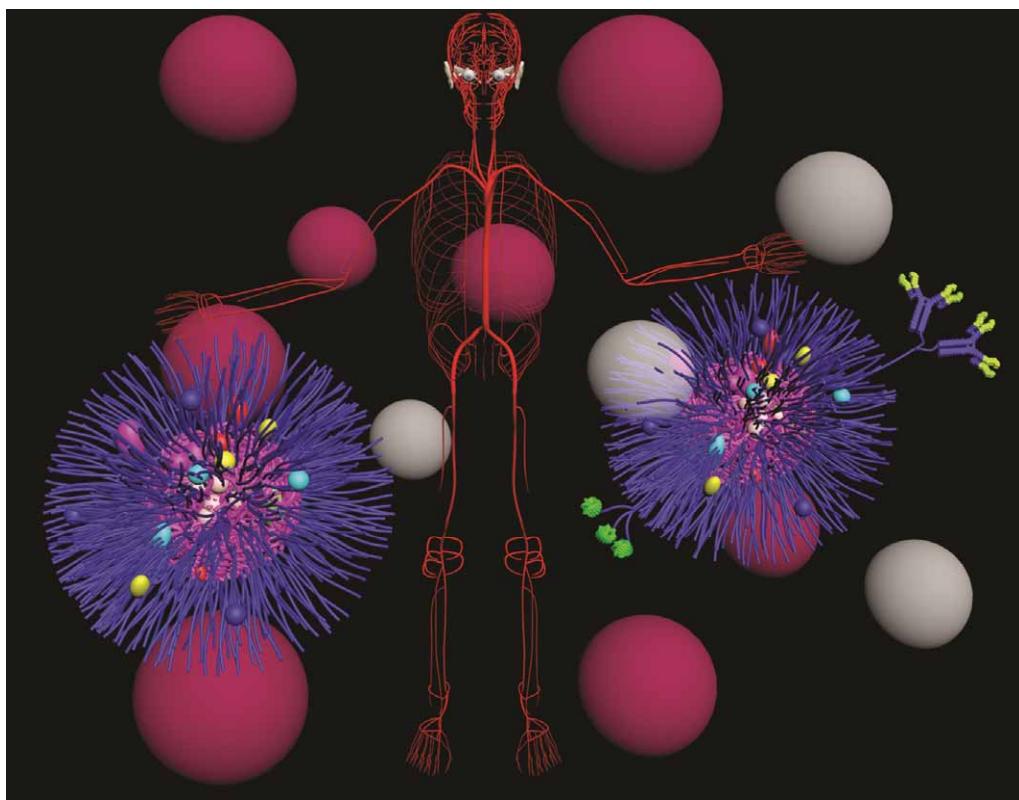


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TUTORIAL REVIEW

Cancer detection using nanoparticle-based sensors†

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This *tutorial review* surveys the latest achievements in the use of nanoparticles to detect cancer biomarkers and cancer cells with a focus on optical and electrochemical techniques. Nanoparticle based cancer diagnostics are becoming an increasingly relevant alternative to traditional techniques. Although some drawbacks exist in relation to the obtained sensitivity the use of nanoparticle-based sensors in biomarker detection or cancer cell detection offers some advantages in comparison to conventional methods. The developed techniques can be interesting and relevant for their use in point-of-care of cancer diagnostics. The methods can be of low cost and in addition easy to be incorporated into user-friendly sensing platforms.

1. General introduction

By the end of the last century, cancer had become the second most likely cause of death worldwide. In 2004, cancer killed 7.4 million people, most of them from developing countries. By 2030, this number is estimated to reach 12 million. Of those who died of cancer, 30% of people could have been saved if their cancer were detected earlier (World Health Organisation,

www.who.int/, 2010). The chance of being cured of cancer increases with early detection and treatment of the disease. Cancer is difficult to diagnose and treat because it generally grows from the heart of organs and is not recognised as a foreign body by our immune system. Since cancer is the uncontrolled growth and spread of cells, it often invades surrounding tissue and can metastasise to distant sites and affect any part of the body.

A biomarker is a “molecule with biologically important intra- or intercellular function, an expression or activity of which either causes or is specifically altered in response to corresponding pathological conditions”¹ (Fig. 1A). Biomarkers of cancer include proteins overexpressed in blood and serum or at the surface of cancer cells that facilitate diagnosis. Cancer biomarkers are present at very low concentration in first stages

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of cancer and are difficult to detect. It is therefore necessary to develop devices which can detect such low concentrations of biomarkers. Furthermore, it would be beneficial to be able to detect and target cancer cells in blood and human tissues.

Cancer biomarkers can be used for:

- screening a healthy population or a high-risk population for the presence of cancer;
- making a diagnosis of cancer or of a specific type of cancer;
- determining the prognosis in a patient;
- monitoring the course of cancer in a patient in remission or while receiving surgery, radiation or chemotherapy.

However, there is no test which answer meets all of these requirements. No biomarker has been established as an “ideal” cancer screening tool because of the lack of sensitivity and specificity of the current tests. Even highly sensitive and specific tests have low predictive values due to the low prevalence of cancer.

Nanomedicine is the application of nanotechnologies to medicine. It is the use of nanomaterials of less than 100 nm in at least one dimension for drug screening, gene and drug delivery, detection, diagnosis and monitoring of disease. Nanomedicine is an exciting new field which may offer new ways to detect cancer biomarkers at low concentrations and to detect and target cancer cells in very deep sites. Nanoparticles (NPs) are organic or inorganic, metallic, magnetic or even polymeric particles with a diameter of less than 100 nm. They can be useful in cancer diagnosis because of their size, charge, stability, and relationship with water, and also because of the ability to functionalise them. Because of their small size and properties, nanoparticles can have long half-lives during circulation in the body and reach very deep sites that other drugs or contrast agents cannot usually access. Moreover they can cross barriers such as blood–brain barrier or gastrointestinal barrier. This can be a major advantage for the detection and visualisation of tumour cells at very early stages of disease. Furthermore, nanoparticles such as Quantum Dots (QDs) and gold nanoparticles (AuNPs) deliver optical signals sufficient for biosensing in cells. Gold nanoparticles can also be used in electrochemical biosensors for cancer biomarkers or



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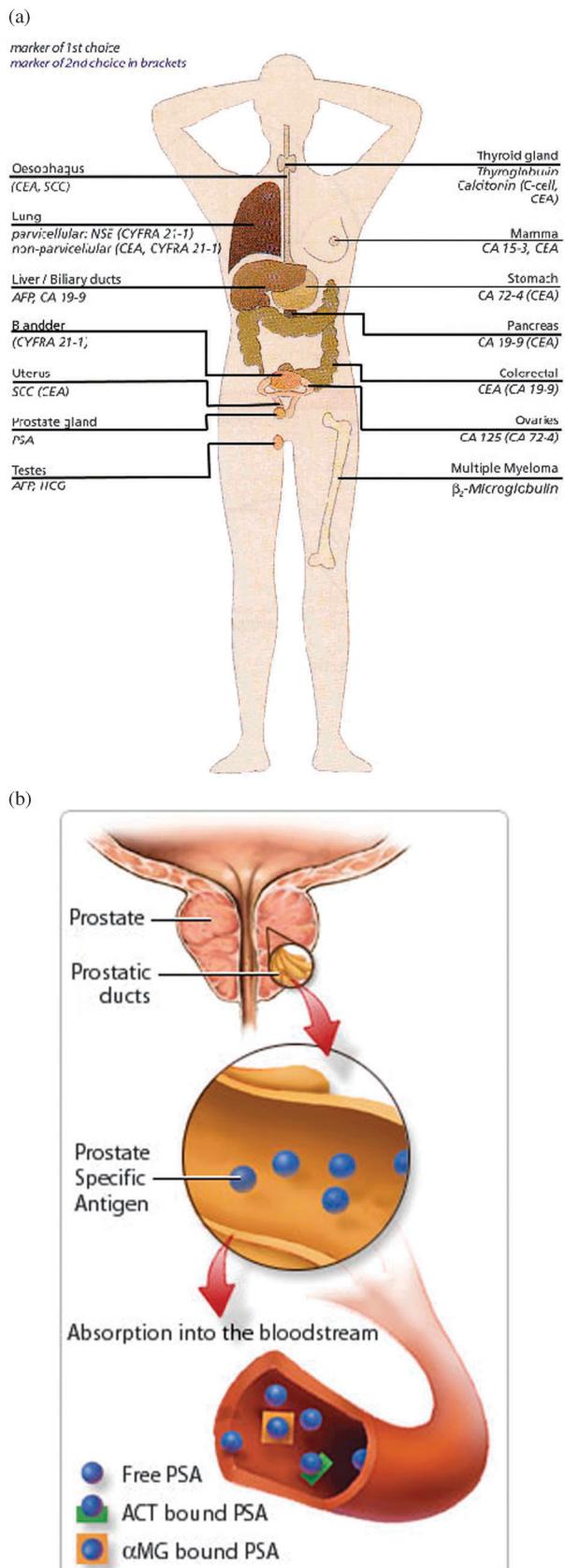


Fig. 1 (A) Cancer biomarkers for different tumours (Medterms, www.medterms.com, 2010). (B) Production of free and attached PSA from prostate to the bloodstream (Testicular Cancer Information & Support, www.tc-cancer.com, 2010).

tumour cells. Nanoparticles can be functionalised to improve their ability to bind and signal the presence of proteins. They can be coated with antibodies, antigens, enzymes specific to a protein, or receptors overexpressed on the surface of tumour cells or specific to cancer biomarkers.

“Biosensors are analytical devices that incorporate a biological material, a biologically derived material or a biomimic intimately associated with or integrated within a physico-chemical transducer or transducing microsystem”.² Biosensors allow the measurement of a specific analyte or group of analytes. The analyte in question can be a cancer biomarker which may reveal a certain stage of a specific disease. The analyte can also be a protein or receptor overexpressed on the surface of the cancer cell. In a biosensor, the analyte (*i.e.* cancer biomarkers or protein on the surface of tumour cells) can be detected through the receptor fixed onto the surface of a biosensor transducer. The specific recognition of the analyte by the receptor results in a signal which is detected by a transducer. These can be electrochemical, optical, thermometric, piezoelectric, magnetic or micromechanical.

The use of nanomaterials may increase the sensitivity of a biosensor and generate higher accuracy and precision. Moreover, nano-sized devices generally allow faster response, because mass transport occurs over smaller distances. Lower power and voltage are needed to achieve the same field in the nano-sized semiconductor and the nano scale should also result in less cost due to the small quantities of material required.

In the following sections, the use of nanoparticles for the detection of cancer biomarkers and cancer cells is reviewed. We will examine the latest trends in cancer diagnostics based on the use of nanoparticles and the different detection strategies ranging from optical to electrical techniques.

1.1 Detection of cancer biomarkers

Advances in genomics, proteomics and molecular pathology have generated many candidate biomarkers with potential clinical value.³ Prostate-specific antigen (PSA), a serine protease belonging to the human kallikrein family, is best known as a prostate cancer biomarker. The expression of PSA is highly restricted to normal and malignant prostate epithelial cells in men, and for this reason, PSA is used extensively as a biomarker to screen for prostate cancer, to detect recurrence after definitive therapy, and to follow response to treatment in the metastatic disease setting.⁴ PSA has two predominant forms in human serum: either free PSA (f-PSA) or PSA complexed to R-1-antichymotrypsin (PSAACT). Total PSA (t-PSA) refers to the sum of f-PSA and PSAACT⁵ (see Fig. 1B). Carcinoembryonic antigen (CEA) is a protein molecule that can be found in many different cells of the body, but is typically associated with certain cancers and the developing foetus. CEA as a cancer biomarker is mostly used for the diagnosis of cancers of the gastrointestinal tract. Alpha-fetoprotein (AFP) is an oncofetal glycoprotein that contains a single glycosylation site at the level of asparagines 232, and is a well-known tumour marker for hepatocellular carcinomas (HCC).⁶ Human chorionic gonadotropin (hCG) is a glycoprotein hormone produced at very high concentrations

by placental trophoblasts. During pregnancy, its concentration in the serum increases rapidly. Elevated expression of hCG β in serum, urine, or tumour tissue is a strong indicator of adverse prognosis in many nontrophoblastic tumours.⁷ Herceptin, HER2 is a member of a family of four transmembrane epidermal growth factor receptor (EGFR) tyrosine kinases and is overexpressed in a variety of human cancer cells such as breast, lung, and ovarian carcinomas.⁸ CA125 is a tumour antigen widely used in monitoring patients with ovarian cancer. The high levels of serum CA125 are thought to be a result of overexpression of the CA125 gene in the malignant cells.⁹

1.1.1 Optical detection techniques

Light absorption. When nanoparticles modified with specific antibodies interact with the target (protein) the resulting nanoparticle aggregation may be measured by light absorption. Another possibility is to measure the colour change produced by enzyme labels loaded onto the surface of nanoparticles.¹⁰ Liu *et al.* developed a nanosensor which detected Carcinoembryonic Antigen (CEA) as model protein. They used AuNPs and magnetic probes to “sandwich” CEA. AuNPs were functionalised with an antibody and with single stranded DNA probes labelled with biotin. SsDNA was the bridge between AuNPs and Horseradish peroxidase (HRP) that enabled the signal amplification using a solution of TMB-H₂O₂ as substrate (see Fig. 2). CEA can also be quantified by measuring the intensity of the signal, the absorbance at 450 nm increasing with the concentration of CEA.¹¹

Zhou *et al.* have shown that enzymes used as catalytic label moieties for signal amplification can be replaced by DNAzyme. They functionalised AuNPs with DNAzyme in substitution of HRP for the detection of α -fetoprotein (AFP). DNAzymes are nucleic acids which have catalytic properties; moreover they are more stable than protein enzymes. DNAzymes can catalyse the reduction of hydrogen peroxide in water in a similar way to HRP, producing chemiluminescence.¹² Zhou and co-workers developed a nanosensor which used a sandwich-type bio-barcode assay (Fig. 3A). MMPs were functionalised with monoclonal antibodies anti-AFP. AuNPs were functionalised with AFP polyclonal antibodies and double stranded DNA (dsDNA). Only one of the strands of DNA was fixed to AuNP, the other strand, complementary to the first one, was the peroxidase-mimicking DNAzyme. AFP was sandwiched between AuNPs and MMPs. After a washing step, a magnetic field was applied in order to release the DNAzyme which had been dehybridised from the other strand into the supernatant. The DNAzyme then reacted with the substrate solution (with 2,2'-Azino-Bis-3-Ethylbenzothiazoline-6-Sulfonic Acid (ABTS) reagent), producing a green colour and an absorbance peak at 410 nm (Fig. 3B).¹³

Chen *et al.* developed a nanosensor to detect thrombin by using fibrinogen-functionalised AuNPs. The absorbance of fibrinogen at 532 nm was measured. When thrombin was added to a solution of fibrinogen-AuNPs, it produced an aggregation of fibrin-AuNPs, fibrinogen was transformed to fibrin in the presence of thrombin. Thus, the absorbance at 532 nm decreased because of the decrease in fibrinogen concentration and the concentration of thrombin could be

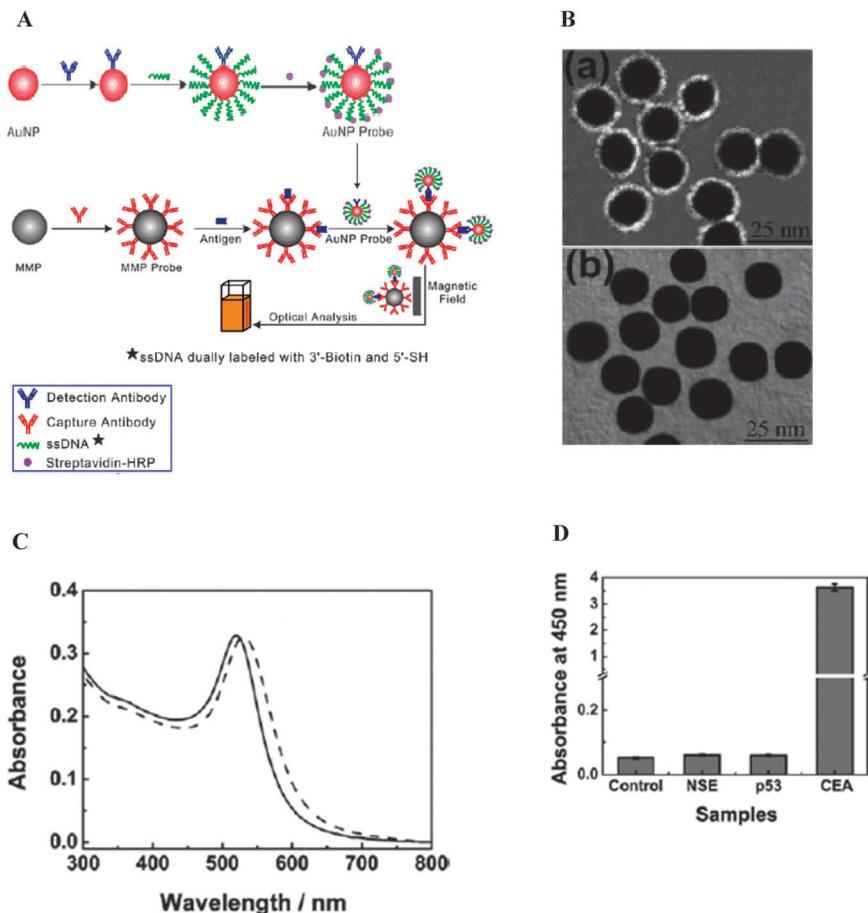


Fig. 2 (A) Illustration of enzyme labelled AuNP probe based immunoassay process (with schematic representation of AuNP and magnetic microparticle probes). (B) TEM images of the enzyme labelled AuNP probes (a) and bare AuNPs (b). (C) UV/Vis spectra of AuNP probes dispersion (dashed line) and AuNPs solution (solid line). (D) The specificity of the AuNP probe-based immunoassay on target protein CEA detection.¹¹

quantified (see Fig. 4) (thrombin–antithrombin, TAT, complex is considered a cancer biomarker).¹⁴

Light scattering. Metal nanoparticles such as AuNPs can be used to scatter light of specific wavelengths. Functionalised nanoparticles can bind specific protein, and depending on their size, composition and the degree of surface plasmon resonance (SPR) they will emit an optical signal. Bao and co-workers developed a bio-barcode assay using AuNPs and magnetic beads for the detection of prostate specific antigen (PSA). MMPs were coated with primary Abs anti PSA. 15 nm AuNPs were coated with secondary biotinylated Abs anti PSA. PSA was sandwiched between MMP and AuNP. After magnetic separation, biotinylated AuNPs which had bound PSA were coated with single stranded oligonucleotides (barcodes). These barcodes were then released and hybridised to a microarray. The microarray was coated by oligonucleotides complementary to the half of barcodes, thus one oligonucleotide forms half of a barcode. The second half of the barcode was then hybridised by another oligonucleotide conjugated with a AuNP probe. Silver was deposited on the surface of AuNP probes, which enhanced the signal.¹⁵ Stoeva *et al.* have shown that it is possible to use this type of bio barcode assay for multiplexed protein detection. PSA, human chorionic

gonadotropin (HCG) and AFP were sandwiched between 30 nm AuNPs and magnetic beads. 30 nm AuNPs were functionalised with detection Abs which recognise target proteins. They were also functionalised with DNA barcodes. These barcodes had a universal oligonucleotide part attached to AuNP and a target reporting part. There were thus three types of functionalised AuNPs, some of them were conjugated with Abs anti PSA, some others with Abs anti HCG and the rest with Abs anti AFP. These three types did not have the same target reporting oligonucleotides, in order to detect and differentiate each biomarker. Biomarkers were sandwiched between AuNPs and MMPs, after magnetic separation, barcodes were released and identified using a chip-based method. Each kind of target reporting oligonucleotides was hybridised on a different slide. They were attached by a universal AuNP probe, which was coated in silver. The silver enhancement enabled a light scattering detection (measurement of gray scale intensity) of each biomarker (see Fig. 5).¹⁶

Surface plasmon resonance. Surface plasmon resonance (SPR) is an optical technique which is based on the detection at the surface of a noble metal such as gold or also on a semiconductor the changes of the dielectric constant induced by molecular adsorption. Choi *et al.* used the SPR peak of

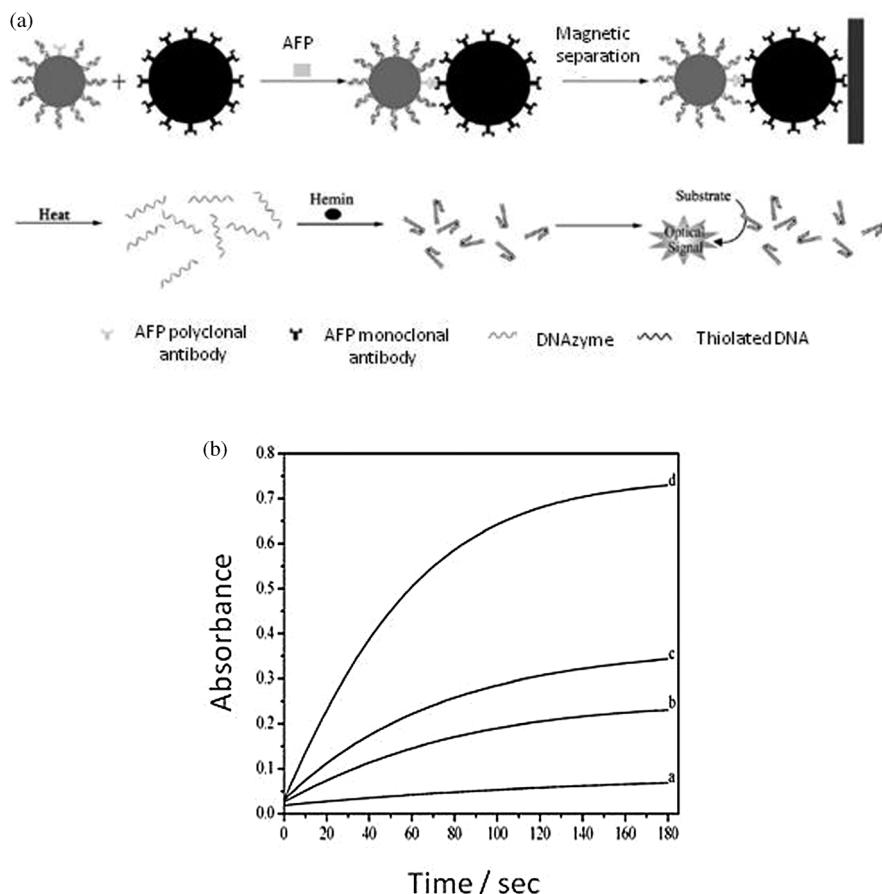


Fig. 3 (A) Immunoassay for AFP detection using peroxidase-mimicking DNAzyme as a catalytic probe. (B) Absorbance changes at 410 nm for different concentrations of AFP: (a) 0, (b) 5, (c) 10, (d) 50 ng mL⁻¹.¹³

AuNPs. They conjugated 20 nm AuNPs with anti PSA Abs to detect PSA. PSA was sandwiched between a gold substrate film functionalised with protein G and anti PSA Abs and modified AuNPs. Thanks to the bond between AuNPs and PSA, SPR angle difference of the complex increased with PSA concentration which facilitated detection of PSA and quantification of the biomarker.¹⁷ Huang and co-workers also developed a sandwich assay using AuNPs for the detection of PSA. They immobilised a PSA specific VHH (intact antigen binding fragment which comes from an antibody), able to bind PSA on a mixed alkanethiol self-assembled monolayer (SAM). PSA was sandwiched by PSA specific VHH molecule (called cAbPSA-N7) and a biotinylated mouse monoclonal Ab. The complex was then attached with streptavidin modified 20 nm AuNPs thanks to biotin–streptavidin interactions (see Fig. 6). Relative resonance units of cAbPSA-N7 were finally measured to detect PSA concentration. AuNPs had the role of signal enhancer for very low concentrations.¹⁸

Fluorescence. A light emission is provoked by the excitation of certain molecules called fluorophores. Quantum dots (QDs) are able to emit light when they are in a state of excitement. Several studies in biological imaging using fluorophores for the diagnostic and imaging of cancer have been reported. When conjugated with target molecules, fluorophores can be attached to cancer biomarkers or cancer cells allowing the

detection of cancer. Gokarna *et al.* developed a device able to detect PSA based on the specificity of the binding of QDs with PSA. They conjugated polyethylene glycol (PEG) coated QDs with anti PSA antibodies (Abs). By laser excitation QDs emitted light which has allowed the detection and quantification of PSA on a microchip image.¹⁹ Jokerst and co-workers integrated fluorophores in microfluidic device to detect and quantify three cancer biomarkers: CEA, CA125 (carbohydrate antigen 125) and HER2. They immobilised capture specific antibodies on agarose beads (which had the role of underlying support structure). Biomarkers bound their specific antibodies, then a detection Ab conjugated QD bound the antigen and allowed the detection of the biomarker by emitting fluorescence (see Fig. 7). This microfluidic device allowed the multiplexed detection of cancer biomarkers from the saliva sample. The sample was injected *via* micropumps and passed through channels. Biomarkers were fixed to channels *via* the sandwich assay and were able to be detected. Jokerst *et al.* compared the efficiency of QD based sandwich assay to standard fluorophore sandwich assays. They demonstrated that the QD based technique is 30 times more efficient than the Alexa Fluor based technique.²⁰

1.1.2 Electrical/electrochemical detection techniques. The binding of a nanoparticle on a cancer biomarker can generate changes in the current, the ohmic response and the potential.

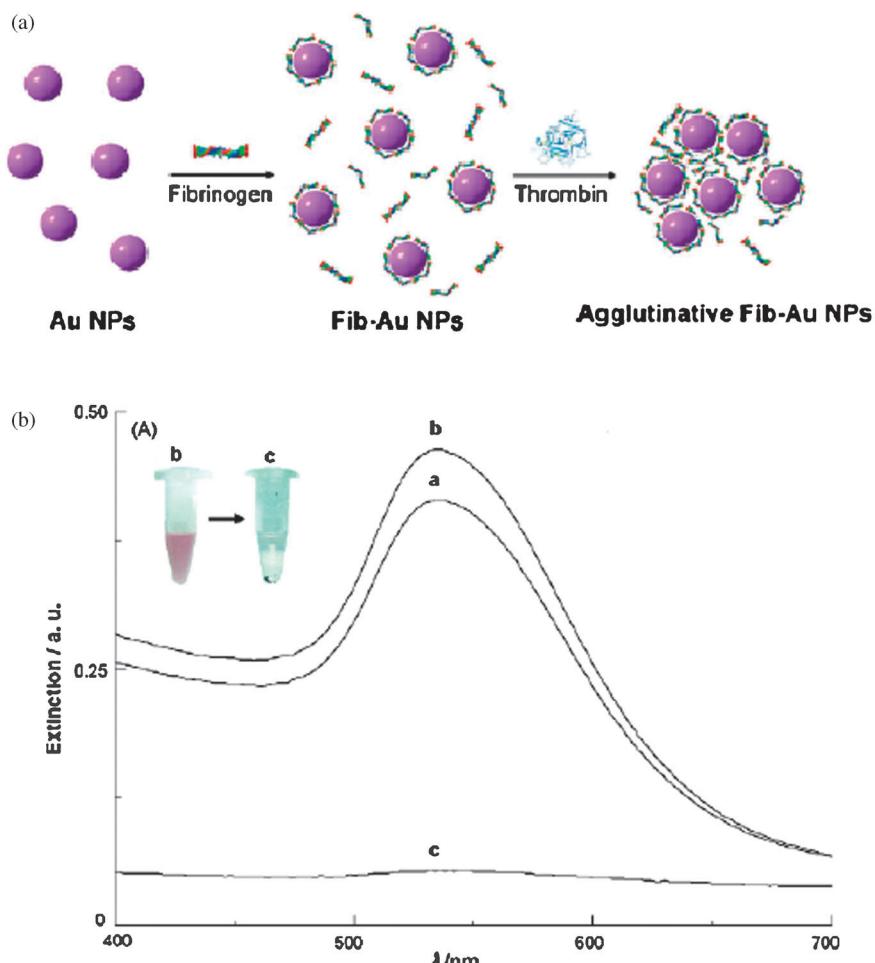


Fig. 4 (A) Schematic representation of thrombin detection by Fib-AuNP sensor based on modulation of the formation of agglutinates from Fib-AuNPs. (B) UV/Vis absorption spectra of solutions of (a) AuNPs in the (b) absence and (c) presence of thrombin (100 pM).¹⁴

These electrical or electrochemical changes make possible the detection and quantification of biomarkers. Due to electrical properties of some nanoparticles, their fixation with the target is followed by a reaction (*i.e.* oxidation–reduction) which can be quantified through the signal generated.

Electrical impedance spectroscopy. Electrical Impedance Spectroscopy (EIS) measures the dielectric properties of a medium as a function of frequency. Tang *et al.* developed an electrochemical immunoassay using AuNPs coated by glutathione (GSH). In order to detect CEA, they functionalised tumour AuNPs with CEA antibodies (CEAAbs), in that way they avoided nonspecific binding. Then these bioconjugates were deposited and immobilised on a gold electrode by electro-copolymerisation with *o*-aminophenol (OAP). When bioconjugates bound CEA the electron transfer resistance of the redox pair increased at the poly-OAP/CEAAb-AuNP/Au electrode. The resulting signal permitted the detection and quantification of CEA by cyclic voltammetry (CV) and electrochemical impedance spectroscopy (EIS).²¹

Electrocatalytic. Some nanomaterials have the property of an electrocatalyser, they help an electrochemical reaction. It is possible to quantify and detect an analyte by measuring the

current changes after application of a potential. Sandwich assay using silver enhanced AuNPs are used in this kind of technique; silver enhanced AuNPs bound to a target analyte provoke a reaction detectable by measuring the current changes. Rusling *et al.* used GSH conjugated AuNPs and magnetic nanoparticles (MNPs) for the detection of PSA. PSA was bound by anti-PSA Abs conjugated to AuNPs of the electrode surface. Then PSA was sandwiched by multi-labelled MNPs bearing secondary PSA Abs. A microelectronic electrode gave a signal due to the reduction of H₂O₂ and allowed the detection of PSA.²² Munge and co-workers also used GSH modified AuNPs for the detection of a cancer biomarker, interleukin 6 (IL-6) in serum (see Fig. 8). The immunoassay was a sandwich type. GSH-AuNPs were immobilised onto pyrolytic graphite and polydiallylammonium bromide. Bovine serum albumin (BSA) and IL-6 Abs were attached to GSH-AuNPs. BSA reduced the non-specific binding of Abs. IL-6 bound Abs and was then sandwiched with secondary Abs labelled by HRP, streptavidin and biotin. This complex enhanced the reduction of H₂O₂ and thus the emitted signal allowed the detection of IL-6. Munge and co-workers compared AuNPs and Single Walled Carbon Nanotubes (SWCNT) for this immunoassay; AuNPs have shown better results and a lower detection limit than SWCNT.²³

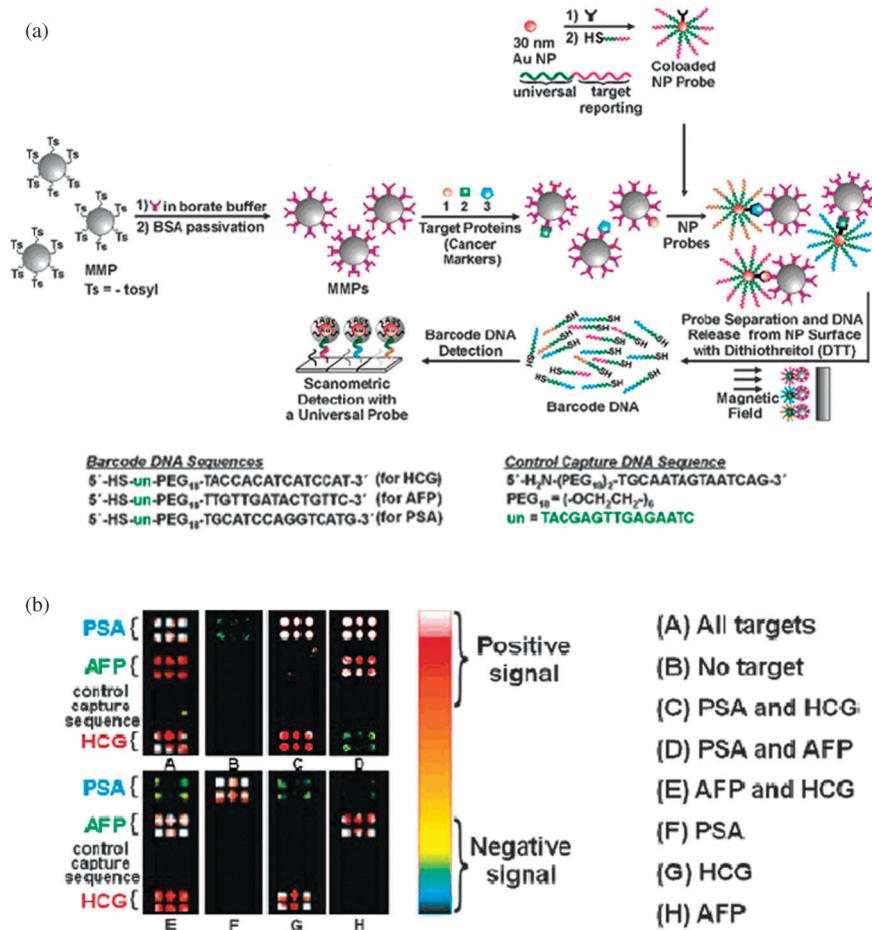


Fig. 5 (A) Bio barcode assay for multiplexed biomarker detection. (B) Scanometric detection of the barcodes.¹⁶

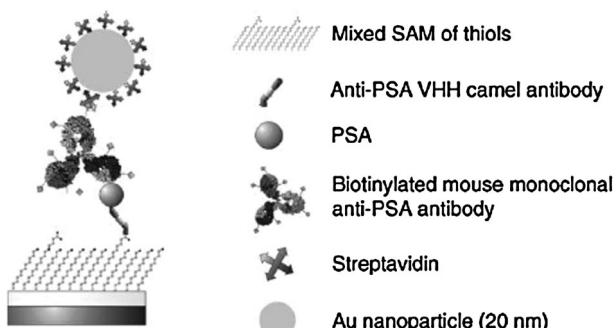


Fig. 6 Schematic representation of the PSA sandwich assay. PSA is captured by Anti-PSA VHH camel Ab fixed on a mixed SAM of thiols. Then PSA is sandwiched with biotinylated mouse monoclonal Ab and incubated 20 min with streptavidin-modified AuNPs.¹⁸

Protein kynase (PTK) activity can be a marker of abnormalities and cancers such as leukaemia. Kerman and Kraatz used an indium tin-oxide (ITO) electrode modified with biotin. Streptavidin attached biotin allowing the immobilisation of biotinylated peptides at the surface of the electrode. PTK catalysed phosphorylation reaction on substrate peptides. PTK transferred the γ -thiophosphate group from ATP-S to the substrate peptides releasing ADP. In that way biotinylated peptides became thiolated which permitted the fixation of

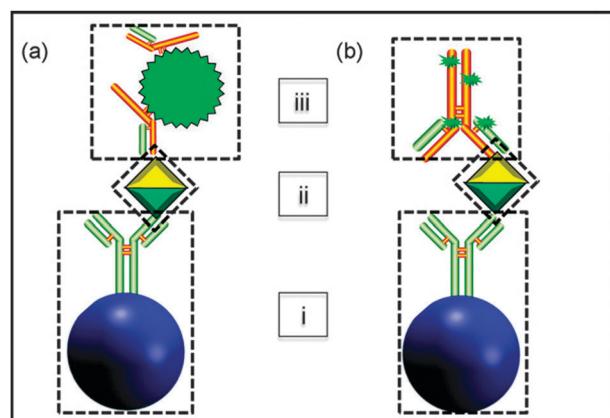


Fig. 7 Representation of immunoassays. (i) Agarose bead as underlying support structure for Ab immobilization. (ii) Antigen. (iii) Fluorophore (a. QD, b. Alexa Fluor) conjugated with detection Ab.²⁰

AuNPs on peptides. The accumulation of AuNPs at the surface of the electrode amplified the signal and allowed the quantification of the PTK activity. The detection method used was square wave voltammetry.²⁴ Previously, Kerman *et al.* also published on the electrochemical detection of PTK activity. They had immobilised peptides as a kinase substrate at the surface of a screen-printed carbon electrode.

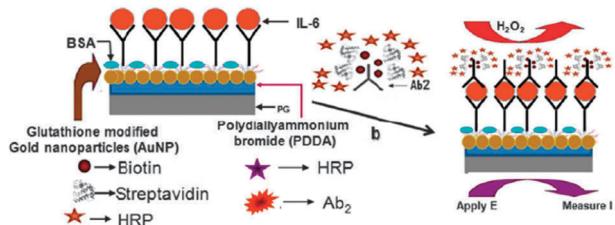


Fig. 8 AuNP immunosensor after treating with simple and multi-labeled Ab₂-biotin-streptavidin-HRP. The final detection step involves immersing the fully prepared immunosensor into an electrochemical cell containing phosphate buffered saline (PBS) buffer and mediator, applying voltage, and injecting a small amount of hydrogen peroxide.²³

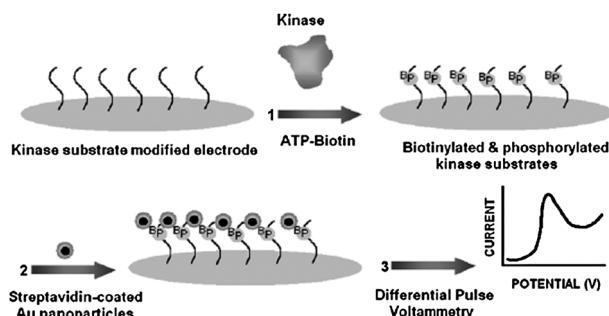


Fig. 9 Representation of the AuNP immunosensor. The phosphorylation and biotinylation of the immobilized peptide on a screen printed electrode (SCPE) (1) is followed by the attachment of streptavidin-coated AuNPs (2). The reduction signal of AuNPs is monitored using differential pulse voltammetry (DPV) (3).²⁵

Protein kinase and ATP-biotin (as co-substrate) were added, allowing *via* the action of the protein, the binding of biotinylated and phosphorylated kinase substrates. Biotinylated peptides were recognised and fixed by streptavidin-modified AuNPs due to the strong affinity of biotin and streptavidin. The reduction signal of AuNPs was then monitored by differential pulse voltammetry (DPV) (see Fig. 9). These immunosensors enabled the detection of PTK activity; moreover they were also used to test the inhibition of PTK activity by different small molecules, simply by adding these molecules to the solution.²⁵

Zhong *et al.* developed a nanogold enwrapped graphene nanocomposite (NGGN) electrochemical assay for the detection of CEA. This immunoassay was sandwich based. AuNPs were deposited onto a glassy carbon electrode (GCE) coated with Prussian Blue (PB). HRP and Anti-CEA secondary Abs were attached on the surface of AuNPs. CEA bound Abs of the complex and was then sandwiched with graphene nanocomposites. The graphene nanocomposites used in this case were nanolabels, which consisted of a chitosan-protected graphene core and a multi-nanogold particle shell. They were conjugated with HRP-Anti-CEA Abs. The sandwich complex enabled the signal amplification by facilitating the electron transfer of HRP(Fe³⁺) to HRP(Fe²⁺) and allowing the reduction of H₂O₂ in water (electron transfer between the analyte and the base electrode) (see Fig. 10A).²⁶ Almost the same team, Yang *et al.* used AuNPs and dsDNA for a CA 15-3 nanosensor. On a gold electrode they deposited a Prussian Blue layer, which promoted the electron transfer. The AuNPs were immobilised on PB. Double stranded DNA was

sandwiched between this first layer of AuNPs and a second one at the top of the complex. The dsDNA enhanced the surface coverage of the biomarker, thus improving the sensitivity of the nanosensor. Anti-CA 15-3 Abs were then attached to the surface on AuNPs. They bound the biomarkers and thus avoided nonspecific binding (See Fig. 10B). Detection of the biomarker was then achieved amperometrically.²⁷

Yuan *et al.* developed an amperometric immunosensor for the detection of CEA biomarker. They immobilised AuNPs on the surface of a bare GCE by electrochemical reduction. They used nickel hexacyanoferrate nanoparticles deposited onto the surface of AuNPs as an electroactive marker. A second layer of AuNPs was deposited on the complex and permitted to attach anti-CEA Abs. BSA was added to block non-specific sites and to improve the signal amplification. CEA was bound by the complex; it could be detected by CV and EIS. This immunoassay offered high sensitivity, a low detection limit, satisfactory regenerations and quantitative immuno-binding reactions. Moreover this nanosensor was inexpensive. These advantages facilitate the use of these nanosensors as diagnostic devices. AuNPs are not the only nanoparticles used for electrochemical detection. Silica NPs (SiNPs) are electroactive and can also be relevant for biomarker detection.²⁸ Hong and co-workers used them and ferrocenecarboxylic acid (Fe-COOH) for the detection of CA 15-3 in an immunoassay. They immobilized Fe-COOH doped SiNPs on a bare Au electrode. Glutaraldehyde was added to enable the fixation of Anti-CA 15-3 Abs. Silica was used in this immunoassay because it prevented the leakage of Fe-COOH and because it was easy to conjugate with Anti-CA 15-3 Abs. BSA was added to block other possible active sites and thus improve the detection (see Fig. 11). Electrochemical changes due to Abs–Antigen interactions were measured by CV and EIS. This system thus enabled a clear and sensitive quantification of CA 15-3.²⁹

Electrochemical techniques can also be used in microchip arrays. Ko *et al.* developed a microchip-based multiplex electro-immunosensing system for the detection of several cancer biomarkers such as AFP, CEA and PSA. They used PDMS and glass substrates on a microfluidic device. Primary Abs-conjugated polystyrene microbeads were immobilised onto the electrode where they bound their specific antigen and afterwards secondary Abs-conjugated with AuNP was added. Silver was deposited at the surface of the AuNPs to enhance the electrical signal (current). Immunoreactions generated an electrical signal measured and monitored by PC-based system (see Fig. 12). This microfluidic device enabled high-throughput processing, the use of small sample volumes and short analysis times.³⁰

Potentiometry. Potentiometric sensors measure the potential difference between the working electrode and the reference electrode when no current is flowing. Wang and co-workers developed a potentiometric immunosensor based on AuNPs/dendrimer/PVC electrode. They immobilised AuNPs on a polyvinyl chloride electrode for the detection of total PSA. A poly(amidoamine) G4 dendrimer was sandwiched between this layer of AuNPs and another. Anti PSA Abs were then adsorbed on the AuNPs surface. Fixation of PSA on Abs

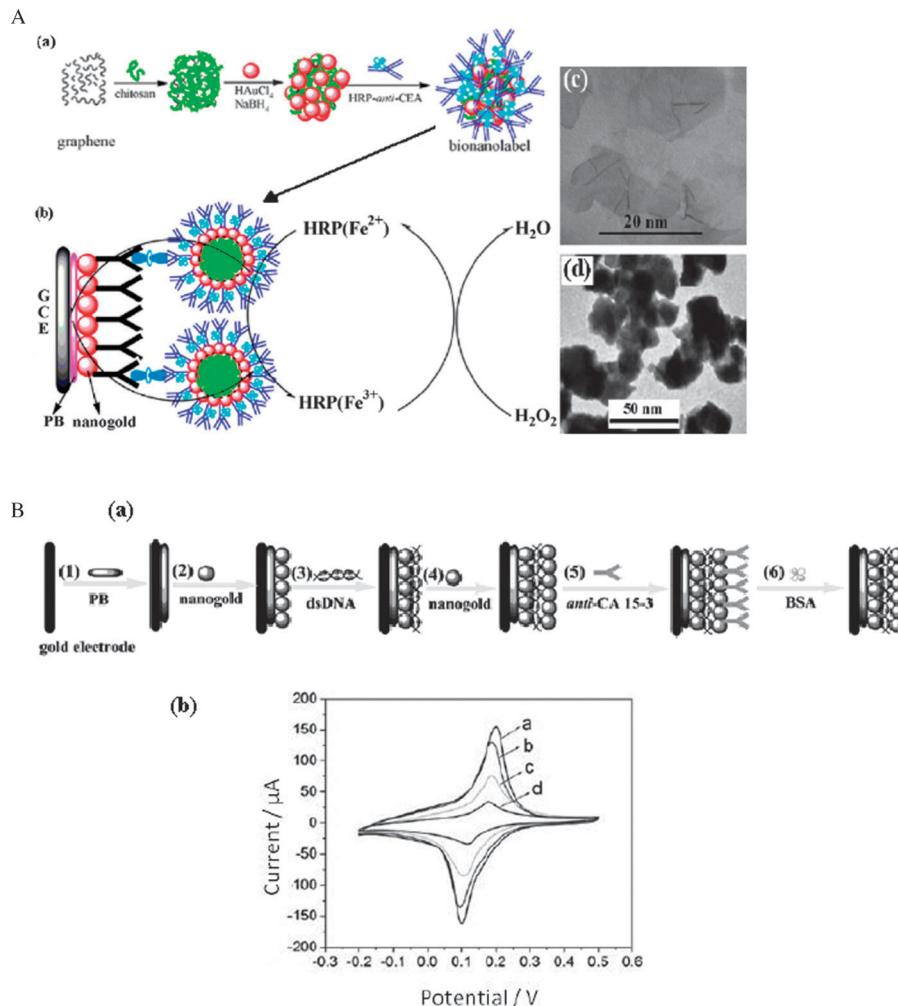


Fig. 10 (A) (a) Fabrication of the HRP-Anti CEA-NGGN nanolabel. (b) Measurement protocol. TEM images of (c) chitosan-protected graphene and (d) the prepared NGGNs.²⁶ (B) (a) Fabrication of the immunosensor. (1) PB electrodeposition on the gold electrode, (2) the electrodeposition of the first-layer AuNPs on the PB-modified electrode, (3) the assembly of dsDNA on the AuNPs/PB-modified electrode, (4) the electrodeposition of the second-layer AuNPs on the DNA/AuNPs/PB-modified electrode, (5) the immobilization of Anti-CA 15-3Abs on the AuNPs/DNA/AuNPs/PB-modified electrode, and (6) the electrode incubated with BSA (b) CVs of the developed immunosensor in pH 6.0 PBS after incubation with various concentration of CA 15-3 at (a) 0 ng mL⁻¹, (b) 3 ng mL⁻¹, (c) 30 ng mL⁻¹ and (d) 150 ng mL⁻¹ at 50 mV s⁻¹.²⁷

provoked a signal. The detection of t-PSA was based on the potential changes due to antibody–antigen interactions (see Fig. 13).³¹

1.2 Detection of cancer cells

Cancer cells can produce on their surface or within the cell an abnormal quantity of proteins, receptors or specific enzymes. These overexpressed components can be detected and bound in order to detect, isolate, quantify and destroy tumour cells. As an example, HER2 is a receptor of tyrosine kinase family. An overexpression of HER2 is present in 25% of invasive breast cancer cells. The overexpression of HER2 can result from gene amplification, increased transcription of the gene and increased translation of HER2 mRNA. The result is high synthesis and expression of HER2 at the cell surface as well as the cancer cells proliferation (CancerNetwork, www.cancernet work.com, 2010) (see Fig. 14) (The small Business Company, www.tsbc.com, 2010). It could thus be relevant to develop a sensor able to detect overexpressed HER2 for the detection of

cancer cells. Several studies have been carried out on detection of tumour cells *via* the attachment of proteins expressed in very high quantity on cancer cells.³²

The detection of cancer cells *via* the use of nanoparticles is reviewed in the following section.

1.2.1 Optical detection techniques

Light scattering. Kah *et al.* used the resonance light scattering properties of AuNPs at their SPR to detect the overexpression of epidermal growth factor receptor (EGFR), a biomarker of cancer. They used anti-EGFR conjugated AuNPs as contrast probes. These modified AuNPs were able to bind cancerous cells (which have too much EGFR on their surface) and be visible on confocal reflectance microscope. They compared the reflectance of nasopharyngeal carcinoma cells (CNE2) and normal human lung fibroblast (NHLF) and have shown that Anti EGFR conjugated AuNPs, because of their unique optical response to light enhance the signal for carcinoma cells. AuNPs bound EGFR which were overexpressed on CEN2.

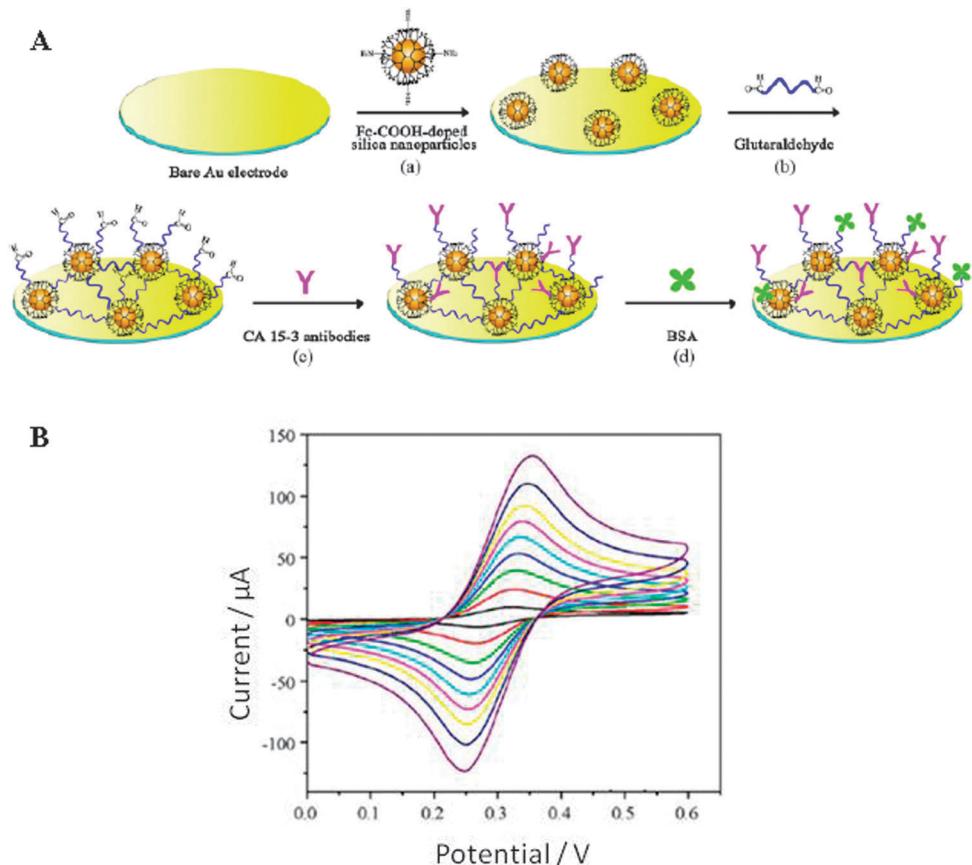


Fig. 11 (A) Fabrication of the immunosensor. (a) Formation of amino functionalized Fc–COOH-doped SiNPs layer, (b) glutaraldehyde cross-linkage, (c) anti-Ca 15-3 loading, and (d) BSA loading. (B) CVs of the immunosensor at different scan rates (from inner to outer): 10, 25, 50, 75, 120, 150, 200 and 300 mV s^{-1} in 0.1 M PBS (pH 7.0).²⁹

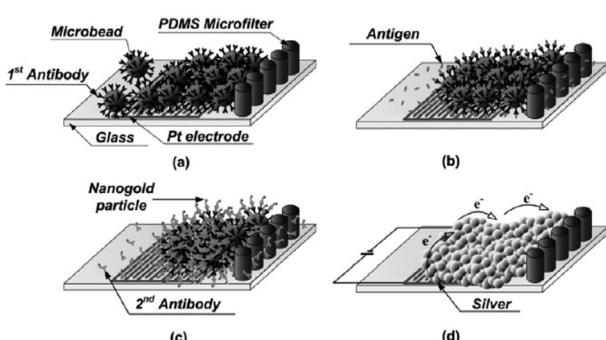


Fig. 12 Representation of the electrical immunoassay. (a) Securing the 1st Ab immobilized microbeads. (b) Reaction between the 1st Ab and the antigen. (c) Reaction with 2nd Ab-conjugated AuNP. (d) Electrical signal amplification using a silver enhancer and detection of the electrical signal resulting from the immunoreaction.³⁰

Excited at their SPR frequency they resonantly scattered the light and allowed the detection of the overexpression of EGFR. The images showed an aggregation of AuNPs on CEN2 surface and not on NHLF surface. In this way the overexpressed EGFR have been detected.³³ Lu and co-workers used oval-shaped AuNPs conjugated with monoclonal anti-HER2 Abs and 56 RNA aptamers for the colorimetric detection of breast cancer cells. Both of anti-HER2 Abs and

56 RNA aptamer were highly specific for the targeting of this type of epidermal growth factor receptor. The surface area of oval-shaped AuNPs offered the possibility to multifunctionalise the NP for a highly specific and sensitive targeting. The nanosensor was tested on SK-BR-3 cell line, a breast cancer cell line. The binding of HER-2 induced a colour change and a two photon scattering intensity change, which facilitated the detection of breast cancer cells. Moreover this assay was specific to this line of breast cancer cells; it allowed the identification of SK-BR-3 tumour cells from among other breast cancer cells.³⁴

Surface plasmon resonance. Lee *et al.* worked on the aggregation of AuNPs. They used DNA complementary to the mutant DNA of EGFR expressed on lung cancer cells. The fixation of mutant DNA to mutant DNA of EGFR on cancer cells forbade the fixation of DNA to AuNPs and caused AuNP aggregates. If the DNA of EGFR was wild, mutant DNA was not able to bind them and consequently they bound AuNPs and no aggregates appeared.³⁵ El-Sayed *et al.* developed a biosensor which permitted the detection of over-expression of EGFR for oral cancer. They conjugated AuNPs with anti EGFR Abs. They demonstrated that 35 nm is the optimum size of AuNPs for this detection. They measured the absorbance peak of AuNPs at 545 nm to compare the over-expression of EGFR on the surface of a non-malignant

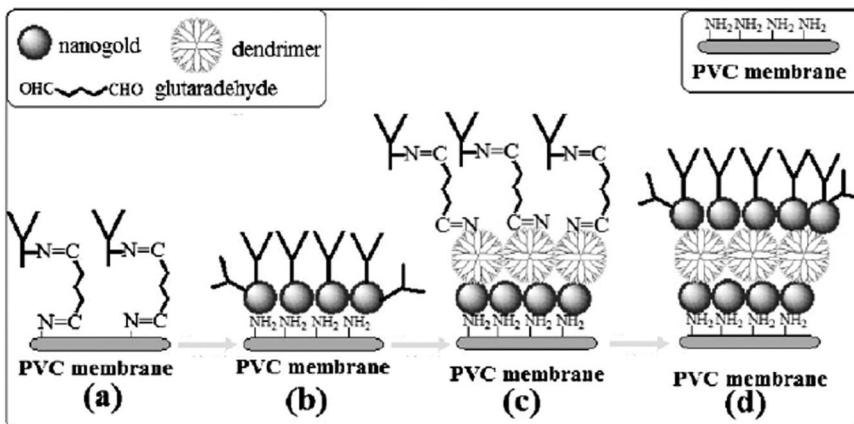
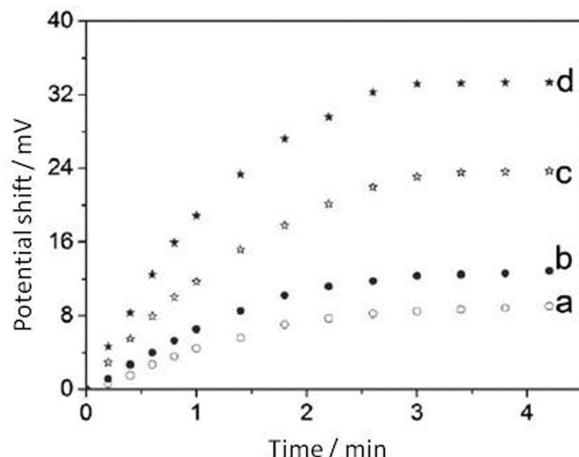
A**B**

Fig. 13 (A) Fabrication of the immunosensor. (a) Anti-PSA/glutaraldehyde-modified aminated PVC electrode. (b) Anti-PSA/nanogold-modified aminated PVC electrode. (c) Anti-PSA/glutaraldehyde/dendrimer/nanogold-modified aminated PVC electrode. (d) Anti-PSA/nanogold/dendrimer/nanogold-modified aminated PVC electrode. (B) Comparison of potential shifts *vs.* immunoreaction time for (a) Anti-PSA/glutaraldehyde-modified aminated PVC electrode, (b) Anti-PSA/nanogold-modified aminated PVC electrode, (c) Anti-PSA/glutaraldehyde/dendrimer/nanogold-modified aminated PVC electrode, (d) Anti-PSA/nanogold/dendrimer/nanogold-modified aminated PVC electrode toward 4.0 ng mL⁻¹ t-PSA under the same conditions.³¹

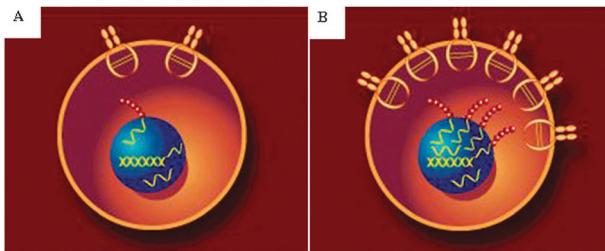


Fig. 14 (A) Normal cells only have a small amount of HER2 on their surface. (B) In 25% of invasive breast cancers, cells have a malfunctioning gene that causes too much HER2 on their surface. (The small Business Company, www.tsbc.com, 2010.)

epithelial cell line and two malignant oral epithelial cell lines. The signal intensity at 545 nm wavelength was higher for malignant cells, which showed that the overexpression of EGFR had been detected and is a marker of oral cancer.³⁶

Fluorescence. Huang *et al.* developed a nanosensor capable of detecting Human Ovarian Tumour (HOT) cells based on an Abs-conjugated silica with a NP fluorescent label. They conjugated fluorescent silica core-shell with Abs anti HER2. The cross-linker used for the conjugation was glutaraldehyde. BSA was also added between silica NPs and anti HER2 Abs in order to decrease the strong hydrophilic hydration and to enhance the Ab linking. HER2 is a member of epidermal growth factor receptor which is expressed at the surface of tumour cells. Anti-HER2 Abs bound the corresponding tumour antigen expressed on the surface of SKOV3, which were HOT cells. The interaction of antigen and antibody provoked an emission of fluorescence from silica NPs. This fluorescence has been analysed by laser scanning microscopy and scanning electron microscopy.³⁷ Zheng and Li worked on the detection of breast cancer cells by analysing the overexpression of telomerase inside the cells. They used an enzymatic sandwich immunoassay for the detection of telomerase. They first immobilised

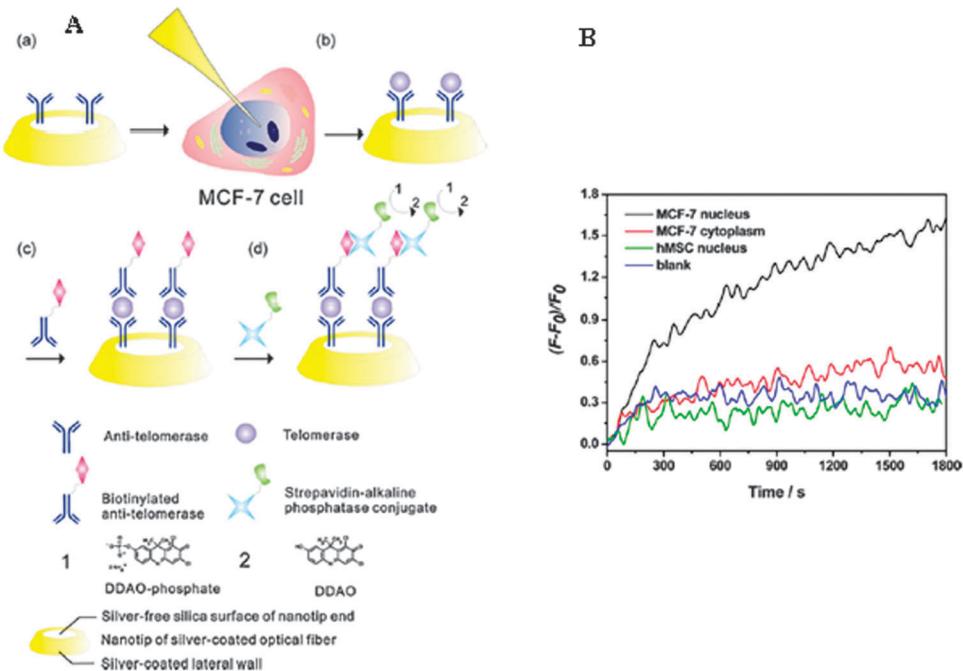


Fig. 15 (A) Single cell telomerase detection by optical fiber nanosensor. (B) Typical fluorescence change obtained upon addition of DDAO-phosphate to nanobiosensors in the detection chamber for four different experimental groups.³⁸

anti-telomerase Abs on a silver coated nanoprobe. Then they injected the bioconjugates inside the nucleus of a line of breast cancer cells, MCF-7. These bioconjugates bound telomerase, then biotinylated anti-telomerase Abs were added which bound the complex. By using the affinity between biotin and streptavidin, streptavidin–alkaline phosphatase conjugates attached to the biotin, and dephosphorylated DDAO phosphate, which induced a fluorescence intensity change (see Fig. 15).³⁸

Xiao and co-workers worked on QD-based biosensor for the detection of HER2 and telomerase. They have shown that IgY (Immunoglobulin Y) is monospecific for the binding of these biomarkers. Conjugated to QDs, IgY was able to bind HER2 and telomerase allowing the imaging and detection of biomarkers by immunohistochemical detection method. In images cells appeared in blue, when the IgY-QDs complexes bound cancer cells a red layer appeared on the surface of cancer cells. For telomerase, blue cancer cells appeared in blue and red. Compared to normal cells, the detection was clear and specific. IgY has also shown a higher specificity to telomerase and HER2 than common mammalian IgG (see Fig. 16).³⁹

Wang *et al.* developed a nanosensor for the capture of circulating tumour cells (CTCs). A silicon nanopillar substrate elaborated with silver enhanced local topographic interactions. On the silicon nanopillar substrate, the authors attached streptavidin which had been bound by biotinylated Abs anti EpCAM. EpCAM are transmembrane glycoproteins which are expressed at the surface of CTCs, negative EpCAM cells do not bind the complex, while positive EpCAM CTCs do, allowing their identification.⁴⁰ Sieuwerts and co-workers worked on the detection of EpCAM expressed at the surface of circulating normal-like breast tumour cells. They demonstrated that in comparison to HER2 positive and luminal

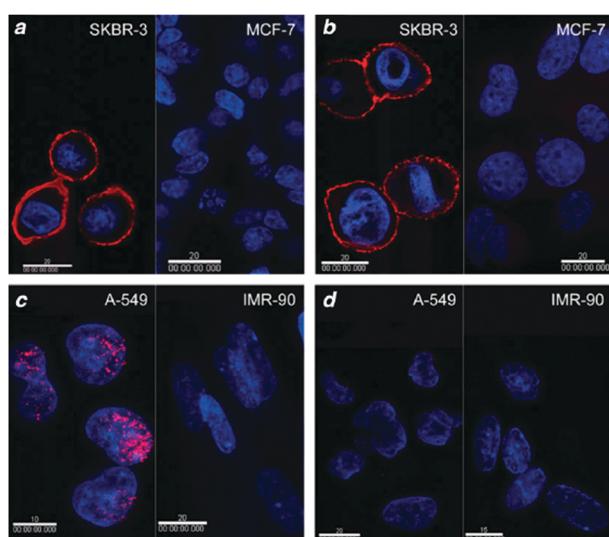


Fig. 16 Immunohistochemical biomarker detection with IgYs and QD fluorophores in positive and negative expressing cell lines. (a) HER2 detection with IgY in high expressing (SK-BR-3, left) and very low expressing (MCF-7, right) breast cancer cells. (b) HER2 detection, same experiment with mammalian IgG antibody CB11. (c) Human telomerase detection with IgY in high expressing (A549, left) and very low expressing (IMR90, right) cells. (d) Human telomerase detection, same experiment.³⁹

cancer cells, normal-like cancer cells were not detected by Cell Search and they were not bound specifically by iron micro-particles conjugated with anti EpCAM Abs.⁴¹ Bifunctional nanocomposites have been used by Ma and co-workers for the detection of lung cancer cells. Superparamagnetic NPs were

coated with silica and then, anti-CEA Abs conjugated water soluble QDs were immobilised onto their surface. SPCA-1, a lung tumour cell line, was identified from the other cells because they expressed on their surface CEA, thus, nanocomposite-based labels targeted these cells. This targeting was controlled by an external magnetic field. Using confocal laser scanning microscopy, it was possible to analyse the signal change of the luminescence intensity.⁴²

1.2.2 Electrical/electrochemical detection techniques.

Electrochemical detection can be carried out for overproduced proteins at the surface of cancer cells.

Electrical impedance spectroscopy (EIS). Ding and co-workers worked on the analysis of glycan expression on living cells. They used single-walled carbon nanohorns (SWNHs) conjugated with concavalin A (a mannose group specific to lectin) to detect cancer cells. Lectin was used to bind cancer cells; it recognised sugar epitopes. The complex single-walled carbon nanohorns-lectin was immobilised on a glassy-carbon electrode. BSA was also added as a blocking layer. Measurements were done by EIS, for the analysis of changes of electron transfer resistance. GCE did not induce any changes in the electron transfer resistance when cells were not attached to the complex. When cells bound lectin, and thus the electrode, the reaction of electron transfer slowed. Lecitin acted as an inert electron transfer blocking layer. Changes were analysed by EIS and thus the presence of cancer cells was detected (see Fig. 17).⁴³

Electrocatalytic. Ding *et al.* also developed a mean of detection of carbohydrate expression on living cells. In this nanosensor they used SWNHs conjugated with arginine-glycine-aspartic acid-serine (RGDS) immobilised on electrode. RGDS were able to capture cells because of their specific binding to the integrin at the surface of cells. Cells thus attached the RGDS-SWNH-electrode. Then AuNPs functionalised with HRP (for amplification signal) and Concavatin A (Con A), a type of lectin, bound immobilized cells due to the affinity between lectin and sugar isotopes (in this paper, mannoses groups expressed at the surface of cancer cells). The fixation of Con A-HRP-AuNPs allowed the enzymatic reaction, and thus the electrochemical signal was analysed for the detection and quantification of mannose expression, biomarker of cancer (see Fig. 18).⁴⁴

De la Escosura-Muniz *et al.* developed an electrocatalytic device for the specific detection of tumour cells. They studied

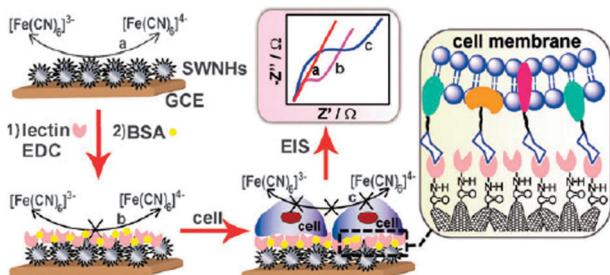


Fig. 17 Representation of the electrochemical label free strategy for the analysis of glycan expression on cell surfaces.⁴³

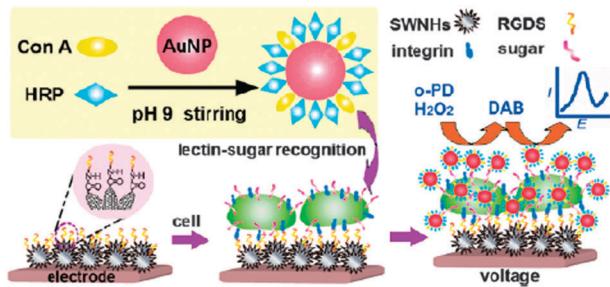


Fig. 18 Representation of the nanoprobe assembly for the mannose groups *in situ* detection on living cells. Abbreviations: o-PD, o-phenylenediamine; DAB, 2,2'-diaminoazobenzene.⁴⁴

the tumoural cells HMy2 which express at their surface HLA-DR molecules recognizable by AuNPs conjugated with Abs anti DR molecules. HMy2 were immobilised on SPCE, they were bound by Abs conjugated AuNPs. AuNPs catalysed the reduction of hydrogen ions, which induced a signal. This signal permitted the quantification of tumour cells and the analysis of cells proliferation. Normal cells which did not express DR molecules on their surface were not attached by AuNPs and thus were not detected. They used two ways for the detection, direct and indirect. In the direct way, they conjugated AuNPs with monoclonal Abs, they bound cells and the detection was done. In the second way, primary Abs were first incubated with cells they bound. Then AuNPs conjugated with secondary Abs bound primary Abs, allowing the catalysis and thus the detection. The second method offered a higher signal due to the amplification mediated by secondary Abs fixed to primary Abs.⁴⁵

Stripping voltammetry. Stripping voltammetry enables the quantification of analytes after their immobilisation on an electrode and after the stripping-based detection of the metal nanoparticles used as labels. Li *et al.* worked on a nanosensor based on the electrochemical detection of breast cancer cells. Breast cancer cells produced at their surface human mucin 1 and CEA. By detecting both of these biomarkers on cells they allowed the differentiation of breast cancer cells from other cancer cells and normal cells. They immobilised MUC 1 aptamers, which were able to bind human mucin 1, on a Au electrode. Cells which produced human mucin 1 at their surface attached the electrode. Then Cadmium sulfide nanoparticles (CdSNPs) conjugated with Abs anti CEA bound these cells only if they expressed CEA at their surface. CdSNPs allowed a change in the electrochemical signal which allowed the detection and quantification of the breast cancer cells (see Fig. 19). In this device, only breast cancer cells were detected because they were the only cells to express both mucin 1 and CEA at their surface. This device thus allowed the specific detection of breast cancer cells at very low detection limits.⁴⁶

1.2.3 Other techniques. Liu *et al.* also used AuNPs optical properties on Aptamer-Nanoparticle Strip Biosensor (ANSB) on a lateral flow assay. A Lateral Flow Assay (LFA), or immunochemical test strip, is a simple separation device which facilitates affinity assays. Liu *et al.* selected aptamers capable of attaching ramos cells *via* SELEX

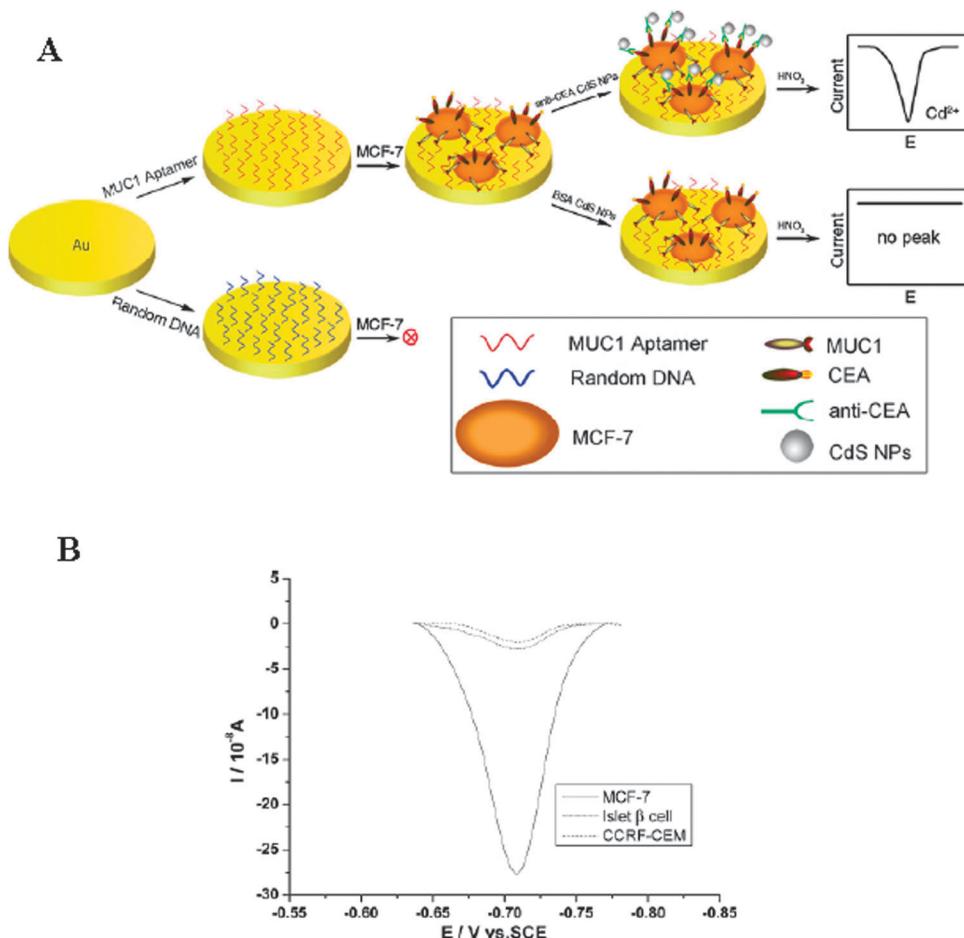


Fig. 19 (A) Representation of the detection of breast cancer cells by the nanosensor through simultaneous recognition of two different cancer biomarkers. (B) Square-wave voltammograms for different kinds of cells, breast cancer cell line MCF-7, islet beta cells and acute leukaemia cells CCRF-CEM.⁴⁶

(systematic evolution of ligands by exponential enrichment) and conjugated them to AuNPs for the detection of tumour cells. Ramos cells were used as model targets; they were bound by thiol aptamer-conjugated AuNPs. Then the complex was captured on the detection zone by biotin-labelled aptamer immobilised on ANSB via streptavidin. This binding produced a characteristic red line due to the optical properties of AuNPs. The excess of thiol aptamer-conjugated AuNPs was then bound on DNA probe complementary with thiol aptamer on the control zone producing another red line (see Fig. 20).⁴⁷

1.3 Conclusion and future perspectives

A general overview of the reported techniques, the role of nanoparticles and the detection techniques used are summarized in Table 1. The work reviewed shows that nanotechnology-based cancer diagnostics are becoming an increasingly relevant alternative to traditional techniques. Nevertheless, most of the current results show a lack of sensitivity or specificity, and relatively high detection limits, which do not permit the early detection of cancer. Moreover there is not an “ideal” biomarker of cancer, a marker which by its presence means cancer and by its absence means absence of cancer. It is important to quantify the concentration of biomarker in blood, tissues or serum at very high sensitivity, very low detection limit

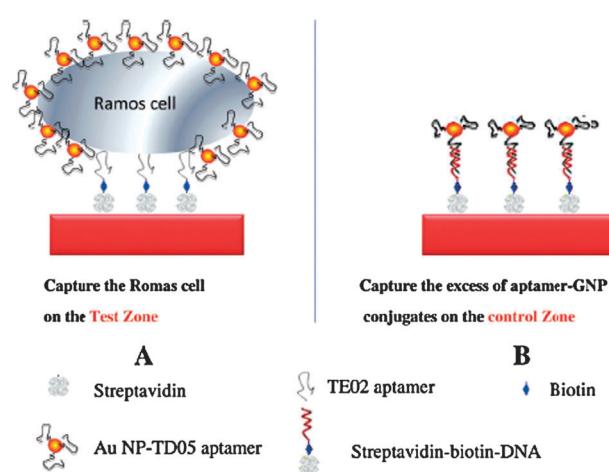


Fig. 20 Representation of the detection of Ramos cells on ANSB: (A) capturing AuNP-aptamer-Ramos cells on the test zone of ANSB through specific aptamer-cell interactions and (B) capturing the excess of AuNP-aptamer on the control zone of ANSB through aptamer-DNA hybridization reaction.⁴⁷

(even at single biomolecule/cell level) and most probably to detect the several cancer biomarkers in a single test. While fluorescent techniques use to offer higher sensitivities and

Table 1 General data on nanoparticle based biosystems for cancer biomarkers and cells detections

	NP	Role of NP	Detection technique	Limit of detection	References
Biomarkers					
CEA	AuNP	Label	Light absorption	12 ng L ⁻¹	11
AFP	AuNP	Catalytic label	Light absorption	0.1 ng mL ⁻¹	13
Thrombin	AuNP	Agglutinates	Light absorption	0.04 pM	14
PSA	Silver enhanced AuNP	Scatter light	Light scattering	1–10 fg mL ⁻¹	15
PSA; HCG; AFP	Silver enhanced AuNP	Scatter light	Light scattering	170 fM	16
PSA	AuNP	Probe capture	SPR	500 fM	17
PSA	AuNP	Signal enhancement	SPR	1 ng mL ⁻¹	18
PSA	QD	Fluorophores	Fluorescence		19
CEA; CA 125; Her-2/Neu	QD	Label/fluorophores	Fluorescence	CEA : 0.02 ng mL ⁻¹	20
CEA	AuNP	Transducer modifier Increase electron transfer resistance	EIS	0.1 ng mL ⁻¹	21
PSA	AuNP	Label/catalyser	Electrocatalytic	0.5 pg mL ⁻¹	22
IL-6	AuNP	Label	Electrocatalytic	10 pg mL ⁻¹	23
Kinase activity	AuNP	Label	Electrocatalytic	10 ng mL ⁻¹	24
Kinase activity	AuNP	Label	Electrocatalytic	5–10 U mL ⁻¹	25
CEA	Nanogold enwrapped graphene nanocomposites	Label	Electrocatalytic	0.01 ng mL ⁻¹	26
CA 15-3	AuNP	Transducer modifier	Electrocatalytic	0.6 ng mL ⁻¹	27
CEA	AuNP	Transducer modifier	Electrocatalytic	0.1 ng mL ⁻¹	28
CA 15-3	Nickel hexacyano- ferrates NP				
CA 15-3	Silica NP	Transducer modifier	Electrocatalytic	0.64 U mL ⁻¹	29
AFP; CEA; PSA	Silver enhanced AuNP	Transducer modifier	Electrocatalytic	—	30
t-PSA	AuNP dendrimer	Transducer modifier	Potentiometry	0.1 ng mL ⁻¹	31
Cells					
Non small cell lung cancer	AuNP	Aggregation	Light scattering	—	33
Breast cancer cells	Oval shaped AuNP	Transducer modifier (induce color change)	Colorimetric/two photon scattering	100 cells mL ⁻¹	34
Nasopharyngeal carcinoma cells	AuNP	Reflectance-based imaging probe/label	SPR	—	35
Epithelial cancer cells	AuNP	Label	SPR	—	36
Ovarian cancer cells	Silica NP	Label/fluorophores	Fluorescence	—	37
Telomerase overexpression on single living cells	Silver coated silica optical fiber	Fluorophores	Fluorescence	—	38
Breast cancer cells	QD	Fluorophores	Fluorescence	—	39
Circulating tumour cells	Silica nanopillar	Fluorophores	Fluorescence	—	40
Lung cancer cells	Silica coated super- paramagnetic NP	Separation	Magnetism	—	42
Dynamic glycan expression on living cells	QD	Fluorophores	Fluorescence	—	43
Dynamic carbohydrates expression on living cells	Carbon nanohorns	Facilitate electron transfer	EIS	1500 cells mL ⁻¹	44
Breast cancer cells	AuNP	Signal amplification	EIS	330 cells mL ⁻¹	46
Cadmium sulfide NP	Cadmium sulfide NP	Transducer modifier Label	EIS	4000 cells/0.7 mL	45
Human tumour cell lines	AuNP	Catalyser/label	Electrocatalytic	—	47
Circulating tumour cells	AuNP	Label	Lateral flow assay	—	

lower detection limits the electrochemical techniques still lack the required analytical performance. The improvement of the sensitivity of electrochemical techniques using catalytic methods as well as other enhancement strategies is being carefully considered so as to avoid time consuming procedures/steps as well as problems related to the assay reproducibility.

Despite the above mentioned drawbacks, the use of nanoparticle-based sensors in biomarker detection or cancer cell detection offers some advantages in comparison to conventional

methods. First, the method is low cost; a very low quantity of the sample is indeed needed and materials, if designed for a mass production technology, could be inexpensive. Secondly, methods based on the use of nanoparticles can be incorporated into user-friendly devices. Furthermore, immunoassays using nanoparticles are very specific and sensitive. Nanoparticles can be conjugated to a specific antibody of the biomarker or of the protein overexpressed at the surface of cancer cells. This enhances the specificity and the sensitivity of the biosensor. Biosensors with nanoparticles are able to attach to biomarkers

or cancer cells, even when these are in low concentration in the serum, at a very early stage of cancer.

In this review we have illustrated different methods to detect cancer biomarkers and cancer cells, with a focus on optical and electrochemical techniques. These techniques can be interesting and relevant for their use in point-of-care of cancer diagnostics. Nevertheless the concentration range of cancer biomarkers and cancer cells in the early stage of the cancer and in the case of metastases should be considered prior to the design of a nanoparticle based sensor and its posterior application.

Abbreviations

Ab	Antibody
ABTS	2,2'-Azino-bis-3-ethylbenzothiazoline-6-sulfonic acid
ACT	Protein anti-chymotrypsin
ADP	Adenosine di-phosphate
AFP	α -Fetoprotein
Ag	Antigen
aMG	Protein α -macroglobulin
ANSB	Aptamer NP strip biosensor
ATP	Adenosine tri-phosphate
AuNP	Gold nanoparticle
BSA	Bovine serum albumin
CA 125	Carbohydrate (or cancer) antigen 125
CA 15-3	Cancer antigen 15-3
CdSNP	Cadmium sulfide NP
CEA	Carcinoembryonic antigen
CNE 2	Nasopharyngeal carcinoma cell
Con A	Concavalin A
CTC	Circulating tumour cell
CV	Cyclic voltammetry
DNA	Deoxyribonucleic acid
DPV	Differential pulse voltammetry
dsDNA	Double stranded DNA
EGFR	Epidermal growth factor receptor
EIS	Electrical impedance spectroscopy
EpCam	Epithelial cell adhesion molecule
GCE	Glassy carbon electrode
GSH	Glutathione
hCG	human chorionic gonadotropin
HER	Herceptin
HOT	Human ovarian tumour
HRP	Horseradish peroxidase
Ig	Immunoglobulin
IL-6	Interleukin 6
ITO	Indium tin-oxide
LFA	Lateral flow assay
LFIA	Lateral flow immunoassay
MMP	Magnetic nanoparticle
mRNA	messenger Ribonucleic acid
Muc	Mucin
NGGN	Nanogold enwrapped graphene nanocomposites
NHLF	Normal human lung fibroblast
NP	Nanoparticle
OAP	σ -Aminophenol
pAb	Polyclonal Ab

PB	Prussian blue
PBS	Phosphate buffered saline
PEG	Polyethylene glycol
PSA	Prostate specific antigen
PTK	Protein kinase
QD	Quantum dot
RGDS	Arginine-glycine-aspartic acid-serine
RNA	Ribonucleic acid
SAM	Self assembly monolayer
SELEX	Systematic evolution of ligands by exponential enrichment
SiNP	Silica NP
SPR	Surface plasmon resonance
ssDNA	single stranded DNA
SWCNT	Single walled carbon nanotubes
SWNH	Single walled carbon nanohorn

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References

- O. Golubitschaja and J. Flammer, *Surv. Ophthalmol.*, 2007, **52**, S155.
- A. P. F. Turner, B. Chen and S. A. Piletsky, *Clin. Chem.*, 1999, **45**, 1596.
- J. A. Ludwig and J. N. Weinstein, *Nat. Rev. Cancer*, 2005, **5**, 845.
- A. M. LeBeau, P. Singh, J. T. Isaacs and S. R. Denmeade, *Biochemistry*, 2009, **48**, 3490.
- L. C. Su, R. C. Chen, Y. C. Li, Y. F. Chang, Y. J. Lee, C. C. Lee and C. Chou, *Anal. Chem.*, 2010, **82**, 3714.
- T. Nakagawa, E. Miyoshi, T. Yakushijin, N. Hiramatsu, T. Igura, N. Hayashi, N. Taniguchi and A. Kondo, *J. Proteome Res.*, 2008, **7**, 2222.
- U. H. Stenman, H. Alftan and K. Hotakainen, *Clin. Biochem.*, 2004, **37**, 549.
- M. G. Anhorn, S. Wagner, J. Kreuter, K. Langer and H. Von Briesen, *Bioconjugate Chem.*, 2008, **19**, 2321.
- S. J. Kaneko, T. Gerasimova, S. T. Smith, K. O. Lloyd, K. Suzumori and S. R. Young, *Gynecol. Oncol.*, 2003, **90**, 29.
- A. Ambrosi, F. Airò and A. Merkoçi, *Anal. Chem.*, 2010, **82**, 1151.
- M. Liu, C. Jia, Y. Huang, X. Lou, S. Yao, Q. Jin, J. Zhao and J. Xiang, *Analyst*, 2010, **135**, 327.
- I. Willner, B. Shlyahovsky, M. Zayats and B. Willner, *Chem. Soc. Rev.*, 2008, **37**, 1153.
- W. H. Zhou, C. L. Zhu, C. H. Lu, X. Guo, F. Chen, H. H. Yang and X. Wang, *Chem. Commun.*, 2009, 6845.
- C. K. Chen, C. C. Huang and H. T. Chang, *Biosens. Bioelectron.*, 2010, **25**, 1922.
- Y. P. Bao, T. F. Wei, P. A. Lefebvre, H. An, L. He, G. T. Kunkel and U. R. Müller, *Anal. Chem.*, 2006, **78**, 2055.
- S. I. Stoeva, J. S. Lee, J. E. Smith, S. T. Rosen and C. A. Mirkin, *J. Am. Chem. Soc.*, 2006, **128**, 8378.
- J. W. Choi, D. Y. Kang, Y. H. Jang, H. H. Kim, J. Min and B. K. Oh, *Colloids Surf., A*, 2008, **313–314**, 655.
- L. Huang, G. Reekmans, D. Saerens, J.- Friedt, F. Frederix, L. Francis, S. Muyldermans, A. Campitelli and C. Van Hoof, *Biosens. Bioelectron.*, 2005, **21**, 483.
- A. Gokarna, L. H. Jin, S. H. Jun, Y. H. Cho, T. L. Yong, H. C. Bong, H. Y. Seong, S. C. Dong and H. L. Jung, *Proteomics*, 2008, **8**, 1809.
- J. V. Jokerst, A. Raamanathan, N. Christodoulides, P. N. Floriano, A. A. Pollard, G. W. Simmons, J. Wong, C. Gage, W. B. Furmaga, S. W. Redding and J. T. McDevitt, *Biosens. Bioelectron.*, 2009, **24**, 3622.

- 21 H. Tang, J. Chen, L. Nie, Y. Kuang and S. Yao, *Biosens. Bioelectron.*, 2007, **22**, 1061.
- 22 J. F. Rusling, G. Sotzing and F. Papadimitrakopoulou, *Bioelectrochemistry*, 2009, **76**, 189.
- 23 B. S. Munge, C. E. Krause, R. Malhotra, V. Patel, J. Silvio Gutkind and J. F. Rusling, *Electrochim. Commun.*, 2009, **11**, 1009.
- 24 K. Kerman and H. B. Kraatz, *Biosens. Bioelectron.*, 2009, **24**, 1484.
- 25 K. Kerman, M. Chikae, S. Yamamura and E. Tamiya, *Anal. Chim. Acta*, 2007, **588**, 26.
- 26 Z. Zhong, W. Wu, D. Wang, D. Wang, J. Shan, Y. Qing and Z. Zhang, *Biosens. Bioelectron.*, 2010, **25**, 2379.
- 27 Y. Yang, Z. Zhong, H. Liu, T. Zhu, J. Wu, M. Li and D. Wang, *Electroanalysis*, 2008, **20**, 2621.
- 28 Y. R. Yuan, R. Yuan, Y. Q. Chai, Y. Zhuo and X. M. Miao, *J. Electroanal. Chem.*, 2009, **626**, 6.
- 29 C. Hong, R. Yuan, Y. Chai and Y. Zhuo, *Anal. Chim. Acta*, 2009, **633**, 244.
- 30 Y. J. Ko, J. H. Maeng, Y. Ahn, S. Y. Hwang, N. G. Cho and S. H. Lee, *Electrophoresis*, 2008, **29**, 3466.
- 31 X. L. Wang, G. H. Tao and Y. H. Meng, *Electroanalysis*, 2009, **21**, 2109.
- 32 C. A. Wilson, E. E. Cajulis, J. L. Green, T. M. Olsen, Y. A. Chung, M. A. Damore, J. Dering, F. J. Calzone and D. J. Slamon, *Breast Cancer Res.*, 2005, **7**, R1058.
- 33 J. C. Y. Kah, M. C. Olivo, C. G. L. Lee and C. J. R. Sheppard, *Mol. Cell. Probes*, 2008, **22**, 14.
- 34 W. Lu, S. R. Arumugam, D. Senapati, A. K. Singh, T. Arbneshi, S. A. Khan, H. Yu and P. C. Ray, *ACS Nano*, 2010, **4**, 1739.
- 35 H. Lee, T. Kang, K. A. Yoon, S. Y. Lee, S. W. Joo and K. Lee, *Biosens. Bioelectron.*, 2010, **25**, 1669.
- 36 I. H. El-Sayed, X. Huang and M. A. El-Sayed, *Nano Lett.*, 2005, **5**, 829.
- 37 S. Huang, R. Li, Y. Qu, J. Shen and J. Liu, *J. Fluoresc.*, 2009, **19**, 1095.
- 38 X. T. Zheng and C. M. Li, *Biosens. Bioelectron.*, 2010, **25**, 1548.
- 39 Y. Xiao, X. Gao, G. Gannot, M. R. Emmert-Buck, S. Srivastava, P. D. Wagner, M. D. Amos and P. E. Barker, *Int. J. Cancer*, 2008, **122**, 2178.
- 40 S. Wang, H. Wang, J. Jiao, K. J. Chen, G. E. Owens, K. i. Kamei, J. Sun, D. J. Sherman, C. P. Behrenbruch, H. Wu and H. R. Tseng, *Angew. Chem., Int. Ed.*, 2009, **48**, 8970.
- 41 A. M. Sieuwerts, J. Kraan, J. Bolt, P. Van Der Spoel, F. Elstrott, M. Schutte, J. W. M. Martens, J. W. Gratama, S. Sleijfer and J. A. Foekens, *J. Natl. Cancer Inst.*, 2009, **101**, 61.
- 42 J. Ma, Q. Fan, L. Wang, N. Jia, Z. Gu and H. Shen, *Talanta*, 2010, **81**, 1162.
- 43 L. Ding, W. Cheng, X. Wang, Y. Xue, J. Lei, Y. Yin and H. Ju, *Chem. Commun.*, 2009, 7161.
- 44 L. Ding, Q. Ji, R. Qian, W. Cheng and J. Huangxian, *Anal. Chem.*, 2010, **82**, 1292.
- 45 A. De La Escosura-Muñiz, C. Sánchez-Espinel, B. Diaz-Freitas, A. González-Fernández, M. Maltez-Da Costa and A. Merkoçi, *Anal. Chem.*, 2009, **81**, 10268.
- 46 T. Li, Q. Fan, T. Liu, X. Zhu, J. Zhao and G. Li, *Biosens. Bioelectron.*, 2010, **25**, 2686.
- 47 G. Liu, X. Mao, J. A. Phillips, H. Xu, W. Tan and L. Zeng, *Anal. Chem.*, 2009, **81**, 10013.