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Enhancement of Early-Stage Cancer Detection by Liquid Biopsy using Nanoparticle Platforms

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ABSTRACT

Cancer has consistently been a public health burden for many decades, responsible for high mortality and morbidity rates worldwide. Effective cancer diagnostic methods are crucial to improving patients' survival and timely implementation of therapeutic and surgical interventions. Current diagnostic technologies are much more efficient in detecting cancer at late stages (stages 3 and 4) than early stages (stages 1 and 2), typically in solid tumor-based cancers. Meanwhile, cancer metastasis is a prominent feature of late stages, which poses a significant barrier to the effectiveness of therapeutic and surgical interventions. Thus, detecting early-stage cancer is crucial when the disease remains within the primary site. Though liquid biopsy has gained attention for noninvasive and low-cost diagnosis, cancer biomarkers present at early stages are at concentrations too low for conventional methods to detect with specificity. The low concentrations of early-stage cancer biomarkers pose a significant barrier in diagnosing patients with high sensitivity and specificity. This drawback can be addressed through an innovative combination of diagnostic nanoparticle platforms with liquid biopsy procedures. The strategy has been demonstrated to remarkably improve the efficiency of detecting early-stage biomarkers at the lowest concentration possible, i.e., decreasing the limit of detection (LOD). This review will assess the utility of various nanoparticle platforms in developing novel diagnostic techniques for early-stage cancer detection.

Keywords: early-stage cancer, gold nanoparticles, quantum dots, magnetic nanoparticles

INTRODUCTION

Nanotechnology or nanoparticle (NP) technology revolves around multidisciplinary sciences like chemistry, biochemistry, physics, biology, and material sciences. These particles can be described as minute entities of varying shapes and sizes (<1 nm to 1000 nm) fabricated from different materials such as lipids, polymers, semiconductors, non-metal, and metals.[1,2] NPs can be synthesized into different architectures, such as hollow particles, multilayer, porous particles, and many more, based on the requirement. In addition, the chemical and biological properties of the NPs can be modified to suit delivery of various therapeutics such as drugs, peptides, genes,[3-5] diagnostics,[6] or imaging agents.[7] It can be that NPs improve a drug's pharmacokinetic profile and decrease any adverse reactions compared to the administration of drugs alone. In addition, they can reduce additional healthcare requirements and costs for any disorder. Modifying the surface with complementary targets can be used as a guided missile against the diseased cell, e.g. cancer cells.[8] There are many FDA-approved drug-loaded NPs for various disorders. Aside from drug delivery and nano-theranostic platform, NPs can also be used for diagnostic purposes using liquid biopsy samples, which is the focus of this review. Liquid biopsy samples may include blood, saliva, sputum, cerebral spinal fluid (CSF), urine, and breast milk.

Using these properties to our advantage, desired NPs have been previously used to enhance the therapeutic potential of their contents, i.e., drugs with poor pharmacokinetic parameters, such as increasing solubility, enhancing bioavailability, modifying release characteristics, and extending their life cycle. The biomedical application of NPs can range from targeted therapies, theranostics, i.e., pairing diagnostic biomarkers with therapeutic agents,[9] to contrast agents for imaging.[10] Based on the modification of NPs for diagnostics, various detection methods can be applied, such as optical, magnetic, mechanical, physical, and biochemical properties that arise from the following factors: the manner of synthesis, size, shape, and surface properties.[11]

Current methods to screen cancers include the Papanicolaou test (cervical cancer),[12] mammography (breast cancer),[13] endoscopy for polyp and occult blood detection for colon cancer,[14] computed tomography (CT), low dose CT (LDCT), X-ray, ultrasound imaging, magnetic resonance imagining (MRI), and tissue biopsy.[15-18] These approaches are either invasive, cause patient discomfort, risk radiation exposure, result in a financial burden, and/or do not meet the criteria required for the early-stage detection.[19] Recently, with the help of liquid biopsy, specific molecules, or biomarkers, have been utilized to detect cancer in the early stage faster and more accurately. Liquid biopsy is a diagnostic procedure that involves the analysis of the patient's fluid sample such as blood (serum or plasma), sputum, urine, breast milk, and cerebrospinal fluid (CSF) for biological markers pertaining to a specific disorder, i.e., cancer.[20-22] Our blood is comprised of abundant biomolecules, which can provide a plethora of information regarding the body's physiological and pathophysiological functioning. Examples of biological materials or biomarkers involve circulating tumor cells (CTCs), platelets, extracellular vesicles (EV), mRNA, miRNA, protein and/or post-translational modified proteins, and cell-free DNA (cfDNA), and in case of cancer, circulating tumor DNA (ctDNA) and many more.[23-28]

Biomarker	Type of Cancer	Reference
miR-21, 25, 155	Lung cancer	96,97
CEA	Lung, ovarian, colon	82,98,99
CA 19-9	Colon	99
PSA	Prostate	100
CA- 125	Lung, ovarian	98,101
miR-27b	Cervical	102
Mucin 1	Pancreatic, colon	93,99
Exosomes	Lung, ovarian, melanoma, glioblastoma	103-106
Long-non-coding RNA GAS5	Lung	107
miR-223	Lung	108
CYFRA 21-1	Lung	109

Table 1. A representative list of biomarkers reported for early-stage cancer diagnosis.

Biomarkers can objectively measure and evaluate normal and abnormal biological processes. Cancer research has been using this method to obtain information regarding the tumorigenic process occurring in the body and to provide the best possible treatment for the patient. Their presence in biological fluids can prove advantageous for detection purposes and cost-effectiveness (Table 1).[23,29-35] However, these biomarkers can sometimes be present at low concentrations for detection, especially in the early stages of any cancer. Using NP platforms, the diagnostic efficiency of the biomarkers can be markedly enhanced such that trace quantities can be detected and quantified (lowering the limit of detection: LOD). Aside from specific protein and nucleic acids-based biomarkers, EV, specifically exosomes, have been previously reported to differentiate between normal and cancer cells based on its cargo. Exosomes are one of the many classifications of EV and are a minute (30-200 nm) lipid vesicle enveloping a variety of cargo such as proteins, nucleic acid, enzymes, peptides, and more. They play a crucial role in cell-to-cell communication in both standard and abnormal conditions.[36,37] This review will focus on how NP technology has been applied in detecting cancer in the early stages (1 or 2) using biomarkers and EVs. In addition, the application of this platform can be used to lower the LOD for selected biomarkers, thereby enabling detection at lowest concentration, leading to an improvement in diagnostic efficiency.

When using NPs for drug delivery purposes, it is of utmost importance that these particles and their respective modifications are non-reactive and biocompatible. However, in the case of diagnosing using a patient sample, such a parameter is not considered necessary since such systems are not being injected into the body. Using this platform, NPs can be tagged or conjugated with the protein or nucleic acid that complements the target protein or nucleic acid in the patient sample. Upon interaction, the NP complexes can emit luminescence, supermagnetism, or fluorescence/exhibit colorimetric changes (Figure 1). These minute particles can offer sensitivity, decrease the LOD, are user-friendly, time-saving, and cost-effective solutions for molecular diagnostics.[7,23] They can be used to detect various disorders, especially early-stage cancer.

Cancer is a heterogeneous complex disease involving abnormal cell growth and proliferation. Its etiology has been associated with external factors (lifestyle, air pollution, exposure to chemicals) and/or genetic (upregulation of oncogenes or downregulation of tumor suppressors).[38-42] Cancer is one of the many significant health problems worldwide, being the second leading cause of death in the United States. Reports have estimated about 2 million new cases, of which 600,000 have led to morbidity in 2022.[43] To tackle high incidences and death rates, early-stage cancer detection is very important,[44] since it can lead to improved outcomes and lower medical costs while

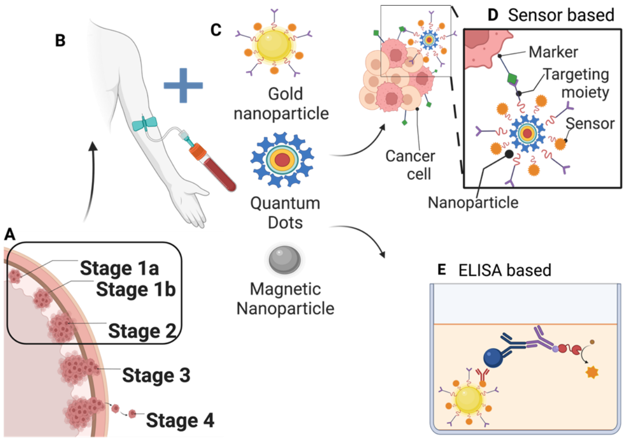

Figure 1. A-B) An illustration of different nanoparticle platforms that are applied in early-stage cancer detection. C-D) Samples for liquid biopsy (blood) can be derived from patients with different stages of cancer. Various nanoparticles such as gold, magnetic nanoparticles, and quantum dots can be applied for diagnostic purposes.

decreasing the need for complicated surgical and therapeutic interventions. Based on their origin, cancers can be segregated into liquid types (lymphoma, leukemia, etc.) and solid (lung, brain, breast, etc.). This review will focus on the early-stage detection of solid type cancers with the help of biomarkers present in bodily fluids. During the early stages of solid type cancers (1 & 2), uncontrolled proliferation occurs within the tissue border of the primary organ site.[30,45] During the metastatic phase, the cancerous cells disseminate and travel to other sites, such as nearby lymph nodes or organs like bone, brain, lung, and liver via lymphatic or blood circulation (stage 3 or 4).[46] The overall survival for patients decreases drastically with disease progression due to the following: inability to cope with the treatment regimen or surgery, drug resistance, relapse, tumor cells spreading and occupying major organs, inability to locate dormant tumors, or untreatable tumors due to their anatomic locations. Hence, the survival of cancer patients depends heavily on early detection before metastasis occurs. Thus, the requirement for a more sensitive, specific, and accurate early-stage detection system is in dire need.[15,23,45,47] This review will discuss the applications of gold, magnetic nanoparticles, and quantum dots in early-stage detection.

GOLD NANOPARTICLES

Gold has been of great use and value over the centuries, both ornamentally and medicinally. For many decades, gold nanoparticles (GNPs) have been widely used for diagnostics, therapeutics, or theranostic purposes.[38] GNPs have been reported to be inert and can be synthesized in desired size and shape. Gold's surface chemistry can be modified to add or conjugate various molecules such as ligands, antibodies, peptide aptamers, and cell-derived materials. This surface functionalization on GNPs allows increased cellular binding affinity through active targeting, which aids in either detecting the desired biomarker or in targeted therapy.[2,48-51] Due to their variable size and shape, GNPs have been reported to have increased the permeability retention (EPR) effect, allowing accumulation in the target tissue or organ. In addition, GNPs are biocompatible and do not cause any significant cytotoxic effect. In the past few decades, GNPs have also emerged as a promising method for

Nanoparticle Type	Type of Cancer	Biomarker of Interest/Specific technique	Reference
Gold nanoparticle	Oral	Microneedles & ultrasound to increase GNP detection	110
Gold nanoparticle	Ovarian	CA-125	111
Gold nanocluster	Oral	Mildly acidic tumor microenvironment would disassemble acid degradable gold nanocluster, which increases detection ability	112
Reduced graphene oxide, multiwalled carbon nanotubes & gold nanoparticles	Cervical	Biosensor tagged with DNA strand complementary to DNA extracted from HPC-18 patients	113
Magnetic nanoparticle	Ovarian cancer	CA-125, beta-2 microglobulin, apolipoprotein A1	114
Quantum Dots	Breast (HER2+)	Exosomes	115
Magnetic Nanoparticle	Colorectal Cancer	Methylation levels of CRC biomarker mSEPT9	116

Table 2. A representative list of nanoparticle platforms for early-stage cancer diagnosis.

optimizing the early detection of cancer.[38,51-54]

When used with different sensor platforms, GNPs widened the detection range and lowered the detection limit for cancerous biomarkers.[55-60] In doing so, these GNP sensors can achieve higher levels of sensitivity and specificity in detecting cancerous biomarkers compared to conventional methods.[55-60] For instance, a sensor was fabricated using the sandwich immunoassay principle to detect cancer biomarkers: prostate-specific antigen (PSA), a common biomarker for detecting prostate cancer. A combination of GNPs (~50 nm) functionalized with capture antibody (GNP-Ab2) and photon-up conversion fluorescent nanoparticle-based optical sensor with detection antibody (UCNPs-Ab1) was used.[58] The GNP-Ab2 and UCNP-Ab1 sandwiched the target antigen present in the human serum sample by forming an immune complex, resulting in fluorescence emission. The design is based on the luminescence resonance energy transfer (LRET) between UCNPs- Ab1 & GNP- Ab2 (Figure 2). The LOD is 1.0 pM compared to the conventional PSA assay which is 2.3 pM, exhibits high specificity and sensitivity of immunoreaction where no interference from larger macromolecules such as IgG has been observed. The PSA sensor has a detection limit of 2.3 pM, after which fluorescent quenching is observed with an increase in target antigen concentration. In contrast, traditional methods have been reported to detect within the range of 0.003 to 0.2 ng/mL.[61] To assess the specificity of the nanoprobe, it was mixed with a pool of macromolecules and ions commonly present in the blood stream that can cause possible interference alongside with PSA antigen, such as human IgG, human serum albumin (HAS), Na+, and K+. The results showed interference from individual molecules and/or ions alone, indicating selectivity towards the target antigen. Furthermore, this sensor proved to be accurate due to its ability to recover more than 96% when spiked with serum sample containing different concentrations of PSA.[58] The specific, sensitive, and accurate immunoreaction between UCNPs-Ab1 and GNPs-Ab2 to its target antigen concluded to be effective system in detecting its respective cancer biomarker for clinical application.

Similarly, GNPs combined with multiwalled carbon nanotubes-graphene and quantum dots, were fixed on a glass carbon electrode and modified by conjugating with PSA antibodies. The analytical performance of this immunosensor exhibited a linear relationship between the change in PSA concentrations (1-10000 pg/mL) and impedance change, where upon binding of the antigen to the electrode surface, there is a decrease in the electron transfer (E-) between the redox probe and the electrochemical double layer leading to an increase in the electron transfer resistance for the probe to access the double layer (Figure 3). It also reduced the LOD to 0.48 pg/mL and, upon exposure to a pool of various macromolecules found in the blood (CEA, alpha fetal protein (AFP), glucose, PSA, and IgG) revealed an increase in the impedance upon binding to PSA. An essential feature of this label-free immunosensor includes long-term stability.[56] In both cases, GNPs were particularly used not only for their surface-modifiable property for immobilizing biomolecules but for their ability to accelerate direct electron transfer between redox probes and electrode surface. Due to which, the sensors were able to detect low levels of PSA and may provide a potential for an early diagnosis of prostate cancer with the help of these highly sensitive and specific sensors.

In another instance, a GNP-based nano-geosensor (GNP-NG) was fabricated by reacting aurous chloride and cystamine HCl solution, followed by the addition of sodium borohydride, resulting in a cysteamine-capped gold nanoparticle, which was then fixed and activated on a glassy carbon working electrode with a well-aligned DNA monolayer (ss-probe) (Figure 3).[62] Previous reports indicated that miR-25 enhanced cell migration and invasion in non-small cell lung cancer and its concentration increases as cancer progresses into advanced stages. In addition, miR-25 has also been correlated with poor patient outcomes.[63,64] The GNP-NG was reported to distinguish between a miR-25 with or without a single base mutation based on the principle of hybridization between the ss-probe and the target miR using electrochemical impedance spectroscopy (EIS). This nano-geosensor upon hybridizing with its target miR, had a decrease in the electron transfer leading to an increase in the charge transfer resistance (Rct). It resulted that the total Rct was directly proportional to the log of miRNA concentration and in addition could target miR-25 with a LOD of 0.25 pM directly from the blood plasma sample without requiring sample extraction or amplification (PCR) in plasma derived from early-stage lung cancer patients. The sensor was also investigated by exposing it to a mixture of molecules that are both complementary, non-complementary, and a one-based mismatched target for its selectivity. The electrochemical signal readings indicated that hybridization of the ss-probe occurred only with the complementary target, indicating that the sensor is sensitive and selective.[62] Traditional methods to detect miR-25 involve miR isolation followed by qPCR for quantitative assessment; such techniques can lead to sample loss, tedious procedures, and are not cost -effective.

In another study, GNPs were synthesized in the shape of a superlattice, which was used to improve conductivity and accelerate electronic transmission and combined with a cationic dye: toluidine blue (TB) and capture miR-21 complementary sequence. The dye was employed to enable the binding of miRNA, because of which combination has been used as a signal amplifier to detect miR-21 concentrations ranging from 100 aM to 1 nM and resulted in a detection limit of 78 aM. The signal can read as a decrease in the current due to the steric hindrance of the electron transfer after the target miR hybridized with the capture sequence. When pooled with other macromolecules found in the serum,

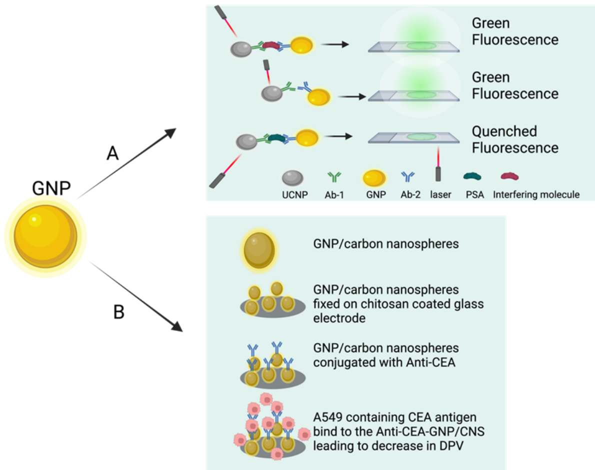

Figure 2. The application of GNPs in detecting cancer-specific biomarkers for early-stage detection using fluorescence and DPV. A) Upconversion nanoparticle tagged with capture anti-PSA and GNP detection anti-PSA. Quenched fluorometric detection of PSA antigen in the serum sample is measured[58] B) GNPs and carbon nanospheres fixed on a glass electrode with anti-CEA tag for detecting CEA protein present on A549 cell surface, measured in terms of changes in DPV[67]

the sensor did not respond to interfering species (CEA, AFP, and CA15-3) (Figure 3). This label-free electrochemical sensor shows promise due to its high sensitivity and selectivity for early-stage detection of cancerous microRNAs in breast cancer patients.[60] Previous reports have stated that miR-21 relative expression increases as the disease progresses compared to miR-21 levels in non-carcinogenic cells.

Aside from miR, conventional protein markers such as carcinoembryonic antigen (CEA) and carcinoma antigen 125 (CA-125) have been used for clinical diagnosis against different cancers such as lung, ovarian, breast, etc.[65,66] Against non-small cell lung cancer (NSCLC), an electrochemical cyto-sensor was synthesized, comprising a self-assembled monodisperse colloidal carbon nanosphere (CNSs) coated with GNPs and placed on a chitosan film-coated glass carbon electrode. This cyto-sensor was immobilized with an antibody to detect NSCLC biomarker; CEA present within the NSCLC cell line, i.e., A549. The dynamic incubation of cyto-sensor with A549 cell lines observed a decrease in the differential pulse voltammetry (DPV), indicating a shielding effect of the A549 cell line. In addition, an inversely proportional relationship was resulting between the DPV and A549 cell density. Aside from A549 cell lines, other carcinogenic cells were assessed against this cytosensor as well, such as MRC-5 cells (human fetal lung) and Hela cells (human cervical cancer). There was no change in the DPV signaling when incubated with MRC-5 and Hela cells when compared to A549 cells, indicating that the cytosensor is specific. This study reported a LOD of 14 cells/mL.[67] In addition, conventional immunoassay methods can measure the concentration of CEA circulating in bodily fluids such as serum or plasma at a concentration range of 2-15 ng/mL[68] (Figure 2).

After careful consideration of the examples provided, it can be concluded that GNPs show excellent promise as an electrochemical sensor for the early detection of cancers, since GNPs aid in electron transfer, conductivity, and stability of biomolecules conjugation.[2] Many researchers have employed aptamers and other ligands to facilitate the detection of cancerous makers aiding in the early-stage detection.[49,69-72] These sensors have been modified to enhance the sensitivity and specificity of cancerous biomarker detection by widening the detection range and lowering the lower detection limit.

QUANTUM DOTS

Quantum dots (QDs) or artificial atoms are semiconductor nanocrystals with optical and electronic properties. These minute structures consist of tunable and efficient photoluminescence with narrow emission, photochemical stability, and core-shell structures. QDs are commonly used in many devices and appliances like computers, phones, etc.[73] These nanostructures have also been utilized in cancer research for molecular imaging. When QDs have tagged biomolecules such as antibodies, ligands, aptamers, etc., complementing the desired target molecule, they can be used to target cancer cells with high sensitivity and specificity.[45,74] For instance, QDs in this study were used for their shift fluorescent properties.[75] PSA is a common biomarker for detecting prostate cancer as mentioned above, and the diagnostic capability can be increased by sandwiching the target antigen between a cadmium selenium/zinc sulfate QD (CdSe/ZnS QDs) conjugated capture anti-PSA antibody and biotinylated anti-PSA with streptavidin and organic dye (Figure 4).[76] Upon forming an immunocomplex, it will emit a fluorescent signal that was analyzed via flow cytometry. In this study, male serum samples from patients with different stages of prostate cancer, benign prostatic hyperplasia, and healthy patients were collected and assessed for their respective PSA levels. A fluorescence shift from the orange to the red region was observed upon incubation with PSA-positive samples, whereas no signal was detected in samples obtained from healthy donors. The lowest LOD detected of free and total PSA concentration using the QD-based microassay resulted in 0.067 and 0.12 ng/mL, with an average detection rate of 89 and 92%, respectively.[76] In another study, glucose -derived CDQs/gold nanocomposites (CDQ/GNC) were used as stabilizing agent and as a reducing agent for immunosensing target antigen; carbohydrate antigen 19-9 (CA19-9) biomarker in pancreatic cancer samples. The CDQ/GNC were immobilized by tagging horseradish peroxidase enzyme labeled CA 19-9

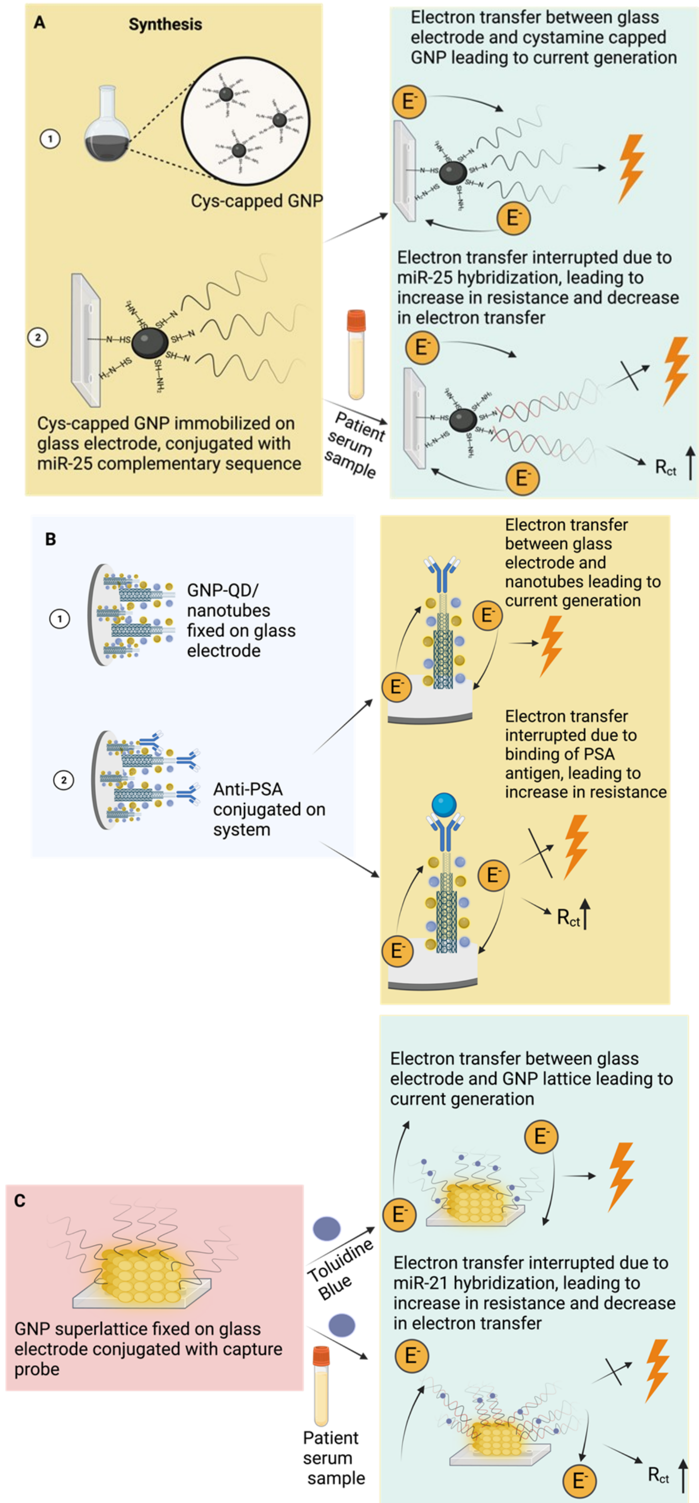

Figure 3. The application of GNPs in detecting cancer-specific biomarkers for early-stage detection using the principles of electrochemistry. A) GNPs fixed on a glass carbon electrode, conjugated with anti-miR-25 (1), which detects target miRNA from human blood plasma by the increase or decrease in R_{ct}[62] B) GNPs, QDs, and multiwalled carbon nanotubes fixed on a glass carbon electrode, followed by conjugating with anti-PSA to detect PSA antigen present in the patient sample measured by an increase or decrease in R_{ct}[56] C) GNPs superlattice fixed on a glass carbon electrode with the anti-miR-21 tag to detect miR-21 in a serum sample and measured by an increase or decrease in R_{ct}[60]

monoclonal antibody. CA 19-9 is a biomarker commonly used to detect pancreatic cancer and is a tumor-associated mucin glycoprotein antigen related to the Lewis blood group protein.[77] This specific biomarker was reported to be presented at the following concentration in respective stages; 1a: <21 U/mL, 1b: 86 U/mL, IIa: 105 U/mL, IIb: 164 U/mL, IV: >180 U/mL.[78] The immune reaction of the sensor occurs by trapping the target antigen with peptide bonds which can be quantified by measuring its fluorescent intensity (Figure 4) upon exposure with various cations, sugars, amino acids, macromolecules (ascorbic acid, uric acid, and caffeine), and tumor markers (CA 27-29, CA 15-3, CA 125, and PSA). Results indicated that the investigated species did not interfere with the CA 19-9 antigen detection and detection limit of 0.007 U/mL with a linear concentration ranging from 0.01-350 U/mL indicated sensitivity.[79]

Using the principle of immunoassay and QDs, a sandwich-type magnetic immunoassay was fabricated to target the cancer biomarker CEA. Aside from being present in NSCLC, this specific biomarker is also present in patients diagnosed with colorectal cancer.[68] Its use in early-stage detection has exhibited great significance, since previous methods for detecting colorectal cancer involved invasive methods such as colonoscopy or tissue biopsy to detect the presence of polyps and lacked sensitivity and accuracy.[65,80-82] In this model, target antigen CEA is extracted with the help of the amino-modified magnetic nanoparticles conjugated with capture anti-CEA, after which the zinc-selenium QD (ZnSe QDs) conjugated with secondary anti-CEA sandwiched the antigen. A permanent magnet will separate the immune reaction, and the single particle was analyzed via single-particle inductively coupled plasma mass spectrometry (SP-ICP-MS) (Figure 5). Compared to the previous immunoassay, the detection limit for CEA exhibited a LOD of 0.006 ng/mL in human serum samples compared to the traditional ELISA (0.025 ng/mL).[83]

MAGNETIC NANOPARTICLES

This class of nanoparticles was previously used as contrast agents for MRI imaging; however, in the past decade, its application has tremendously changed in drug delivery and diagnostics. These particles have been fabricated from either metal (such as iron, cobalt, or nickel) or are an amalgamation of metals and polymers. One of the many advantages of magnetic nanoparticles (MNPs) is their capacity to be manipulated magnetically, based on the metals used in their synthesis, using an external magnetic field.[84,85] Recently, MNPs have been widely used in tumor targeting, especially superparamagnetic iron oxide nanoparticles (SPIONs), used as contrast agents in cancer screening.[86] Regarding cancer biomarker detection, MNPs can range from biomolecule conjugation to bioseparation to biosensing.[87]

Aside from proteins and microRNA in cancer, circulating tumor cells (CTCs) have also played a pivotal role in cancer metastasis and contribute to 90% of cancer-related deaths, especially in ovarian cancer.[88] Conventional methods for early-stage cancer detection cannot be used due to the low concentration of CTCs; however, this drawback was addressed with the help of QDs. This sensor operates as such: (i) attachment of biotin-bovine serum albumin-folic acid (BSA-FA) in

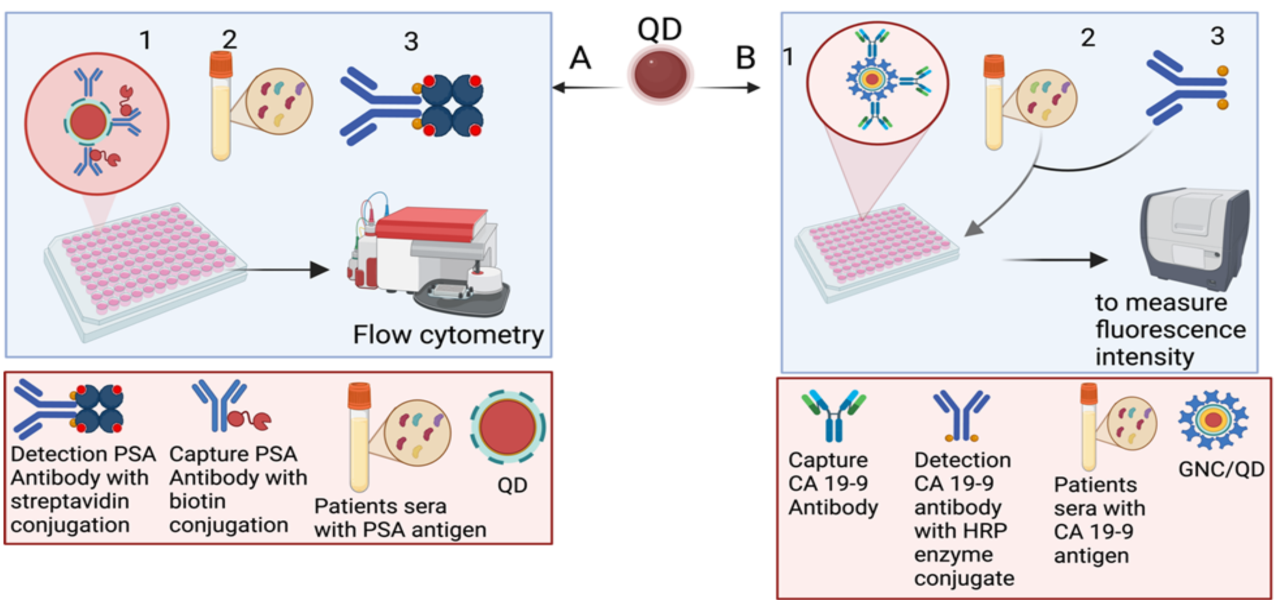

Figure 4. The application of QDs in detecting cancer-specific biomarkers for early-stage detection. A) QDs with capture anti-PSA and biotin with detection anti-PSA/streptavidin dye sandwiched PSA in a 96 well place and quantified via flow cytometry[76] B) CA 19-9 is sandwiched between GNC/QD with anti-CA 19-9/ HRP enzyme and biotinylated detection anti-CA 19-9. Fluorometric detection of CA 19-9 was measured[79]

combination with streptavidin-coated magnetic nanoparticles. For targeting specifically CTCs, BSA is conjugated with biotin-folic acid (FA). The biotin-BSA -FA binds to the CTCs with the help of the complementary binding between FA and Folate receptor (FR), which are commonly overexpressed on CTCs. (ii) The next step is coupling streptavidin-coated SPIONs with the biotin on the biotin-BSA-FA-CTC complex, forming a SPIONs-SA-biotin-CTC. This unique complex is then isolated with the help of an external magnetic field (Figure 6). This sensor has been reported to have a capture efficiency below 20% in FR-negative cells such as A549. In contrast, cells such as SKOV3 with FR overexpression showed a capture efficiency close to 80%, indicating that this sensor is specific to cancer cells with FR overexpression. In addition, this sensor can detect CTCs in whole blood, thereby suggesting that macromolecules (albumin and other glycoproteins) and ions do not interfere with the system's operation.[89]

Similarly, poly-dopamine-coated iron oxide (Fe3O4) nanoparticles have been developed to detect and isolate pancreatic cancer cells through Mucin 1 (MUC1) receptor detection. MUC1 are transmembrane glycoproteins reported to have been highly expressed in malignant tumors and precancerous lesions. Overexpression of this glycoprotein reduces the adhesion of cancer cells in the outer matrix, facilitating their metastasis in cancers such as pancreatic, lung, breast, or prostate.[90-92] This study highlights the Fe3O4 nanoparticle modification that enables the detection of MUC1 in pancreatic cancer cells. Initially, dopamine-coated Fe3O4 nanoparticles were synthesized, followed by labeling with 6- carboxyfluorescein tagged hairpin DNA sequences (H1-FAM and H2-FAM). The MUC1 aptamer/hybridization chain reaction (HCR) trigger probe (Apt-tri probe) was conjugated to the MUC1 receptor on the cancer cell to form a complex. When in the presence of the labeled nanoparticle, i.e., dopamine-coated Fe3O4 nanoparticles), quenched FAM will hybridize the Apt-tri probe bound to the MUC1 on the cell's surface. After which, the FAM molecules will open as the dopamine-coating nanoparticle pulls them off from the membrane receptor. The hairpin structure will be opened by hybridizing the trigger and FAM (Figure 5). The fluorescent intensity will be analyzed within the cell and counted, resulting in a LOD as low as 41 cells/mL. Other cell lines, such as HepG2 and HPDE-C7 cells, were treated to this probe and resulted in lowered fluorescent intensity when compared to PANC-1 cells. This indicates that the modified nanoparticle is sensitive and specific to cells overexpressing MUC1. The probe's results were later confirmed with traditional western blot and immunohistochemistry (IHC), indicating the expression of MUC1 on cancer cells. This particular system's sensitivity and detection ability lies with the expression of the MUC1 receptors and can be applied to a wide variety of cancers with a dominant gene expression.[93]

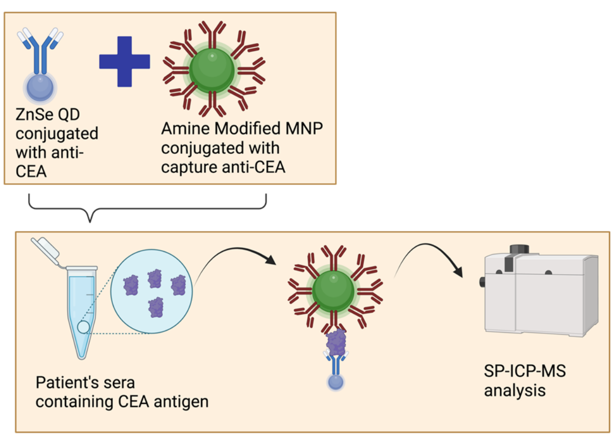

Figure 5. The application of MNPs and QDs combined in detecting cancer-specific biomarkers for early-stage detection. Amine-modified MNPs conjugated with anti-CEA and a ZnSe QD with the detection anti-CEA tag sandwiched CEA antigen in the serum and analyzed via ICP-MS[83]

CONCLUSION

This review showcases a small portion of the bigger picture of how nanoparticle platforms can be used for early-stage cancer diagnosis, which addresses the drawbacks involved in traditional diagnostic methods. These platforms have been reported to have increased sensitivity and specificity against an early-stage cancer diagnosis. The biomarkers in liquid biopsy samples for early-stage cancer detection are typically not present in sufficient levels for conventional methods; the nanoparticle technology can provide a boost at detecting these biomarkers at the lowest concentration. In addition to gold, magnetic, and quantum dots, there are numerous nanoparticles systems such as lipid-based, iron, biomimetic (cellular protein or parts like membrane-coated nanoparticles), silica, and polymer-based, which when tagged or used in combination to detect a specific biomarker to a specific cancer subtype, can improve the diagnostic efficiency. [45,84,94,95] Biosensors can either detect cancer cells or specific molecular biomarkers related to those cells. In contrast, immunoassays can increase the sensitivity of conventional biomarkers such as CEA, miRNA, and many more,[65] making them efficient for clinical use. This review discussed how various nanoparticles can be used to detect different types of cancers at an early stage with the help of different types of biomarkers present in body fluids. Further research and testing are required for these biosensors or immunoassays in more extensive and diverse populations to meet the regulatory guidelines. However, this system can improve overall patient outcomes through early detection.

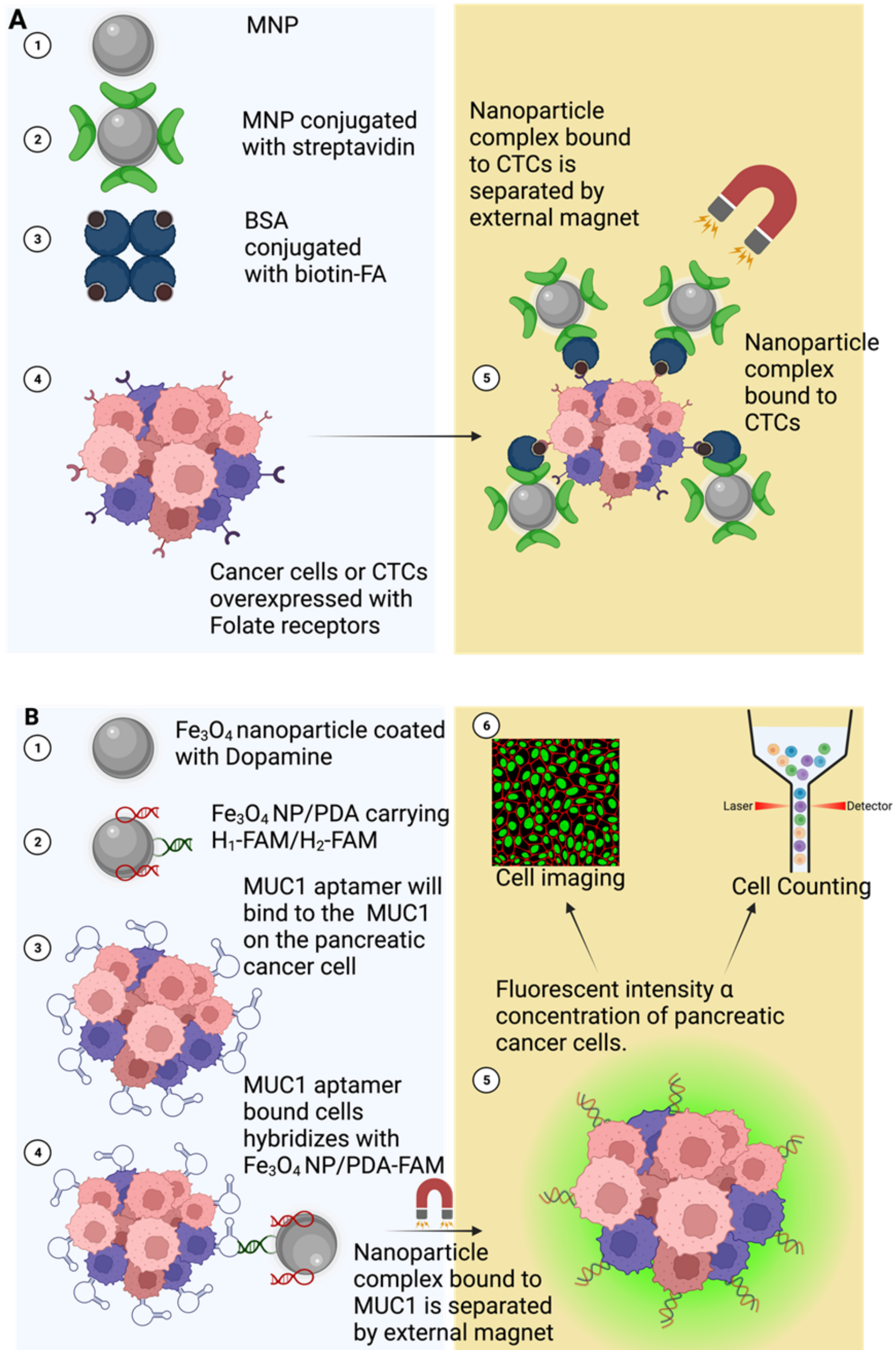

Figure 6. The application of MNPs in detecting cancer-specific biomarkers for early-stage detection. A) Fe_3O_4 magnetic NPs, conjugated with streptavidin (1) and BSA with biotin-folic acid (FA) (2). The BSA-FA binds to FR on CTCs, followed by the binding of modified MNPs to the overall complex on CTCs (3). An external magnet will separate the complex (4) from other non-specific cells, and captured cells are analyzed.[89] B) Anti-MUC1 aptamer will bind to MUC1 overexpressed cancer cells (1). Fe_3O_4 MNPs, coated with PDA with FAM tag, will bind to the cell-bound aptamer via hybridization (2) and will be separated by an external magnet (3), cleaving Fe_3O_4/PDA. The FAM-aptamer enables the FAM chain to open causing cancer cells to emit fluorescence (4), which can be analyzed qualitatively (cell imaging) and quantitively (cell counting).[93]

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CONFLICTS OF INTEREST

All authors declare no conflicts of interest.

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