorbed through a rough metal surface such as gold or silver or structures such as plasmonic magnetic nanotubes, as shown in Figure 4, (27). The mechanism largely depends on the combination of surface and molecular target.

When the studies are examined, SERS for the detection of exosomes such as HER 2 (43,44,45), CD9 (46), miRNA-21 (47). Shin et al. (48) reported the specific surface protein compounds of exosomes were analyzed to diagnose cancer. In his study, CD9, CD81, EpCAM and EGFR exosomal biomarkers obtained from lung cancer cell lines were compared with Raman bands. The results of the research has contributed to studies on exosomal surface protein markers for cancer diagnosis.

Another study done by Tian and his coworkers (46) for the diagnosis of liver cancer, it was reported that cholesterol-labeled DNA anchor modified gold nanostars were used for exosome detection from HepG2 (hepatocellular carcinoma) cell line. Target exosomes are captured via magnetic beads and can be deposited on the silica surface. It has been reported that there is a linear relationship between exosome concentration and SERS signal, reaching the detection limit of 27 particles/μL.

2. Electrochemical Biosensor

Electrochemical biosensors are sensors that convert the biochemical cases (enzyme-substrate reaction etc.) that occur during the reaction of the biomolecule with the analyte into electrical signals (voltage, impedance etc.). A typical electrochemical biosensor platforms is illustrated in Figure 5. In this type of sensor, the electrode is used as a support for the immobilization of biomolecules (antibody, aptamers etc.) and electron movement (49).

Electrochemical biosensors are mostly preferred because of their portability, low cost, easy to use and small size (50). However, this type of biosensors has some disadvantages such as sensitivity to temperature change and short shelf life. The glucose sensor used for blood glucose measurement is a typical example of an electrochemical biosensor. Electrochemical biosensors include amperometric (39,51), voltammetric (52,53) and impedimetric (54,55) biosensors. In amperometric sensors, a constant potential is applied

to the electrochemical cells and then the associated current is obtained due to a reduction or oxidation reaction. They measure the concentration-induced current through an electrochemical electrode coated with biologically active material. However, voltammetric biosensors are an electrochemical method that measures current as a function of the potential applied to the electrode.

Li et al. (54) has worked on an impedimetric electrochemical biosensor designi for the detection of tetraspanin and syntenin exosome-specific markers. The exosome detection limits were found to be 1.9 x10^5 particles/mL, and 3–5 pM for tetraspanin and syntenin, respectively.

An aptemer-based electrochemical biosensor was designed by Zhou et al. (56). In the design, the aptamer targeting CD63 was immobilized on the gold surface. They have reported that 100 times lower detection limit (106 particles/mL) was obtained compared to commercial diagnostic kits.

3. Electrochemiluminescence Biosensors

Electrochemiluminescence (ECL) is a method developed to take advantage of electrochemical and chemiluminescent techniques. Directly or indirectly, chemiluminescence is produced when electrically stimulated. In 2021 Liu et al. (57) has developed an electrochemiluminescence biosensor for the detection of phosphatidylserine -positive exosomes for early detection of ovarian cancer. A phosphatidylserine specific binding peptide was immobilized on Au nanoflowers for recognition and capture of exosomes. The engineered biosensor has been reported to outperform most existing methods, with a low limit of detection of up to 39 particles μL^{-1}.

An ECL aptasensor has been developed to detect exosomes from MCF 7 cells by Qiao et al (58). They have reported that the exosomes from breast tumor cells can be detected in the range of 3.4×10^5 to 1.7×10^8 particle/mL under optimum conditions. Also, in the study, known concentration of exosomes isolated from culture medium were added to exosome-free plasma to prove that the produced ECL is clinically applicable. It has been reported that recovery rates vary between 69% and 106% as indicated in Table 1. For example, 4.25 x10^6 exosomes has been added per ml into exosome-free plasma, and 4.12 x10^6 exosomes detected in this complex environment by the ECL sensor with a recovery rate of 96%.

Li et al (59) designed an ECL aptasensor to detect MUC1 protein in breast cancer cells (MCF- 7, SK-BR-3 and MDA-MB-231) and cancer-derived exosomes. CD63 aptamer has been modified on the glass-carbon electrode surface to capture cells and exosomes (Fig. 6). It has been noted to get linear responses in the range of 7.53 - 753μg/mL with a limit of detection of 0.83μg/mL MCF-7 cells, and in the range of 3.22×10^{-4} - 156μg/mL with a limit of detection of 2.73×10^{-4} μg/mL exosomes, respectively. In addition, the expression of MUC1 protein on the surface of MDA-MB-231 and SK-BR-3 cells, and their exosomes have been also analyzed. İt has been indicated the is consistent of MUC1 expression of breast cancer cells and exosomes. They concluded that the designed ECL sensor can also be used in clinical studies.

Conclusion

Early diagnosis and effective treatment methods are among the most important challenges in the fight against cancer. It is known that exosomes, which are responsible for intercellular communication, play a role in many pathological phenomena, including metastasis. We can say that exosomes are a new generation biomarker that has been very popular recently in the early diagnosis of cancer and evaluation of response to treatment. In this study, different biosensor studies developed for the detection of cancer-derived exosomes have been compiled. In the light of the information gathered from the studies examined, the following conclusions were reached:

Figure 5. Scheme of Electrochemical Biosensore. The sample (analyte) to be detected binds to the recognition elements and creates an electrochemical reaction (potentiometry, amperometry, conductometry, impedance). The electrode changes the signal to obtain a readable output.

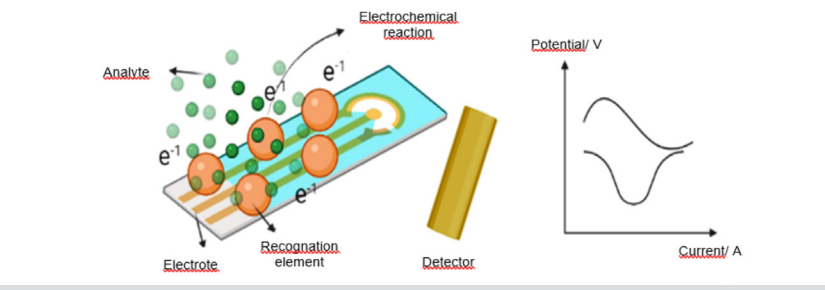
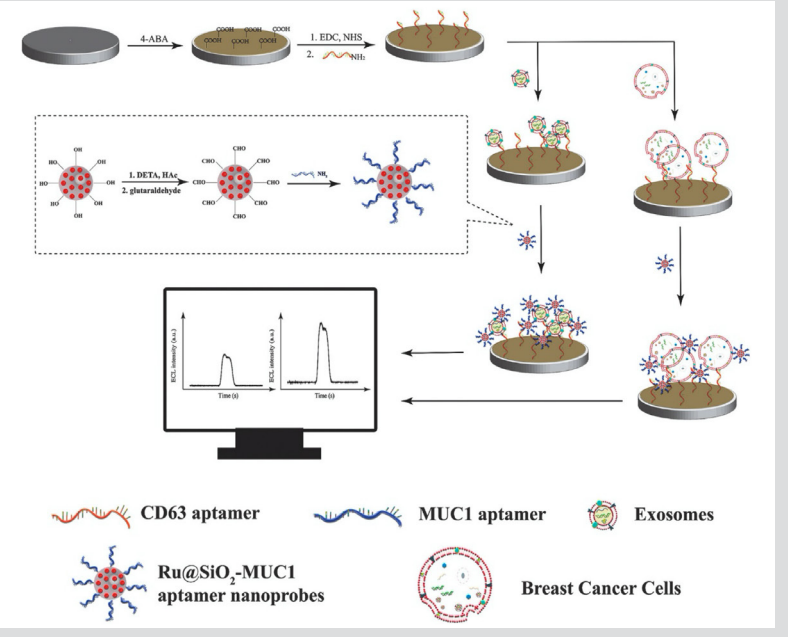


Table 1. Exosome detection in exosome-free plasma (58)

Added (particle/mL)	Count (particle/mL)	Recovery (%)
4.25×10^6	4.12×10^6	96.97
1.7×10^6	1.66×10^6	97.65
8.5×10^5	7.75×10^5	91.18
4.25×10^5	4.53×10^5	106.6

- Rapid and reliable detection of cancer-derived exosomes will facilitate early detection of cancer, which is the second leading cause of death worldwide. We can say that there are high- sensitivity devices that can be used especially in regions with low resources such as underdeveloped and developing countries, in the home healthcare environment and in emergency situations. Increasing interest in point-of-care tests increases the need for fast, reliable and sensitive biosensors.
- It is very promising that new nanomaterials that can be easily synthesized and designed and used in exosome detection are designed. With these advances in nanomaterials, new types of biosensors have been developed with significant advantages such as high sensitivity, high efficiency and fast operation in the detection of exosomes. In most cases, biosensor signals are directly dependent on the stability of nanomaterials. Therefore, we can say that there is a need for studies to increase stability.
- Studies have shown that it is important to measure exosome markers at a very low detection limit in body fluids such as serum, plasma, and urine.
- With the development of biosensor studies, rapid and early detection of cancer types whose detection usually coincides with the metastatic stage and whose survival rate is known to be low will be achieved. It is possible to say that the cost per test will be significantly reduced due to reasons such as reduced reagent consumption, less sample volume requirement and being fully automated. We can say that biosensor studies that allow multiplex exosome detection will increase in the coming years and will find use in the clinic.

Figure 6. ECL aptasensor for the detection of MUC1 protein on breast cancer cells and their derived exosomes (59). For design, a Glassy carbon electrode (GCE) has treated in HCl solution containing 4-ABA to obtain a 4-ABA/GCE electrode. After activating the carboxylic acid group with the 4-ABA/GCE electrode, CD63 aptamers have been added to the medium and immobilized to the surface via NH2-COOH. After washing with PBS, exosome/MCF 7 cells have been added to the medium. Then, 6.5μL Ru@SiO2 - MUC1 aptamer nanoprobes have been dropped onto the surface.



Received Date/Geliş Tarihi: 14.06.2021
Accepted Date/Kabul Tarihi: 09.07.2021

Referance

1. Villarreal-Gómez, L. J., Soria-Mercado, I. E., Hernandez-Gómez, M., & Giraldi, R. G. (2015). Detection of molecular markers of cancer through the use of biosensors. Biol Med (Aligarh) S,2,
2. Jainish, P., & Prittesh, P. (2017). Biosensors and biomarkers: promising tools for cancer diagnosis. Int J Biosen Bioelectron, 3(4), 00072.
3. Makaju, S., Prasad, P. W. C., Alsadoon, A., Singh, A. K., & Elchouemi, A. (2018). Lung cancer detection using CT scan images. Procedia Computer Science, 125, 107-114.
4. Schoots, I. G. (2018). MRI in early prostate cancer detection: how to manage indeterminate or equivocal PI-RADS 3 lesions?. Translational andrology and urology, 7(1), 70.
5. Babayan, A., & Pantel, K. (2018). Advances in liquid biopsy approaches for early detection and monitoring of cancer. Genome medicine, 10(1), 1-3.
6. Zhu, C. Z., Ting, H. N., Ng, K. H., & Ong, T. A. (2019). A review on the accuracy of bladder cancer detection methods. Journal of Cancer, 10(17), 4038.
7. Weinstein, D., Leininger, J., Hamby, C., & Safai, B. (2014). Diagnostic and prognostic biomarkers in melanoma. The Journal of clinical and aesthetic dermatology, 7(6), 13.
8. Zhang, Y., Liu, Y., Liu, H., & Tang, W. H. (2019). Exosomes: biogenesis, biologic function and clinical potential. Cell & bioscience, 9(1), 19.
9. Lorencova, L., Bertok, T., Bertokova, A., Gajdosova, V., Hroncekova, S., Vikartovska, A., ...Tkac, J. (2020). Exosomes as a Source of Cancer Biomarkers: Advances in Electrochemical Biosensing of Exosomes. ChemElectroChem
10. Logozzi, M., Mizzoni, D., Angelini, D. F., Di Raimo, R., Falchi, M., Battistini, L., & Fais, S. (2018). Microenvironmental pH and exosome levels interplay in human cancer cell lines of different histotypes. Cancers, 10(10), 370.
11. McNicholas, K., & Michael, M. Z. (2017). Immuno-characterization of exosomes using nanoparticle tracking analysis. In Exosomes and Microvesicles (pp. 35-42). Humana Press, New York, NY.
12. Lyu, T. S., Ahn, Y., Im, Y. J., Kim, S. S., Lee, K. H., Kim, J., ... & Cho, J. A. (2021). The characterization of exosomes from fibrosarcoma cell and the useful usage of Dynamic Light Scattering (DLS) for their evaluation. Plos one, 16(1), e0231994.
13. Raghu, D., Christodoulides, J. A., Christophersen, M., Liu, J. L., Anderson, G. P., Robitaille, M., ... & Raphael, M. P. (2018). Nanoplasmonic pillars engineered for single exosome detection. PloS one, 13(8), e0202773.
14. Nikishin, I., Dulimov, R., Skryabin, G., Galetsky, S., Tchevkina, E., & Bagrov, D. (2021). ScanEV–A neural network-based tool for the automated detection of extracellular vesicles in TEM images. Micron, 145, 103044.
15. Khodashenas, S., Khalili, S., & Moghadam, M. F. (2019). A cell ELISA based method for exosome detection in diagnostic and therapeutic applications. Biotechnology letters, 41(4), 523-531.
16. Ma, M., Li, B., Zhang, M., Zhou, L., Yang, F., Ma, F., ... & Zhang, X. (2020). Therapeutic effects of mesenchymal stem cell-derived exosomes on retinal detachment. Experimental eye research, 191, 107899.
17. Jankovičová, J., Sečová, P., Michalková, K., & Antalíková, J. (2020). Tetraspanins, More than Markers of Extracellular Vesicles in Reproduction. International Journal of Molecular Sciences, 21(20), 7568.
18. Jiang, C., Yao, Y., Cai, Y., & Ping, J. (2019). All-solid-state potentiometric sensor using single-walled carbon nanohorns as transducer. Sensors and Actuators B: Chemical, 283, 284- 289.
19. Kokkinos, C., & Economou, A. (2020). Recent advances in voltammetric, amperometric and ion-selective (bio) sensors fabricated by microengineering manufacturing approaches. Current Opinion in Electrochemistry, 23, 21-25.
20. Girigoswami, K., & Akhtar, N. (2019). Nanobiosensors and fluorescence based biosensors: An overview. International Journal of Nano Dimension, 10(1), 1-17.
21. Liu, B., Zhuang, J., & Wei, G. (2020). Recent advances in the design of colorimetric sensors for environmental monitoring. Environmental Science: Nano, 7(8), 2195-2213.
22. Pol, L., Acosta, L. K., Ferré-Borrull, J., & Marsal, L. F. (2019). Aptamer-based nanoporous anodic alumina interferometric biosensor for real-time thrombin detection. Sensors, 19(20), 4543.
23. Pohanka, M. (2018). Overview of piezoelectric biosensors, immunosensors and DNA sensors and their applications. Materials, 11(3), 448.
24. Gaddes, D., Reeves, W. B., & Tadigadapa, S. (2017). Calorimetric biosensing system for quantification of urinary creatinine. ACS sensors, 2(6), 796-802.
25. Williams, T. C., Pretorius, I. S., & Paulsen, I. T. (2016). Synthetic evolution of metabolic productivity using biosensors. Trends in biotechnology, 34(5), 371-381.
26. Chen, N., Guo, W., Lin, Z., Wei, Q., & Chen, G. (2018). Label-free sensitive luminescence biosensor for immunoglobulin G based on Ag6Au6 ethisterone cluster-estrogen receptor α aggregation and graphene. Talanta, 185, 243-248.
27. Pang, Y., Wang, C., Lu, L., Wang, C., Sun, Z., & Xiao, R. (2019). Dual-SERS biosensor for one-step detection of microRNAs in exosome and residual plasma of blood samples for diagnosing pancreatic cancer. Biosensors and Bioelectronics, 130, 204-213.
28. Sina, A. A. I., Vaidyanathan, R., Wuethrich, A., Carrascosa, L. G., & Trau, M. (2019). Label-free detection of exosomes using a surface plasmon resonance biosensor. Analytical and bioanalytical chemistry, 411(7), 1311-1318.
29. Kim, T. J., Türkcan, S., & Pratx, G. (2017). Modular low-light microscope for imaging cellular bioluminescence and radioluminescence. Nature protocols, 12(5), 1055-1076.
30. Hananya, N., Reid, J. P., Green, O., Sigman, M. S., & Shabat, D. (2019). Rapid chemiexcitation of phenoxy-dioxetane luminophores yields ultrasensitive chemiluminescence assays. Chemical science, 10(5), 1380-1385.
31. Viter, R., Savchuk, M., Starodub, N., Balevicius, Z., Tumenas, S., Ramanaviciene, A., ... & Ramanavicius, A. (2019). Photoluminescence immunosensor based on bovine leukemia virus proteins immobilized on the ZnO nanorods. Sensors and Actuators B: Chemical, 285, 601-606.
32. Thacharodi, A., Jeganathan, C., & Thacharodi, D. (2019). Biomonitoring of heavy metal pollution by bioluminescent bacterial biosensors. Indian J Sci Technol, 15, 12-20.
33. Zhang, P., He, M., & Zeng, Y. (2016). Ultrasensitive microfluidic analysis of

circulating exosomes using a nanostructured graphene oxide/polydopamine coating. Lab on a Chip, 16(16), 3033-3042.

34. Zhang, H., Wang, Z., Zhang, Q., Wang, F., & Liu, Y. (2019). Ti3C2 MXenes nanosheets catalyzed highly efficient electrogenerated chemiluminescence biosensor for the detection of exosomes. Biosensors and Bioelectronics, 124, 184-190.

35. Huang, L., Wang, D. B., Singh, N., Yang, F., Gu, N., & Zhang, X. E. (2018). A dual- signal amplification platform for sensitive fluorescence biosensing of leukemia-derived exosomes. Nanoscale, 10(43), 20289-20295.

36. Piliarik, M., Vaisocherová, H., & Homola, J. (2009). Surface plasmon resonance biosensing. Biosensors and Biodetection, 65-88.

37. Grasso, L., Wyss, R., Weidenauer, L., Thampi, A., Demurtas, D., Prudent, M., ... & Vogel, H. (2015). Molecular screening of cancer-derived exosomes by surface plasmon resonance spectroscopy. Analytical and bioanalytical chemistry, 407(18), 5425-5432.

38. Hosseinkhani, B., van den Akker, N., D'Haen, J., Gagliardi, M., Struys, T., Lambrichts, I., et al., 2017. Direct detection of nano-scale extracellular vesicles derived from inflammation- triggered endothelial cells using surface plasmon resonance. Nanomed. Nanotechnol. Biol. Med. 13 (5), 1663–1671.

39. Park, J., Lin, H.-Y., Assaker, J.P., Jeong, S., Huang, C.-H., Kurdi, A., et al., 2017. Integrated kidney exosome analysis for the detection of kidney transplant rejection. ACS Nano 11 (11), 11041–11046.

40. Xia, Y., Liu, M., Wang, L., Yan, A., He, W., Chen, M., ... & Chen, J. (2017). A visible and colorimetric aptasensor based on DNA-capped single-walled carbon nanotubes for detection of exosomes. Biosensors and Bioelectronics, 92, 8-15.

41. Yang, Y., Li, C., Shi, H., Chen, T., Wang, Z., Li, G., 2019. A pH-responsive bioassay for paper-based diagnosis of exosomes via mussel-inspired surface chemistry. Talanta 192, 325–330.

42. Oliveira-Rodríguez, M., Serrano-Pertierra, E., García, A.C., Martín, S.L., Mo, M.Y., Cernuda-Morollon, E., et al., 2017. Point-of-care detection of extracellular vesicles: sensitivity optimization and multiple-target detection. Biosens. Bioelectron. 87, 38–45.

43. Zong, S., Wang, L., Chen, C., Lu, J., Zhu, D., Zhang, Y., et al., 2016. Facile detection of tumor-derived exosomes using magnetic nanobeads and SERS nanoprobes. Analytical Methods 8 (25), 5001–5008.

44. Weng, Z., Zong, S., Wang, Y., Li, N., Li, L., Lu, J., et al., 2018. Screening and multiple detection of cancer exosomes using an SERS-based method. Nanoscale 10 (19), 9053–9062.

45. Kwizera, E.A., O'Connor, R., Vinduska, V., Williams, M., Butch, E.R., Snyder, S.E., et al., 2018. Molecular detection and analysis of exosomes using surface-enhanced Raman scattering gold nanorods and a miniaturized device. Theranostics 8 (10), 2722–2738.

46. Tian, Y. F., Ning, C. F., He, F., Yin, B. C., & Ye, B. C. (2018). Highly sensitive detection of exosomes by SERS using gold nanostar@ Raman reporter@ nanoshell structures modified with a bivalent cholesterol-labeled DNA anchor. Analyst, 143(20), 4915-49-22.

47. Ma, D., Huang, C., Zheng, J., Tang, J., Li, J., Yang, J., et al., 2018. Quantitative detection of exosomal microRNA extracted from human blood based on surface-enhanced Raman scattering. Biosens. Bioelectron. 101, 167–173.

48. Shin, H., Jeong, H., Park, J., Hong, S., & Choi, Y. (2018). Correlation between cancerous exosomes and protein markers based on surface-enhanced Raman spectroscopy (SERS) and principal component analysis (PCA). ACS sensors, 3(12), 2637-2643.

49. Seifati, S.M., Nasirizadeh, N., Azimzadeh, M., 2017. Nano-biosensor based on reduced graphene oxide and gold nanoparticles, for detection of phenylketonuria-associated DNA mutation. IET Nanobiotechnol. 12 (4), 417–422

50. Im, H., Yang, K.S., Lee, H., Castro, C.M., 2018. Nanotechnology Platforms for Cancer Exosome Analyses. Diagnostic and Therapeutic Applications of Exosomes in Cancer. Elsevier, pp. 119–128.

51. Doldan, X., Fagúndez, P., Cayota, A., Laíz, J., Tosar, J.P., 2016. Electrochemical sandwich immunosensor for determination of exosomes based on surface markermediated signal amplification. Anal. Chem. 88 (21), 10466–10473.

52. Boriachek, K., Islam, M.N., Gopalan, V., Lam, A.K., Nguyen, N.T., Shiddiky, M.J.A., 2017. Quantum dot-based sensitive detection of disease specific exosome in serum. Analyst 142 (12), 2211–2219

53. Yadav, S., Boriachek, K., Islam, M.N., Lobb, R., M€oller, A., Hill, M.M., et al., 2017. An electrochemical method for the detection of disease-specific exosomes. ChemElectroChem 4 (4), 967–971.

54. Li, Q., Tofaris, G.K., Davis, J.J., 2017. Concentration-normalized electroanalytical assaying of exosomal markers. Anal. Chem. 89 (5), 3184–3190.

55. Kilic, T., Valinhas, A.T.D.S., Wall, I., Renaud, P., Carrara, S., 2018. Label-free detection of hypoxia-induced extracellular vesicle secretion from MCF-7 cells. Sci. Rep. 8 (1), 9402.

56. Zhou Q, Rahimian A, Son K, Shin DS, Patel T, Revzin A. Development of an aptasensor for electrochemical detection of exosomes. Methods. 2016;97:88–93.

57. Liu, X., Wang, Q., Chen, J., Chen, X., & Yang, W. (2021). Ultrasensitive electrochemiluminescence biosensor for the detection of tumor exosomes based on peptide recognition and luminol-AuNPs@ g-C3N4 nanoprobe signal amplification. Talanta, 221, 121379.

58. Qiao, B., Guo, Q., Jiang, J., Qi, Y., Zhang, H., He, B., ... & Shen, J. (2019). An electrochemiluminescent aptasensor for amplified detection of exosomes from breast tumor cells (MCF-7 cells) based on G-quadruplex/hemin DNAzymes. Analyst, 144(11), 3668-3675.

59. Li, R., An, Y., Jin, T., Zhang, F., & He, P. (2021). Detection of MUC1 protein on tumor cells and their derived exosomes for breast cancer surveillance with an electrochemiluminescence aptasensor. Journal of Electroanalytical Chemistry, 882, 115011.