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Potential of Surface Plasmon Resonance Biosensors in cancer detection

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Highlights

- 67 papers on SPR biosensors for cancer markers have been reviewed
- Molecular cancer markers, micro RNA, vesicles, exosomes and cancer cells are reviewed
- Validated SPR biosensors for cancer markers are briefly characterized
- SPR biosensors are able to contribute to the discovery of new cancer markers
- Seven new markers are proposed, two of them having high sensitivity and selectivity

Abstract

A review is made of 71 papers on surface plasmon resonance biosensors, published between 2005 and 2020, mostly in the last decade. The reviewed papers are divided into two groups, depending on the validation of the developed biosensor. Validated biosensors are briefly characterized, while those that are not validated are listed in a table. Focus is placed on applications of SPR biosensors in testing the effectiveness of cancer markers and in the discovery of new cancer markers. Seven new markers are proposed, two of them having high sensitivity and diagnostic selectivity as determined by ROC curves. Papers concerning the determination of micro RNA and large particles such as vesicles, exosomes and cancer cells are also reviewed.

Keywords: Surface plasmon resonance; circulating cancer markers; biosensors; liquid biopsy; body fluids; validation; ROC curves;

1. Introduction

Biosensors are the subject of enormous expectations and are gradually gaining in diagnostic importance. These expectations are connected with what is called ‘liquid biopsy’, i.e. diagnosis based on analysis of body fluids such as blood, urine and saliva, and the possibility of early diagnosis of various cancers or other diseases. An ideal biosensor should react exclusively to the target marker despite the presence of numerous similar proteins, glycoproteins, etc. in the analyzed body fluid. Moreover, the biosensor’s dynamic response range should include the concentrations of the marker found in the body fluid both of persons with the disease and of the healthy population. It is also

expected that the precision of measurement of the marker concentration will be sufficient to distinguish between samples below and above a threshold concentration value (called the 'cut-off').

Most cancer markers are large molecules (proteins or glycoproteins), such as PSA, CEA or CA-125/MUC16. More rarely, exosomes, circulating tumor cells or micro RNA are used as cancer markers. Apart from cancer detection, markers are expected to enable determination of the type and stage of cancer. To date, none of the known markers, in combination with methods for their determination, are regarded as fully satisfactory diagnostic tools. The discovery of new markers is desirable, as well as the development of new determination tools. Surface plasmon resonance (SPR) biosensors may be such a tool. The aim of this review is to analyze papers concerning biosensors for the detection of different kinds of cancer with the use of SPR techniques, and to classify them in terms of the maturity of the proposed tools for cancer diagnosis.

2. Most frequently used SPR techniques in cancer marker detection

The SPR effect occurs at the metal–dielectric interface when the energy of incident light is coupled into surface plasmons, which cause weakening of the total internal reflection of the light beam. The effect occurs at a specific incident angle and wavelength. A minimum (SPR dip) appears on the reflection spectrum. When the marker being determined is immobilized on the metal surface, there occurs a change in the refractive index and a shift of the minimum, as well as a rotation of the angle of incident light if a polarized beam is used. A glass slide covered with a thin gold layer is usually used as a biosensor base. Alternatively, a glass prism covered with a thin gold layer can be used. However, marker detection based on the SPR effect can also be carried out using gold or silver nanoparticles immobilized on a glass slide or optical fiber, or simply suspended in solution. An example of the last technique, with spectrophotometric recording, is found in a study of CA-125 determination with suspended gold nanorods [1]. Usually, a biosensor consists of a receptor attached to the gold surface via a linker.

Generally, biosensors are used with one of three SPR techniques: a fluidic version (SPR), nonfluidic SPR imaging (SPRi), and localized SPR (LSPR). To date, the most frequently used SPR technique in cancer detection is the fluidic version of SPR. In this version, measurement is performed with the biosensor in contact with flowing solution. Such a measuring system is shown in Fig 1. A biosensor is created *in situ* during the measurement by sequential introduction of a receptor and a solution containing the determined marker, pushed by a running buffer. Usually, a chip covered with approximately 50 nm of gold is preliminarily covered with a linker. Carboxylated dextran is frequently used as a linker. A suitable antibody covalently bonded to the linker is usually used as a receptor. In such cases, the EDC/NHS protocol is applied for the *in situ* creation of amide bonds between carboxy groups of the linker and amine groups of the antibody. Subsequently, a sample containing the analyte is directed to the biosensor.

Entrapment of the analyte by the antibody causes changes in the SPR signal and creates an analytical signal. Finally, the chip sensor is cleaned to prepare it for the next measurement. Subsequent measurements can be performed rapidly, as in flow-injection analysis. An example of a recorded curve (sensogram) is shown in Fig. 2.

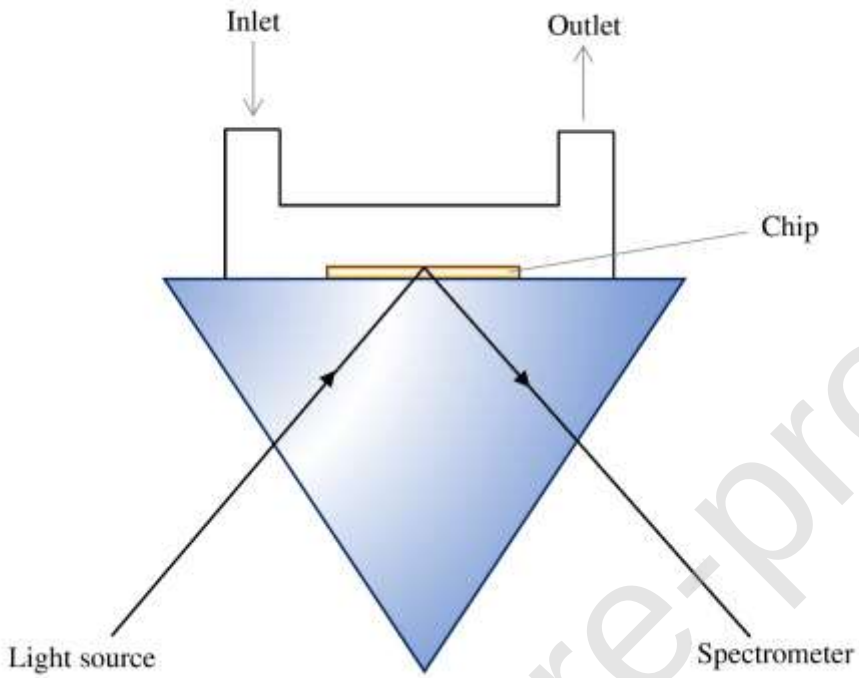

Fig. 1. Scheme of fluidic SPR

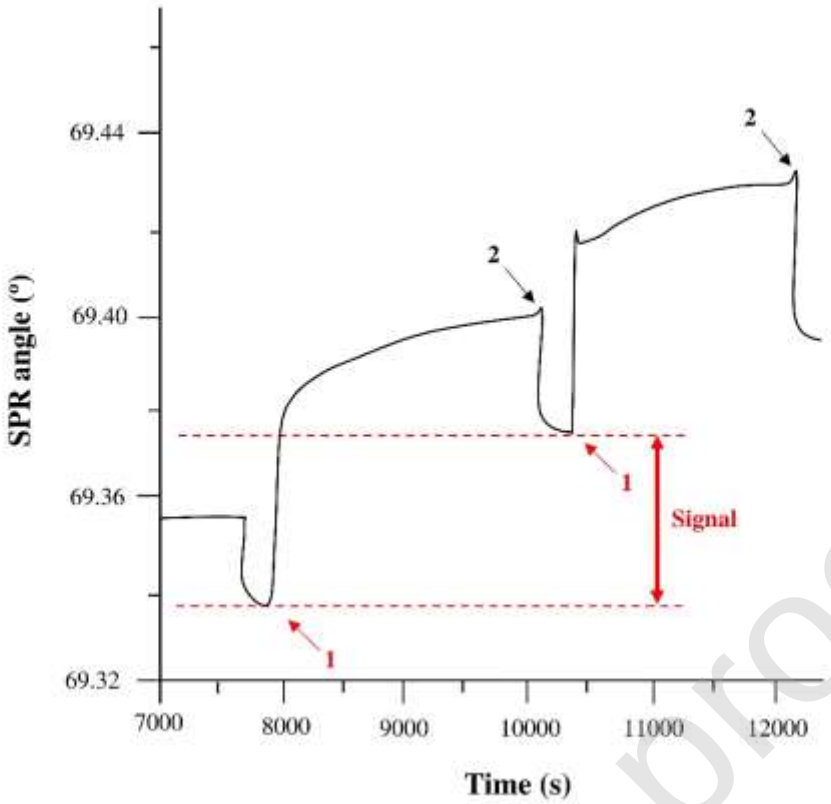

Fig. 2. A typical sensorgram of the SPR biosensor. (reproduced from [2])

Amplification of the analytical signal is used when higher sensitivity of measurement is required. This can be done by injecting a secondary antibody after a marker (e.g. interleukin 6) is entrapped by a primary antibody, with the formation of a sandwich structure [3]. Stronger enhancement of the analytical signal can be achieved by the introduction of functionalized gold nanoparticles. Two optional solutions are shown in Fig. 3, using the example of CEA determination [4].

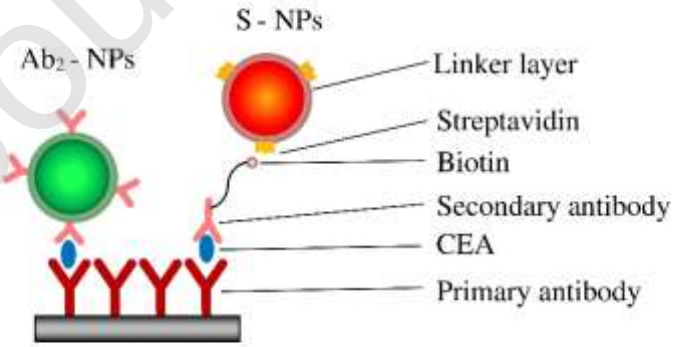

Fig. 3. Scheme of AuNP containing sandwich biosensors. Adapted from [4]

Nonfluidic measurement is usually performed in a stationary arrangement, with an array of separated measuring points. An example of nonfluidic SPR imaging measurement is shown in Fig. 4. The biosensor is prepared *ex situ* by sequential immobilization of a linker and a receptor, and finally the analyzed solution. The SPR measurement is performed following the gentle removal of solution from the biosensor surface, which is the major difference compared with fluidic measurements. The reflected light is directed to a CCD camera and converted into an image.

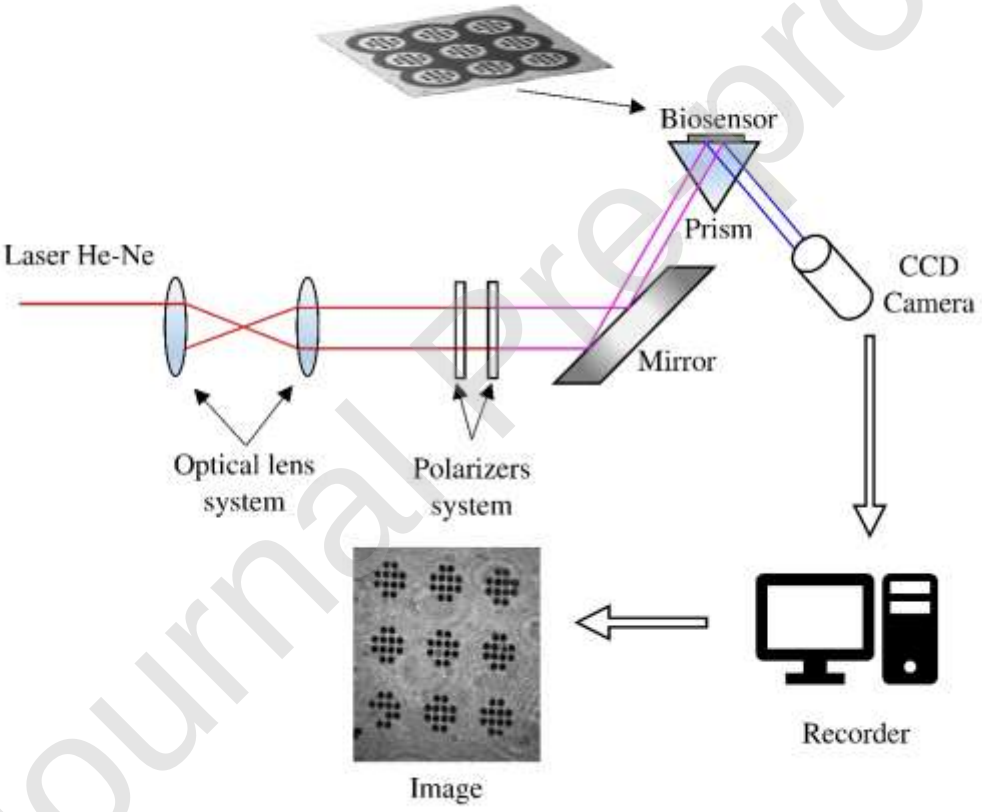

Fig. 4. Example of nonfluidic measurement. Reproduced with permission from [5].

Localized SPR measurement is performed with gold or silver nanoparticles fixed onto a glass slide or optical fiber (see Fig. 5). The red shift of the absorption maximum serves as the analytical signal in LSPR (see Fig. 6).

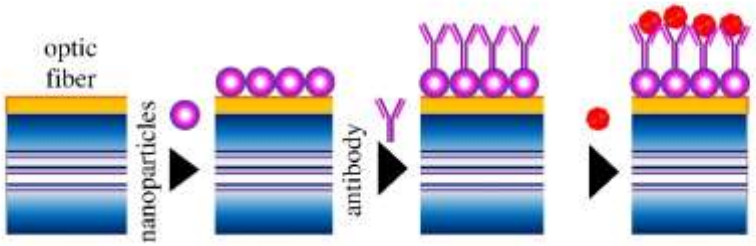

Fig. 5. Example of LSPR biosensor

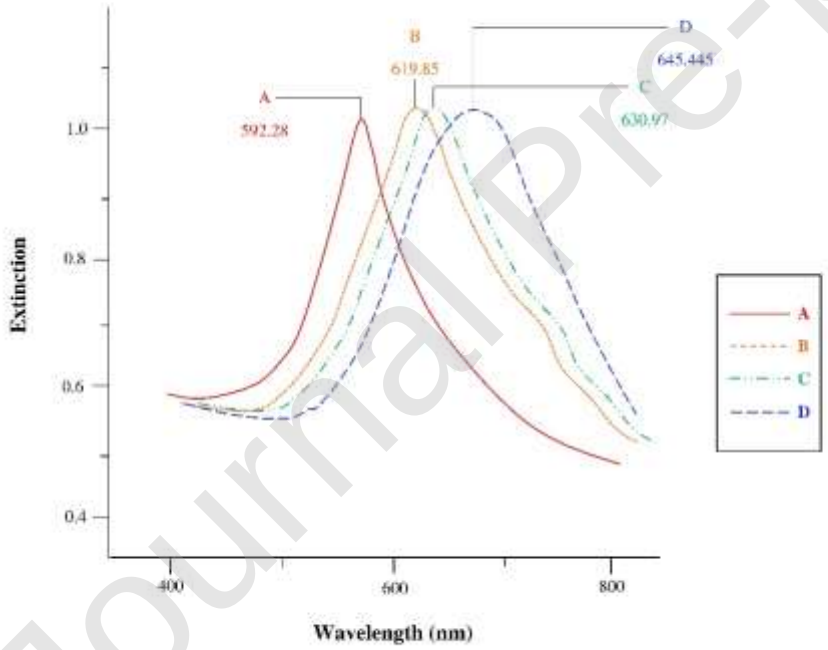

Fig. 6. Example of analytical signal in LSPR (reproduced from [6])

3. Criteria for selection of biosensors as ready for use in cancer diagnosis

Not all papers on the determination of cancer markers by SPR techniques include sufficient characterization in terms of quantitative marker determination. A fully developed biosensor and the related analytical procedure should be ready to undergo clinical investigation for subsequent use in

diagnosis. A crucial factor is the selectivity of the biosensor; or more precisely, analytical selectivity (as the former term has dual meanings). The marker being analyzed should be determined in a body fluid, such as blood serum, in the presence of a large number of similar substances. This is usually achieved by the use of a suitable antibody as a receptor in the sensing element of a biosensor. Alternatively, a marker's inhibitor or a suitable aptamer may serve as a receptor. Apart from analytical selectivity, a biosensor and its related analytical procedure should enable determination of the marker within a concentration range that includes the concentrations found both in cancer samples and in healthy population samples. Insufficient analytical sensitivity of a biosensor is frequently overcome by the formation of a sandwich structure, usually containing nanoparticles. A significant factor in successful marker determination is the linearity of the calibration graph. Generally, the nature of the formation of an analytical signal determines the Langmuirian dependence of that signal on the marker concentration (see Fig. 7). Any biosensor has a limited number of receptor sites. The gradual occupation of active sites causes a plateau to form on the calibration graph. Fortunately, the initial section of the graph is pseudolinear, and it can be successfully used for calibration purposes. The Langmuirian curve can be linearized in semi-logarithmic coordinates. Here, however, the logarithm of the concentration is obtained instead of the concentration itself, and the results may be subject to unacceptable error. A final test for a biosensor is its validation by the determination of a marker in natural samples, such as blood serum, with parallel determination of the marker in the same samples by a standard method. The longer the series of natural samples analyzed, the better the validation.

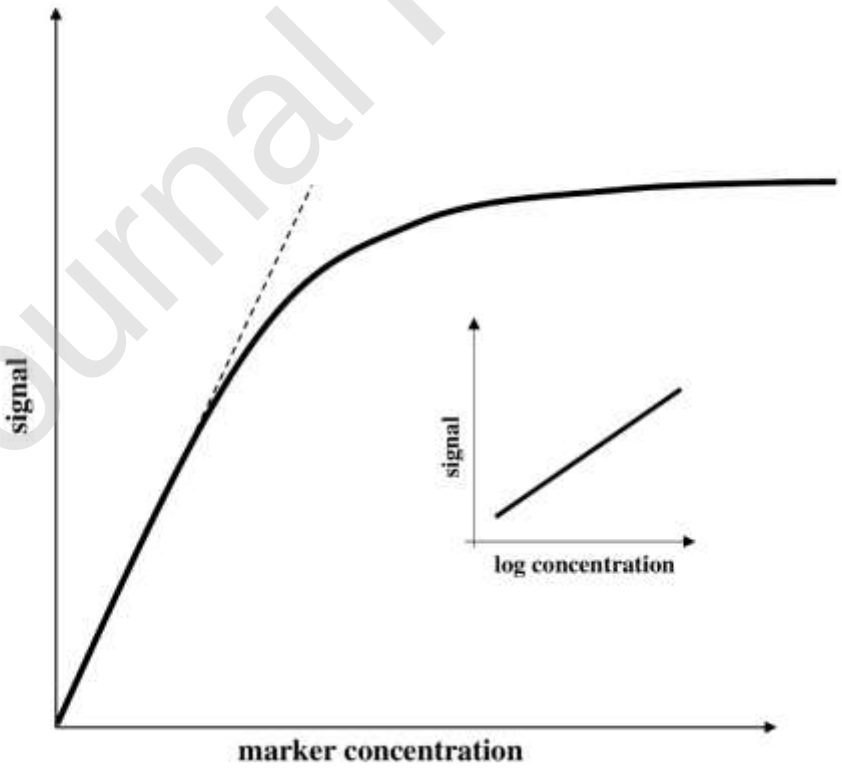

Fig. 7. Typical dependence of analytical signal on marker concentration (calibration graph)

4. Validated SPR biosensors

Only validated biosensors and related analytical procedures are ready to undergo clinical investigation for subsequent use in diagnosis. The biosensor and related analytical procedure are validated by the determination of the range of dynamic response of the analytical signal, precision, recovery and interferences, comparison of the results with another procedure such as ELISA, and examples of marker determination in natural samples (e.g. blood plasma). Highly desirable is the use of the biosensor and analytical procedure for investigation of the marker in significant series of clinical samples, including long control series of samples from healthy donors.

Less than 20 cancer markers can be determined by SPR techniques using validated biosensors and related analytical procedures. These are listed in Table 1. Multiple biosensors have been developed for three markers: CEA, PSA and CA-125. Carcinoembryonic antigen (CEA) is one of the biomarkers most commonly used to detect colon cancer. Increases in CEA levels have been found in breast cancer, gastric cancer, intrahepatic cholangiocarcinoma, pancreatic cancer and male genitourinary tract tumor. The marker is a high-molecular-weight oncofetal glycoprotein (180–200 kDa) and is 60% composed of carbohydrates. The normal concentration of the CEA marker in serum is lower than 5 ng mL^{-1}. A biosensor used with a fluidic SPR system for the determination of CEA in blood serum was developed by Springer and Homola [7]. The sensing element was an anti-CEA antibody covalently immobilized on a gold chip surface via a carboxy-terminated linker using the EDS/NHS protocol. This simple biosensor had a limit of detection of 8 ng mL^{-1}, which is insufficiently low for colon cancer diagnosis. The same authors, in a series of subsequent works [7,8,4], gradually developed a biosensor fully acceptable for colon cancer diagnosis. The analytical signal was enhanced by the application of functionalized gold nanoparticles (AuNP). A secondary anti-CEA antibody was covalently attached to AuNP, and a sandwich was formed with CEA between the primary and secondary antibody (see Fig. 3). This biosensor enables the determination of CEA concentrations above 0.1 ng mL^{-1}, which covers the concentration range of healthy individuals. A similar approach to CEA determination was employed by Li et al. [9]. A direct biosensor used with a fluidic SPR system demonstrated insufficient sensitivity. This biosensor used a CM5 chip covered with carboxylated dextran and mouse anti-CEA antibody covalently immobilized by means of the EDS/NHS protocol. Better but still insufficient results were given by a biosensor with a sandwich structure with secondary anti-biotin conjugated CEA antibody (a different clone). Significant and sufficient enhancement of the analytical signal was achieved by the subsequent attachment of AuNP conjugated with streptavidin via biotin–streptavidin interactions (see Figure 8). CEA-spiked human serum samples were used for the determination of precision and recoveries. Even stronger enhancement of a CEA analytical signal was achieved with the use of AuNP and CdSe/ZnS quantum dots [10]. First, a layer of AuNP was immobilized on a gold chip surface via hexanedithiol linker. A primary anti-CEA antibody was then immobilized *in situ*. After

CEA capture, the CdSe/ZnS quantum dots conjugated with secondary anti-CEA antibody were introduced. A biosensor for CEA determination used with the nonfluidic SPRi technique was developed by [11]. The biosensor consists of anti-CEA antibody immobilized via cysteamine linker. Despite its simple construction, the biosensor enables CEA determination in the blood of both cancer patients and healthy individuals, without signal enhancement. The biosensor was validated by comparison with the standard electrochemiluminescence method on a series of colorectal cancer blood samples.

Prostate-specific antigen (PSA) is a biomarker used for the diagnosis of prostate cancer. It is a serine protease and a 34 kDa glycoprotein. PSA is present in blood serum either in free form or as a complex with α-1-antichymotrypsin, which is a PSA inhibitor. The complex has a molecular weight of 96 kDa. The total PSA concentration refers to the sum of free and complex PSA. Normal total PSA concentration is below 4 ng mL^{-1}. Apart from the total concentration, free PSA is also determined in serum. Several biosensors for the determination of total PSA used with fluidic SPR have been developed. Huang et al. [12] developed three biosensors: a direct immunosensor, a sandwich biosensor and a colloidal gold-enhanced sandwich assay. The direct immunoassay consists of camel anti-PSA antibody immobilized via a 16-mercapto-1-hexadecanoic acid linker. The carboxyl group of the linker reacts with amino groups in the antibody by means of the EDS/NHS protocol. However, the analytical signal of this immunosensor is insufficient for the determination of PSA in human serum. The signal was enhanced by attaching a secondary antibody to the captured PSA, but this was still insufficient. Therefore, the authors attached AuNP to the already formed sandwich via a biotin–streptavidin link. Such a multilayer sandwich created an analytical signal sufficient for total PSA determination in blood serum samples. The multilayer sandwich is created *in situ* by successive injection of the analyzed sample, the secondary anti-PSA antibody conjugated with biotin, and finally AuNP covered with streptavidin. A similar technique for total PSA determination was applied by Uludag and Tothull [13]. The direct immunoassay for the determination of total PSA consists of mouse anti-PSA antibody immobilized by the EDS/NHS protocol via a mercaptoundecanoic acid linker. The analytical signal of this biosensor is too weak for PSA determination in human serum. Therefore, after PSA is captured from the analyzed serum, AuNP covered with secondary anti-PSA antibody is injected, which causes sufficient enhancement of the analytical signal. Jiang et al. [14] developed a dual-channel biosensor for simultaneous determination of total PSA (tPSA) and free PSA (fPSA). A direct immunosensor was used for the determination of tPSA, and a biosensor with AuNP-enhanced signal for the determination of fPSA. Anti-tPSA and anti-fPSA antibodies were used in the respective cases. The authors developed four different biosensors for fPSA determination. The best sensitivity was achieved with an "asynchronous competitive inhibition assay", where the sample containing fPSA was injected onto a surface containing immobilized anti-fPSA antibody, and subsequently AuNP covered with fPSA (fPSA@AuNP) was injected. Erturk et al. [15] developed a microcontact imprinting biosensor for total PSA determination using a fluidic SPR technique. The biosensor was validated against determination

of tPSA in blood serum by ELISA. The results demonstrated good agreement between the two methods.

Table 1. Validated biosensors and related analytical procedures.

Marker	SPR type	Type of cancer	Reference
CEA	SPR Fluidic	Colon cancer	[7]
CEA	SPR Fluidic NP	Colon cancer	[7]
CEA	SPR Fluidic NP	Colon cancer	[8]
CEA	SPR Fluidic NP	Colon cancer	[4]
CEA	SPR Fluidic	Colon cancer	[9]
CEA	SPR Fluidic NP	Colon cancer	[9]
CEA	SPR Fluidic NP	Colon cancer	[10]
CEA	SPRi Non fluidic	Colon cancer	[11]
PSA	SPR Fluidic	Prostate cancer	[12]
PSA	SPR FluidicNP	Prostate cancer	[12]
PSA	SPR Fluidic	Prostate cancer	[13]
PSA	SPR FluidicNP	Prostate cancer	[13]
Total-PSA	SPR Fluidic	Prostate cancer	[14]
Free-PSA	SPR FluidicNP	Prostate cancer	[14]
PSA	SPRi Fluidic	Prostate cancer	[15]
CA 125	SPR Fluidic	Ovarian cancer	[16]
CA125	SPR Fluidic	Ovarian cancer	[17]
CA125	SPRi Non fluidic	Ovarian cancer	[18]
AFP	SPR FluidicNP	Hepatocellular carcinoma	[10]
CYFRA 21-1	SPR FluidicNP	Non-small cell lung cancer	[10]
HER 2	SPR FluidicNP	Breast cancer	[19]
Rac1	SPR Fluidic	Non-small cell lung cancer	[20]
Rac1b	SPR Fluidic	Non-small cell lung cancer	[20]
5LOX	SPR Fluidic	Breast cancer	[21]
CDK4	SPR Fluidic	Lung, head and neck cancers	[22]
HE4	LSPR Non fluidic	Ovarian cancer	[6]
Laminin-5	SPRi Non fluidic	Bladder cancer	[23]
Collagen IV	SPRi Non fluidic	Breast cancer	[24]

MMP1	SPRi Non fluidic	Bladder cancer	[25]
20S immune-proteasome	SPRi Non fluidic	Leukemia	[26]
Cathepsin L	SPRi Non fluidic	Ovarian cancer	[27]
Aromatase	SPRi Non fluidic	Bladder cancer	[28] [29]
Cytokeratin 17	LSPR Non fluidic	Lung cancer	[30]

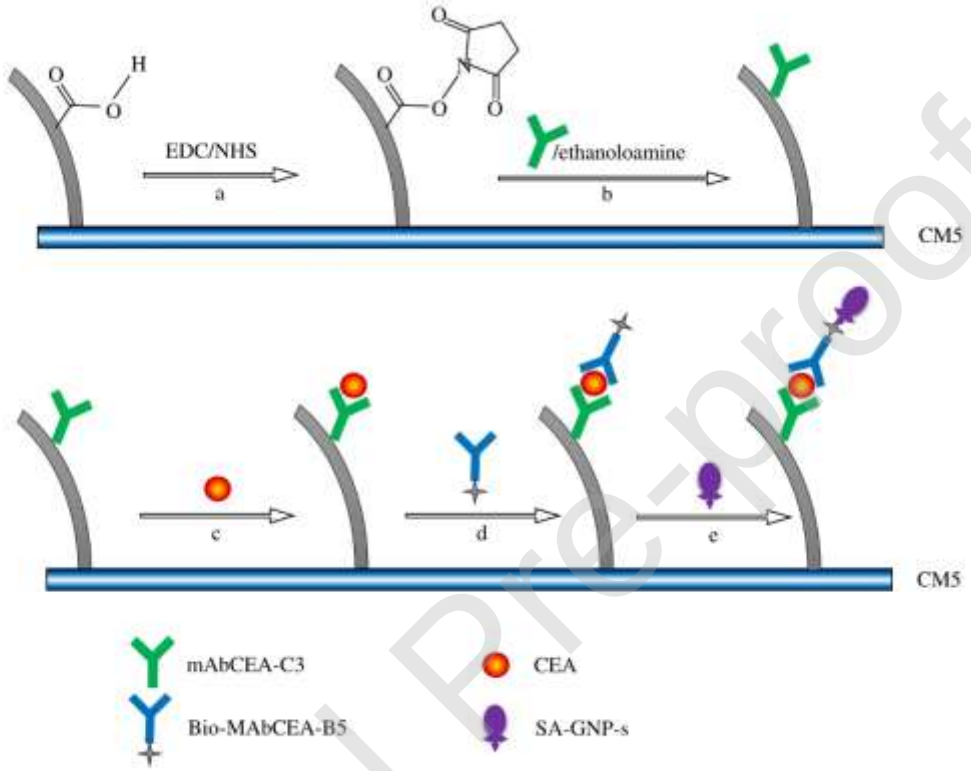

Fig. 8. The methodology of CEA detection using SA–GNPs enhanced sandwich format (reproduced from [9]).

Carcinoma antigen 125 (CA-125), also known as mucin 16 (MUC16), is an ovarian tumor cell marker widely used as a biomarker in epithelial ovarian carcinoma. CA-125 is a macromolecule consisting of over 22,000 amino acids with masses ranging from approximately 190 kDa to 2700 kDa. Unlike other markers, CA-125 concentration is usually expressed in activity units (U/ml). The threshold activity (cut-off value) above which the result is considered positive is fixed at a value of 35 U/ml. Suwansa-ard et al. [16] developed a biosensor for use with the fluidic SPR technique for the determination of CA-125 in human serum. The sensing part of the biosensor contains anti-CA-125 antibody attached to a gold surface via 11-mercaptoundecanoic acid linker by means of the EDS/NHS protocol. The biosensor was validated by parallel determination of CA-125 in series of blood serum samples with a routinely used enzyme-linked fluorescent assay. A similar solution was developed by Pal et al. [17]. A biosensor was formed using a CM5 chip containing carboxylated dextran, by means

of the EDS/NHS protocol. The biosensor was validated by parallel determination of CA-125 in blood serum by fluorescence spectroscopy. Recently, a biosensor used with the nonfluidic SPRi technique for the determination of CA-125/MUC16 was developed by Szymanska et al. [18]. Anti-CA-125 antibody was immobilized via a cysteamine linker using the EDS/NHS protocol. The marker was determined in two series of real samples: blood serum from patients with ovarian cancer and patients with endometrial cysts. The method was validated by parallel determination of the samples using the chemiluminescent Architect i2000 method.

α-fetoprotein (AFP) is an important marker of hepatocellular carcinoma and other chronic liver diseases. AFP is a glycoprotein consisting of 591 amino acids and a carbohydrate moiety. It may be present in monomeric, dimeric and trimeric forms. Wang et al. [10] developed a biosensor for use with the fluidic SPR technique which enables the determination of AFP in blood serum. The biosensor consists of a primary anti-AFP antibody attached to a gold chip surface via a hexanedithiol linker using the EDS/NHS protocol, and CdSe/ZnS quantum dots conjugated with a secondary anti-AFP antibody injected after the AFP from the analyzed serum is captured. The biosensor and related method were validated by parallel AFP determination in serum by the electrochemiluminescence method.

Cytokeratin fragment 21-1 (CYFRA 21-1) is a promising marker for non-small-cell lung cancer. The marker is a soluble fragment of cytokeratin 19. Wang et al. [10] developed a biosensor for the determination of CYFRA 21-1 in blood serum, which was validated by parallel CYFRA 21-1 determination in serum by the electrochemiluminescence method. The biosensor's construction was similar to that of the biosensor for AFP determination, and used primary and secondary anti-CYFRA 21-1 antibodies.

Human epidermal growth factor receptor 2 (HER2), also known as receptor tyrosine-protein kinase (ERBB2) or cluster of differentiation 340 (CD 340), is a membrane-spanning protein having both intracellular and extracellular domains. The extracellular domain of HER2 can be cleaved by matrix metalloproteases, and may enter the serum of patients with breast cancer. This fragment of the intact HER2 has a size of 105 kDa and is known as soluble p 105. The blood HER2 extracellular domain concentration of breast cancer patients is usually higher than 15 ng mL^{-1}. A biosensor for HER2 determination using the fluidic SPR technique was developed by Eletxigerra et al. [19]. The analytical signal was enhanced with the use of AuNP. A primary anti-HER2 antibody was attached to a gold chip surface via mercaptoundecanoic acid linker using the EDS/NHS protocol. After injection of a sample containing HER2, a biotinylated secondary anti-HER2 antibody was injected, followed by commercially available streptavidin-decorated AuNP. The analytical signal enhancement is sufficient for HER2 determination in human serum.

Rac1, also known as Ras-related C3 botulinum toxin substrate 1, is a signaling protein of approximately 21 kDa involved in tumorigenesis, cancer invasion and metastasis. Rac1b is a hyperactive variant of Rac1. Both proteins are potential markers of non-small-cell lung cancer. Sahu

et al. [20] developed biosensors for the determination both of Rac1 and Rac1b in blood serum. Both biosensors are used with the fluidic version of SPR. The commercially available CM5 chip was used in the construction of the biosensors. This chip is covered by carboxylated dextran. In the case of the Rac1 biosensor, anti-Rac1 antibody was immobilized using the EDS/NHS protocol; anti-Rac1b antibody was used similarly in the case of the Rac1b biosensor. Both biosensors were validated using western blot analysis as the reference method.

Arachidonate 5-lipoxygenase (5-LOX) is an enzyme consisting of 673 amino acids (approximately 78 kDa). It is a potential breast cancer marker. A biosensor for the determination of 5-LOX, used with the fluidic SPR technique, was developed by Kumar et al. [21]. Anti-5-LOX antibody was immobilized on the CM5 chip surface using the EDS/NHS protocol. The serum level of 5-LOX in breast cancer patients was reported in this study for the first time.

Cyclin-dependent kinase 4 (CDK4) has been found to be overexpressed in many types of cancer and seems to be a key factor in promoting the initiation and development of tumors. Banerjee et al. [22] developed a biosensor for the determination of CDK4 using the fluidic SPR technique. Using the EDS/NHS protocol, anti-CDK4 antibody was immobilized on the CM5 chip surface. The method was validated by parallel determination of CDK4 by the western blot technique.

Human epididymis secretory protein 4 (HE4) is an ovarian cancer marker. It is a protein consisting of 124 amino acids, which after glycosylation have a size of 25 kDa. Unlike the majority of markers, HE4 concentration is usually expressed in picomole L^{-1}. The threshold concentration (cut-off value) is 70 picomole L^{-1} for premenopausal women and 90–140 picomole L^{-1} for postmenopausal women. Yuan et al. [6] developed a biosensor for HE4 determination used with the LSPR technique. The sensing part of the biosensor was an anti-HE4 antibody immobilized on a nanosilver chip via 11-mercaptoudecanoic linker by means of the EDS/NHS protocol. The analytical signal is a shift of the maximum on the absorption spectrum (see Fig. 6). The results were validated by parallel HE4 determination using ELISA.

Laminin 5 is an emerging cancer marker. It is a component of basement membrane. Laminin 5 is a large molecule of size 400 kDa. A biosensor for the determination of laminin 5 was developed by Sankiewicz et al. [23]. The biosensor is used with the nonfluidic SPRi technique. The anti-laminin 5 antibody was immobilized on each of an array of gold measuring points via cysteamine linker using the EDS/NHS protocol. The laminin 5 concentration in the plasma of bladder cancer patients is approximately three times higher than in the case of healthy donors. The biosensor was validated by parallel laminin 5 determination using ELISA.

Collagen IV is a potential breast cancer marker. The protein is a major component of the basement membrane which also surrounds the cancer vasculature. It may therefore be a marker of angiogenic activity, which is significantly enhanced in the case of cancer. The size of collagen IV is 160–190 kDa. Sankiewicz et al. [24] developed a biosensor for the determination of collagen IV. The biosensor is used with the nonfluidic SPRi technique. The sensing part of the biosensor consists of

monoclonal mouse anti-collagen IV antibody immobilized on the free gold surface of an array chip via cysteamine linker using the EDS/NHS protocol. Series of blood plasma samples from breast cancer patients and healthy donors were used for validation of the biosensor in parallel with ELISA determination.

Matrix metalloproteinase 1 (MMP-1), also called collagenase 1, is an endoprotease overexpressed in many tumor cells. This enzyme, of size 42.7 kDa, degrades collagen type I, II, III, V, VII, VIII, and X. A biosensor for the determination MMP-1, used with the nonfluidic SPRi technique, was developed by Tokarzewicz et al. [25]. The sensing part of the biosensor is the anti-MMP-1 antibody bound to an array of gold active points on a chip via cysteamine linker by the EDS/NHS protocol. The biosensor was validated by parallel determination of MMP-1 in the plasma of bladder cancer patients and healthy subjects using ELISA.

20S immunoproteasome (20Si) is one of two forms of 20S proteasome, and a marker of leukemia. It is a barrel-shape protein complex of size approximately 700 kDa. Sankiewicz et al. [26] developed a biosensor for the determination of 20Si, used with the nonfluidic SPRi technique. The sensing part of the biosensor is the inhibitor ONX 0914 {N-[2-(4-morpholinyl)acetyl]-L-alanyl-O-methyl-N-[(1S)-2-[(2R)-2-methyl-2-oxiranyl]-2-oxo-1-(phenylmethyl)ethyl]-L-tyrosinamide} immobilized on an array of gold measuring points via 1-octadecanethiol linker. The thiol group of the linker is bound to the gold, while the inhibitor interacts with the alkyl chain via hydrophobic interactions. The biosensor was validated by the determination of 20Si in plasma samples of leukemia patients and healthy donors with parallel determination by another method.

Cathepsin L is a potential marker of ovarian cancer. It is a cysteine endopeptidase of size 24.2 kDa. A biosensor for cathepsin L determination was developed by Tokarzewicz et al. [27]. The sensing part of the biosensor consists of the RKLLW-NH2 inhibitor (Arg-Lys-Leu-Leu-Trp-NH2) immobilized on an array of gold measuring points via 1-octadecanethiol linker. The inhibitor is bound to the linker via hydrophobic interactions. The biosensor was validated by cathepsin L determination in the blood plasma of healthy persons and in the plasma of patients suffering from ovarian tumor and ovarian cyst.

Aromatase (also known as CYP19A1) is a potential bladder cancer marker. It consists of 503 amino acids and has a molecular weight of 58 kDa. Two biosensors for aromatase determination were developed by Gorodkiewicz et al. [29]. One of them consists of the anti-aromatase antibody bonded to the gold biosensor surface via cysteamine linker using the EDS/NHS protocol. The other biosensor contains exemestane inhibitor as the sensing element, which is bonded to the gold chip surface via octadecanethiol linker. The inhibitor is bound to the linker via hydrophobic interactions. The biosensor containing antibody as the sensing element was validated by aromatase determination in a series of plasma samples from patients with bladder cancer and in reference samples from healthy subjects.

Cytokeratin 17 is an acidic type I cytokeratin and is a lung cancer marker. A catheter-embedded biosensor for the detection of cytokeratin 17 in lung tissue has been developed by

Loyez et al. [30]. A gold-coated tilted fiber Bragg grating with immobilized cytokeratin 17 antibody was used. The biosensor was validated by cytokeratin 17 detection using immunohistochemistry in lung cancer tissue.

Overall, Table 1 lists 33 validated biosensors and related analytical procedures for the determination of 18 biomarkers of nine different types of cancer. In the majority of cases, suitable antibodies were used as sensing elements bound to the chip surface via a linker. Biosensors used with the fluidic SPR technique incorporated linkers containing a carboxyl end group for bonding with amine groups of antibodies by the EDS/NHS protocol. In turn, biosensors used with the nonfluidic SPRi technique contained cysteamine linker with an amine end group for bonding with carboxyl groups of the antibody, again by the EDS/NHS protocol. In two cases an inhibitor of the marker was used as the sensing element of the biosensor in place of the antibody. In several cases, a sandwich structure containing AuNP was used to achieve the necessary sensitivity. More details concerning the construction of biosensors and SPR measuring techniques are given in recent reviews ([31-34]).

5. Applications of SPR biosensors in testing the effectiveness of cancer markers and discovering new cancer markers

The determination of cancer markers in body fluids is a secondary problem; a primary problem is the discovery of new markers. There is still a shortage of biosensors offering near 100% sensitivity and specificity, i.e. respectively 100% of true positive and 0% false positive results. An illustration of the relationship between true positive and false positive results is provided by receiver operating characteristic curves (ROC curves). An example of such curves obtained with a biosensor used with the fluidic SPR technique for the determination of the marker 5-LOX in blood serum is shown in Fig. 9. Breast cancer samples and control samples are partly overlapping, with a cut-off value of 4.32 ng μL^{-1}. Based on this cut-off value, the sensitivity of the biomarker is only 80%, which means that 20% of breast cancer is undiscovered, and its specificity is 83.3%, which means that 16.7% of samples are wrongly determined as positive. The area under the ROC curve (AUC) is **a** summary indicator of a marker's predictive value. In the case of 5-LOX this value is 0.86.

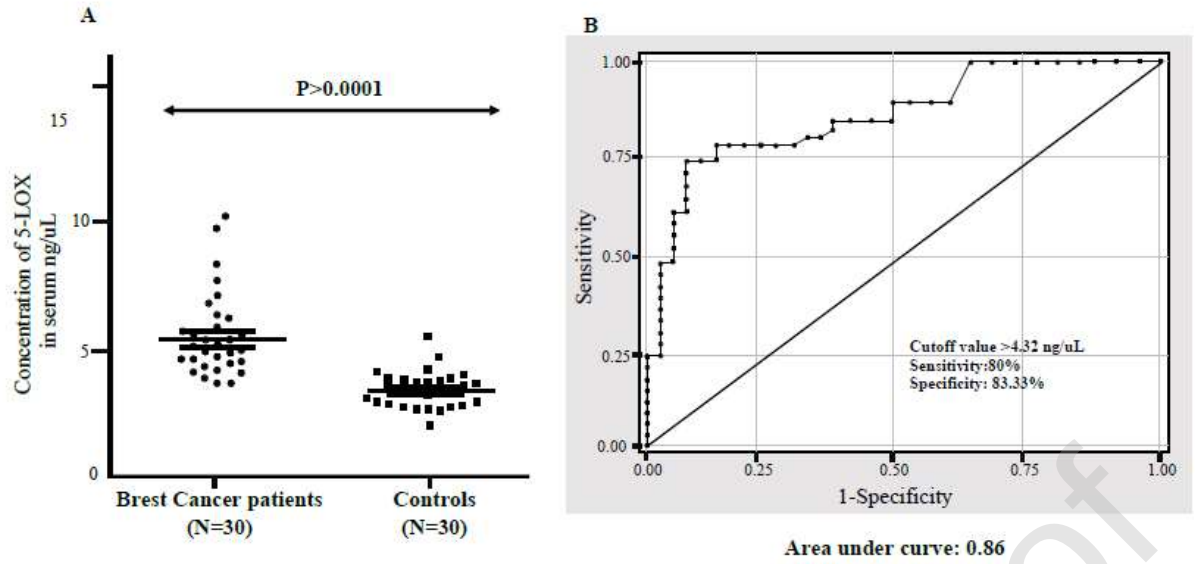

Fig. 9. (**A**) Scatter graph showing serum concentrations of 5-LOX in breast cancer patients and controls. (**B**) ROC analysis showing the area under the curve for 5-LOX to differentiate breast cancer patients from healthy individuals (reproduced from [21]).

Several SPR biosensors have been used for testing the effectiveness of new potential cancer markers. Their characteristics are listed in Table 2.

Table 2. Diagnostic effectiveness of newly proposed cancer markers as tested by SPR biosensors

Marker	**Type of cancer**	**SPR technique**	**Sensitivity [%]**	**Specificity [%]**	**Cut off value µg x mL^{-1}**	**AUC**	**Reference**
5-LOX	Breast cancer	Fluidic SPR	80	83.3	4.32	0.86	[21]
Rac 1	NSCLC	Fluidic SPR	75.3	90.4	1.57	0.88	[20]
Rac 1b	NSCLC	Fluidic SPR	80.5	96.2	0.43	0.92	[20]
CDK4	Lung cancer	Fluidic SPR	71	64	29	0.75	[22]
CDK4	HNSCC	Fluidic SPR	63	62	28	0.67	[22]
Podo-planin	Bladder cancer	Non fluidic SPRi	72.5	69.5	0.00474	0.82	[35]

Cystatin C	Bladder cancer	Non fluidic SPRi	87	92	0.54	0.93	[36]
Aroma-tase	Bladder cancer	Non fluidic SPRi	100	100	0.0174	1	[29]

NSCLC – non small cell lung cancer, HNSCC - Head and neck squamous cell carcinoma

Eight new markers for different types of cancer have been proposed, in combination with related analytical methods for marker determination. New markers for breast cancer, non-small-cell lung cancer, lung cancer, bladder cancer, and head and neck squamous cell carcinoma are proposed. In most cases their sensitivity and specificity are not ideal; however, generally none of the currently used cancer markers has 100% sensitivity and specificity. Thus, all of them deserve further investigation with larger numbers of samples. Very good selectivity (96.2%) and good sensitivity (80.2%) are exhibited by Rac1b as a marker for non-small-cell lung cancer. Aromatase as a bladder cancer marker exhibits exclusive sensitivity and selectivity. The results of aromatase concentration for BC patients and healthy donors are shown in Figure 10A. The gap between the lowest result for bladder cancer (BC) patients (17.41 ng/ml) and the highest value from the set of results for healthy individuals (7.74 ng/ml) is almost 10 ng/ml, and there are no overlapping results within these two groups. The ROC curve corresponding to these results is shown in Fig. 10B (curve a).

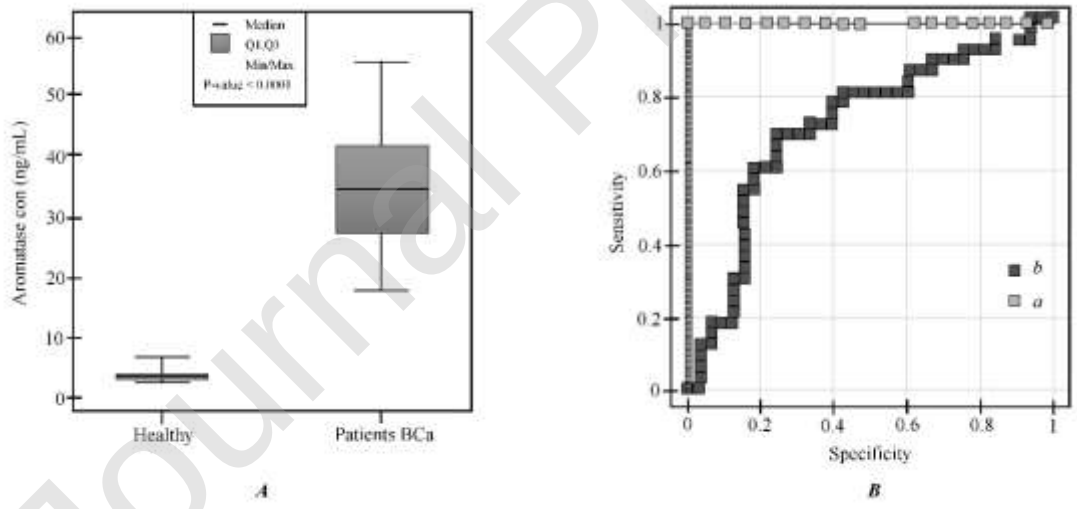

Fig. 10. (A)Concentration of aromatase in blood plasma from healthy individuals and patients with bladder cancer (BCa) tumors. (B) (curve a) Receiver operating characteristic curve of blood plasma aromatase concentrations for healthy individuals and patients with bladder cancer. Cut-off value, 17.41 ng/ml. BCa, bladder cancer. (curve b) Receiver operating characteristic curve of plasma aromatase concentrations for patients with muscle-invasive and non muscle-invasive BCa. Cut-off value, 37.32 ng/ml. BCa, bladder cancer. All curves reproduced from [29].

The promising parameters found for aromatase as a BC marker are only a beginning. Many more samples should be analyzed to confirm the true sensitivity and selectivity of this marker.

Apart from BC discovery, aromatase can also be a marker of the muscle-invasive stage of BC, which is a more advanced stage of the disease. The corresponding ROC curve is shown in Fig. 10B (curve b). The sensitivity of aromatase as a marker of the muscle-invasive stage of BC is 60%, and its specificity is 81%, which means that only 60% of muscle-invasive tumors are found, and 19% of non-muscle-invasive tumors are erroneously classified as muscle-invasive tumors.

The biosensors reviewed above are ready for use in clinical and diagnostic applications. However, such factors as the availability of suitable instruments, the possibility of creating a portable device, the cost of a single measurement, conditions of biosensor storage, etc. should also be taken into consideration.

6. Non-validated SPR biosensors

There are numerous papers devoted to the development of new designs of SPR biosensors and/or related measuring techniques, in which the detection of a cancer marker is only an illustration of a developed biosensor's capability. The first part of Table 3 lists such papers. In other papers, the developed SPR biosensors are partially characterized in terms of analytical parameters, but without sufficient validation. These papers appear in the second part of Table 3. All of these technical solutions may be validated in the future. All cancer markers cited in Table 3 are already used for cancer detection as determined by other methods. Thus, the papers listed in Table 3 represent new analytical proposals for the determination of markers that are already in use.

Table 3. Non-validated SPR biosensors

Marker	**SPR type**	**Type of cancer**	**Reference**
Biosensors used only for the detection of a marker			
HER2	SPR Fluidic	Breast cancer	[37,38]
BCR1/BCR2	LSPR	Breast cancer	[39]
CEA	SPR Fluidic	Colon cancer	[40]
CEA	SPR Fluidic NP	Colon cancer	[41]
CEA and EGFR	SPR non fluidic	Lung cancer	[42]
Cytokeratin 17	SPR Non fluidic	Lung cancer	[43]
CEA antibody	SPR Fluidic	Multiple cancers	[44]
p53	LSPR	Head and neck cancer	[45]
Cytochrome C	SPRi Non fluidic	Apoptosis	[46]

Biosensors partially characterized with analytical procedures			
E-cadherin	SPR non fluidic	Breast cancer	[47]
DNA mutation	Fluidic SPR	Breast cancer	[48]
Cytokeratins	LSPR	Lung cancer	[49]
Cytokeratin 19	Fluidic SPR	Non-small cell lung carcinoma	[50]
PSA	Fluidic SPR	Prostate cancer	[51]
PSA	Fluidic SPR	Prostate cancer	[12]
PSA	LSPR	Prostate cancer	[52]
PSA	Fluidic SPR	Prostate cancer	[53]
PSA	Non fluidic SPR NP	Prostate cancer	[54]
PSA	Fluidic SPR	Prostate cancer	[55]
CA19-9	Fluidic SPR	Colorectal cancer, pancreatic cancer	[2]
CD166/ALCAM	Fluidic SPR	Pancreatic cancer	[56]
VEGF	LSPR	Multiple cancers	[57]
IL-8	Fluidic SPR	Multiple cancers	[58]
IgG kappa/ IgG lambda	Fluidic SPR	Leukemia	[59]

7. Other circulating markers

Micro RNAs (miRNAs) are non-coding RNAs regulating gene expression through base-pairing with complementary sequences of messenger RNA (mRNA) molecules. As a result, these mRNA molecules are silenced. miRNAs are small molecules containing approximately 22 nucleotides. They are emerging cancer markers [60]. The detection of miRNA by SPR requires signal amplification, due to the small size of the molecules, which results in a low SPR signal. Various approaches are used to amplify the miRNA signal in SPR. Hong et al.[61] developed a competition assay for the determination of miRNA 200b. The SPR analytical signal is created by the replacement of gold nanoparticles conjugated with surrogate DNA by the analyte (miRNA 200b). The conjugated nanoparticles are attached to the biosensor surface by a suitable DNA via MUA linker. Wang et al. [62] used graphene oxide–gold nanoparticle hybrids for miRNA141 SPR signal enhancement. Two thiol-modified DNA oligonucleotide probes, containing sequences complementary to the target miRNA-141, were used; the capture DNA was immobilized on a gold sensor surface, while the other (called assistant DNA) was fixed on the graphene oxide–gold nanoparticle hybrids. These hybrids were used for signal amplification. One section of the miRNA-141 is bound to the capture DNA, while the other section is bound to the assistant DNA, forming a sandwich structure. The developed method ensures highly sensitive and selective miRNA-141 determination in prostate carcinoma cell lines (22Rv1), hepatocellular carcinoma cell lines (SMMC-7721), colon cancer cell lines (LoVo), and

cervical cancer cell lines (HeLa). A similar solution was applied by Nie et al. [63] for miRNA-141 determination in the cell lysates from cancer cell lines 22Rv1, SMMC-7721, LoVo and HeLa. Li et al. [64] used mismatched catalytic hairpin assembly amplification coupling with programmable streptavidin aptamer for miRNA- 21 determination. Two hairpin DNAs were used.

Exosomes are emerging biomarkers. These small extracellular vesicles, secreted by cells, are present in body fluids (blood, urine, breast milk, saliva) and transfer DNAs, RNAs, proteins, and lipids from parent cells to recipient cells for cell-cell communication. Exosomes are involved in the regulation of immune responses and in cancer development. There are several papers on SPR determination of exosomes as potential cancer markers. Zhu et al. [65] applied antibody microarrays specific to the extracellular domains of exosome membrane proteins in a fluidic system in conjunction with the SPRi technique.

Park et al. [66] applied nanohole-based surface plasmon resonance for the detection of transmembrane (EpCAM and CD63) and intravesicular (AKT1) proteins contained in exosomes' lysates. The exosomes originated from three ovarian cancer cell lines and one benign cancer cell line. Apart from the atypical nanohole SPR, which is more sensitive than usual SPR, gold nanoparticles were used to enhance the analytical signal.

Ibn Sina et al. [67] used a custom-made fluidic SPR platform for quantification of the proportion of cancer-related exosomes within the total exosome population isolated from patient serum, which may potentially provide information on the stage of the disease. A two-step strategy was applied, involving initial isolation of the total exosome population using tetraspanin biomarkers (CD9, CD63) and subsequent detection of exosomes containing the HER2 marker (breast cancer) by the formation of a sandwich with anti-HER2.

Liao et al. [68] developed a multistage signal amplification strategy for the determination of SMMC-7721 exosomes derived from hepatic carcinoma. First, a DNA tetrahedron probe is immobilized on a gold surface. Then the aptamer ZY-sls, which is complementary to the probe, is attached. This aptamer captures the SMMC-7721 exosome from solution. For SPR signal enhancement, the SMMC-7721 exosome captures the CD63 aptamer-linked polydopamine-functionalized gold nanoparticle aggregate, and finally, for additional signal enhancement, the sandwich is treated with $HAuCl_4$. Concentrations as low as 5.6×10^5 particles mL^{-1} can be determined.

Reiner et al. [69] used a magnetic nanoparticle-enhanced grating coupled SPR assay for specific detection of different small lipid extracellular vesicles secreted from mesenchymal stem cells. Pre-incubated vesicles were captured by magnetic nanoparticles via lipid-binding annexin V and cholera

toxin B chain immobilized on the nanoparticles' surface. An antibody specific for the tetraspanin protein CD63 was used for sensitive detection of extracellular vesicles.

Circulating tumor cells (CTCs) are also potential cancer markers. They are released from the cancer into the blood. However, the concentration of CTCs in blood is extremely low. Therefore, CTC accumulation is applied, as well as SPR signal enhancement. Mousavi et al. [70] used a gold nanoslit SPR platform for sensitive detection of the lung cancer cell lines CL1–5. The CL1–5 cells were initially accumulated on functionalized magnetic nanoparticles.

Jia et al. [71] detected the breast cancer cell line MCF-7 using histidine-tagged arginine-glycine-aspartic acid peptide as a linker. The breast cancer cells MCF-7 were captured via the interaction between human mucin-1 and a gold surface modified with a mucin-1-selective aptamer. The SPR signal was enhanced by binding of NiO nanoparticles via the histidine tag on the peptide.

8. Conclusions

SPR biosensors are able to compete with other methods in the determination of cancer markers, and to contribute to the discovery of new cancer markers. Some of the new markers exhibit very promising diagnostic sensitivity and specificity; for example, the marker Rac1b in the detection of non-small-cell lung cancer, and the marker aromatase in the detection of bladder cancer. Some of the biosensors described in the reviewed papers were validated, while others were only superficially characterized. Greater attention should be paid to the validation of newly developed SPR biosensors. It is recommended that analysts developing biosensors co-operate more closely with clinicians. Without such co-operation, access to long series of clinically characterized body fluid samples seems unrealistic. Apart from biosensors for the detection of particular kinds of cancer, biosensors should be developed for the determination of a stage of cancer or progress in cancer therapy. Considering the limited diagnostic specificity and selectivity of biosensors, panels consisting of two or several biosensors should be developed.

Conflicts of Interest: The authors declare no conflict of interest.

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9. References

[1]K.Zhang, X.Shen, Cancer antigen 125 detection using the plasmon resonance scattering properties of gold nanorods, Analyst, 138(2013)1828.

[2] J.W.Chung, R.Bernhardt, J.C.Pyuna, Additive assay of cancer marker CA 19-9 by SPR biosensor, Sensors Actuators B 118 (2006) 28–32.

[3] T.-H. Chou, C.-Y. Chuang, C.-M.Wu, Quantification of Interleukin-6 in cell culture medium using surface plasmon resonance biosensors, Cytokine 51 (2010) 107–111.

[4] M.L. Ermini, X. Chadtová Song, T. Špringer, J. Homola, Peptide Functionalization of Gold Nanoparticles for the Detection of Carcinoembryonic Antigen in Blood Plasma via SPR-Based Biosensor. Front. Chem. 7(2019) 40. doi: 10.3389/fchem.2019.00040

[5] A.Sankiewicz, L.Romanowicz, M.Pyc, A.Hermanowicz, E.Gorodkiewicz, SPR imaging biosensor for the quantitation of fibronectin concentration in blood samples, J. Pharmac. Biomed. Anal. 150(2018) 1-8.

[6] J.L.Yuan, R.Q.Duan, H.Yang, X.G.Luo, M.R.Xi, Detection of serum human epididymis secretory protein 4 (HE4) in patients with ovarian cancer using a label-free biosensor based on localized surface plasmon resonance. Int. J. Nanomed. 7(2012) 2921−2928.

[7] T.Špringer, J.Homola, Biofunctionalized gold nanoparticles for SPR-biosensor-based detection of CEA in blood plasma, Anal. Bioanal. Chem. 404(2012)2869–2875.

[8] T.Springer, X.Chadtová-Song, M.L.Ermini, J.Lamačová, J.Homola, Functional gold nanoparticles for optical affinity biosensing, Anal. Bioanal. Chem. 409(2017) 4087–4097.

[9] R.Li, F.Feng, Z.-Z.Chen, Y.-F.Bai, F.-F.Guo, F.Y.Wu, G.Zhou, Sensitive detection of carcinoembryonic antigen using surface plasmon resonance biosensor with gold nanoparticles signal amplification, Talanta 140(2015)143–149.

[10] H.Wang, X.Wang, J.Wang, W.Fu, C.Yao, A SPR biosensor based on signal amplification using antibody-QD conjugates for quantitative determination of multiple tumor markers, Sci. Reports | 6(2016) 33140**.**

[11] B.Szymanska, Z.Lukaszewski , K.Hermanowicz-Szamotowicz, E.Gorodkiewicz, An immunosensor for the determination of carcinoembryonic antigen by Surface Plasmon Resonance Imaging, Bioanal.Chem., 609(2020)113964.

[12] L.Huang, G.Reekmans, D.Saerens, J.-M.Friedt, F.Frederix, L.Francis, S.Muyldermans, A.Campitelli, C.Van Hoof, Prostate-specific antigen immunosensing based on mixed self-assembled monolayers, camel antibodies and colloidal gold enhanced sandwich assays, Biosens. Bioelectron. 21 (2005) 483–490.

[13] Y.Uludag, I.T.Tothill, Cancer Biomarker Detection in Serum Samples Using Surface Plasmon Resonance and Quartz Crystal Microbalance Sensors with Nanoparticle Signal Amplification, Anal. Chem., 84(2012) 5898−5904.

[14] Z.Jiang, Y.Qin, Z.Peng, S.Chen, S.Chen, C.Deng, J.Xiang, The simultaneous detection of free and total prostate antigen in serum samples with high sensitivity and specificity by using the dual-channel surface plasmon resonance, Biosens. Bioelectron. 62 (2014) 268–273.

[15] G.Erturk, H.Ozen, M.A.Tumer, B.Mattiasson, A.Denizli, Microcontact imprinting based surface plasmon resonance (SPR) biosensor for real-time and ultrasensitive detection of prostate specific antigen (PSA) from clinical samples. Sens. Actuators, B 224(2016) 823−832.

[16] S.Suwansa-ard, P.Kanatharana, P.Asawatreratanakul, B.Wongkittisuksa, C.Limsakul, P.Thavarungkul, Comparison of surface plasmon resonance and capacitive immunosensors for cancer antigen 125 detection in human serum samples, Biosens. Bioelectron. 24 (2009) 3436–3441.

[17] M.K.Pal, M.Rashid, M.Bisht, Multiplexed magnetic nanoparticle-antibody conjugates (MNPs-ABS) based prognostic detection of ovarian cancer biomarkers, CA-125, β- 2M and ApoA1 using fluorescence spectroscopy with comparison of surface plasmon resonance (SPR) analysis, Biosen. Bioelectron., 73 (2015) 146–152.

[18]B.Szymanska, Z.Lukaszewski, K.Hermanowicz-Szamotowicz, E.Gorodkiewicz, A biosensor for determination of the circulating biomarker CA125/MUC16 by Surface Plasmon Resonance Imaging, Talanta, 206(2020)120187.

[19] U.Eletxigerra, J.Martinez-Perdiguero, R.Barderas, J.M.Pingarron, S.Campuzano, S.Merino, Surface plasmon resonance immunosensor for ErbB2 breast cancer biomarker determination in human serum and raw cancer cell lysates, Anal. Chim. Acta 905(2016)156-162.

[20] V.Sahu, A.Gupta, R.Kumar, T.Gupta, A.Mohan, S.Dey, Quantification of Rac1 and Rac1b in serum of non small cell lung cancer by label free real time assay. Clin. Chim. Acta. 460(2016) 231−235.

[21] R.Kumar, A.K.Singh, M.Kumar, S.Shekhar, N.Rai, P.Kaur, R.Parshad, S.Dey, Serum 5-LOX: a progressive protein marker for breast cancer and new approach for therapeutic target, Carcinogenesis, 37(2016) 912–917.

[22] J.Banerjee, R.Pradhan, A.Gupta, R.Kumar, V.Sahu, A.D.Upadhyay, P. Chaterjee, S.Dwivedi, S.Dey, A.B.Dey , CDK4 in lung, and head and neck cancers in old age: evaluation as a biomarker, Clin. Transl. Oncol. 19(2017) 571–578.

[23] A.Sankiewicz, L.Romanowicz, P.Laudanski, B.Zelazowska-Rutkowska, B.Puzan, B.Cylwik, E.Gorodkiewicz, SPR imaging biosensor for determination of laminin-5 as a potential cancer marker in biological material, Anal. Bioanal. Chem. 408(2016b) 5269–5276.

[24] A.Sankiewicz, Z.Lukaszewski, K.Trojanowska, E.Gorodkiewicz, Determination of collagen type IV by Surface Plasmon Resonance Imaging using a specific biosensor, Anal. Biochem. 515(2016c) 40-46.

[25] A.Tokarzewicz, L.Romanowicz, I.Sveklo, E.Gorodkiewicz, The development of a matrix metalloproteinase-1 biosensor based on the surface plasmon resonance imaging technique, Anal. Methods**.** 8(2016) 6428-6435.

[26] A.Sankiewicz, A.Markowska, Z.Lukaszewski, B.Puzan, E.Gorodkiewicz, Methods for 20S Immunoproteasome and 20S Constitutive Proteasome Determination Based on SPRI Biosensors, Cell Molec. Bioengin. xxxxxx

[27] A.Tokarzewicz, L.Romanowicz, A.Sankiewicz, A.Hermanowicz, K.Sobolewski, E.Gorodkiewicz, A New Analytical Method for Determination of Cathepsin L Based on the Surface Plasmon Resonance Imaging Biosensor, Int J Mol Sci. 20(2019) 2166.

[28] E.Gorodkiewicz, A.Sankiewicz, P.Laudanski, Surface plasmon resonance imaging biosensors for aromatase based on a potent inhibitor and a specific antibody: Sensor development and application for biological material. Cent. Eur. J. Chem. 12(2014) 557‑567.

[29] T.Guszcz, B.Szymańska, R.Kozlowski, Z.Lukaszewski, P.Laskowski, E.Gorodkiewicz, Plasma aromatase as a sensitive and selective potential biomarker of bladder cancer and its role in tumorigenesis, Oncol. Let., **19(2020)262-268**.

[30] M.Loyez, J.-C.Lorrieu, S.Chevineau, M.Remmelink, D.Leduc, B.Bondue, P.Lambert, J.Deviere, R.Wattiez, C.Caucheteur, *In situ* cancer diagnosis through online plasmonics, Biosenos.Bioelectron., 131(2019)104-112.

[31] J.-F.Masson, Surface Plasmon Resonance Clinical Biosensors for Medical Diagnostics,, ACS Sens. 2(2017) 16−30.

[32] E.Gorodkiewicz, Z.Lukaszewski, Recent Progress in Surface Plasmon Resonance Biosensors (2016 to Mid-2018), Biosensors. *8*(4)(2018)132.

[33] A.R.Ferhan, J.A.Jackman, J.H.Park, N.-J.Cho, D.-H.Kim, Nanoplasmonic sensors for detecting circulating cancer biomarkers, Adv. Drug Deliv. Rev. 125(2018) 48–77.

[34] D.Wang, J.F.C.Loo, J.Chen, Y.Yam, S.-C.Chen, H.He, S.K.Kong, H.P.Ho, Recent Advances in Surface Plasmon Resonance Imaging Sensors, Sensors. 19(2019)1266.

[35] A.Sankiewicz. T.Guszcz, R.Mena-Hortelano, K.Zukowski, E.Gorodkiewicz, Podoplanin serum and urine concentration in transitional bladder cancer, Cancer Biomark. 16(2016) 343-350.

[36] A.Tokarzewicz, T.Guszcz, A.Onopiuk, R.Kozlowski, E.Gorodkiewicz, Utility of cystatin C as a potential bladder tumor biomarker confirmed by surface plasmon resonance technique, Indian J. Med. Res. 218(2018) 46-50.

[37] M.Loyez, M.Lobry, E.M.Hassan, M.C.DeRosa, C.Caucheteur, R.Wattiez, HER 2 breast cancer biomarker using a sandwich optical fiber assay, Talanta, 221(2021)121452.

[38] J.P.Monteiro, J.H.de Oliveira, E.Radovanovic, A.G.Brolo, E.M.Girotto, Microfluidic Plasmonic Biosensor for Breast Cancer Antigen Detection, Plasmonics, 11(2016) 45–51.

[39] B.Hossain, M.Islam, L.F.Abdulrazak, M.Rana, T.B.A.Akib, M.Hassan, Graphene-Coated Optical Fiber SPR Biosensor For BRCA1 And BRCA2 Breast Cancer Biomarker Detection: A Numerical Design-Based Analysis, Photon. Sens. **10(2020)67–79**.

[40] J.Xu, Y.Chen, Surface plasmon resonance sensing with adjustable sensitivity based on a flexible liquid core coupling unit, Talanta, 184(2018) 468-474.

[41] T.Springer, L.Ermini, B.Spackova, J.Jablonku, J.Homola, Enhancing Sensitivity of Surface Plasmon Resonance Biosensors by Functionalized Gold Nanoparticles: Size Matters, Anal. Chem., 86(2014)10350−10356.

[42] P.K.Teotia, R.S.Kaler, 1-D grating based SPR biosensor for the detection of lung cancer biomarkers using Vroman effect, Optics Communic. 406(2018) 188–191.
[43] C.Ribaut, M.Loyez, J.-C.Larrieu, S.Chevineau, P.Lambert, M.Remmelink, R.Wattiez, C.Caucheteur, Cancer biomarker sensing using packaged plasmonic optical fiber gratings: Towards in vivo diagnosis, Biosens. Bioelectr. 92(2017) 449–456.
[44] J.Ladd, H.L.Lu, A.D.Taylor, V.Goodell, M.L.Disis, S.Y.Jiang, Direct detection of carcinoembryonic antigen (CEA) autoantibodies in clinical human serum samples using a surface plasmon resonance sensor. Colloids Surf., B, 70 (2009) 1−6.
[45] W.Zhou, Y.Y.Ma, H.A.Yang, Y.Ding, X.G.Luo, A label free biosensor based on silver nanoparticles array for clinical detection of serum p53 in head and neck squamous cell carcinoma. Int. J.Nanomed. 6(2011) 381−386.
[46] I.Stojanović, Y.van Hal, T.J.G. van der Velden, R.B.M. Schasfoort, L.W.M.M.Terstappen, Detection of apoptosis in cancer cell lines using Surface Plasmon Resonance imaging, Sens. Bio-Sens. Res. 7 (2016) 48–54.
[47] D.Vergara, M.Bianco, R.Pagano, P.Priore, P.Lunetti, F.Guerra, S.Bettini, S.Carallo, A.Zizzari, E.Pitotti, L.Giotta, L.Capobianco, C.Bucci, L.Valli, M.Maffia, V.Arima, A.Gaballo, An SPR based immunoassay for the sensitive detection of the soluble epithelial marker E-cadherin, Nanomedicine: Nanotechnol. Biol. Med. 14(2018)1963–1971.
[48] L.G.Carrascosa, A.Calle, L.M.Lechuga, Label-free detection of DNA mutations by SPR: application to the early detection of inherited breast cancer, Anal. Bioanal. Chem. 393(2009)1173–1182.
[49] M.Loyez, J.Albert, C.Caucheteur, R.Wattiez, Cytokeratins Biosensing Using Tilted Fiber Gratings, Biosensors, 8(2018) 74.
[50] N.F.Chiu, T.-L.Lin, C.-T.Kuo, Highly sensitive carboxyl-graphene oxide-based surface plasmon resonance immunosensor for the detection of lung cancer for cytokeratin 19 biomarker in human plasma, Sens. Actuators B, 265(2018)264–272.
[51] F.Duan, S.Zhang, L.Yang, Z.Zhang, L.He, M.Wang, Bifunctional aptasensor based on novel two-dimensional nanocomposite of MoS2 quantum dots and g-C3N4 nanosheets decorated with chitosan-stabilized Au nanoparticles for selectively detecting prostate specific antygen, Anal. Chim. Acta. 1036 (2018)121-132.
[52] H.S.Jang, K.N.Park, C.D.Kang, J.P.Kim, S.J.Sim, K.S.Lee, Optical fiber SPR biosensor with sandwich assay for the detection of prostate specific antigen**,** Optics Commun. 282 (2009) 2827–2830.
[53] C.Cao, S.J.Sim, Double-Enhancement Strategy: A Practical Approach to a Femto-Molar Level Detection of Prostate Specific Antigen-α1- Antichymotrypsin (PSA/ACT Complex) for SPR Immunosensing, J. Microbiol. Biotechnol., 17(2007),1031–1035.

[54] J.-W.Choi, D.-Y.Kang, Y.-H.Jang, H.-H.Kim, J.Mind, B.-K.Oh, Ultra-sensitive surface plasmon resonance based immunosensor for prostate-specific antigen using gold nanoparticle–antibody complex, Coll. Surf. A: Physicochem. Eng. Asp. 313–314 (2008) 655–659.

[55] J.Breault-Turcot, H.-P.Poirier-Richard, M.Couture, D.Pelechacz, J.-F.Masson, Single chip SPR and fluorescent ELISA assay of prostate specific antigen, Lab Chip, 15(2015) 4433.

[56] H.Vaisocherova, V.M.Faca, A.D.Taylor, S.Hanash, S.Jiang, Comparative study of SPR and ELISA methods based on analysis of CD166/ALCAM levels in cancer and control human sera, Biosen. Bioelectron. 24 (2009) 2143–2148. https://doi.org/10.1016/j.bios.2008.11.015

[57] N.Cennamo, M.Pesavento, L.Lunelli, L.Vanzetti, C.Pederzolli, L.Zeni, L.Pasquardini, An easy way to realize SPR aptasensor: A multimode plastic optical fiber platform for cancer biomarkers detection, Talanta, 140 (2015) 88 – 95.

[58] C.-Y.Yang, E.Brooks, Y.Li, P.Denny, C.-M.Ho, F.Qi, W.Shi, L.Wolinsky, B.Wu, D.W.T.Wong, C.D.Montemagno, Detection of picomolar levels of interleukin-8 in human saliva by SPR, Lab. Chip, 5(2005) 1017–1023.

[59] O.Krupin, C.Wang, P.Berini, Detection of leukemia markers using long-range surface plasmon waveguides functionalized with Protein G. Lab Chip, 15(2015) 4156−4165.

[60] A.Lujambio, S.W.Lowe, The microcosmos of cancer, Nature, 482(2012)347–355.

[61] L.Hong, M.Lu, M.-P.Dinel, P.Blain, W.Peng, H.Gu, J.-F.Masson, Hybridization conditions of oligonucleotide-capped gold nanoparticles for SPR sensing of microRNA, Biosens. Bioelectron. 109(2018)230–236.

[62] Q.Wang, Q.Li, X.Yang, K.Wang, S.Du, H.Zhang, Y.Nie, Graphene oxide–gold nanoparticles hybrids-based surface plasmon resonance for sensitive detection of microRNA, Biosens. Bioelectron. 77(2016)1001–1007. https://doi.org/10.1016/j.bios.2015.10.091

[63] W.Nie, Q.Wang, X.Yang, H.Zhang, Z.Li, L.Gao, Y.Zheng, X. Liu, K.Wang, High sensitivity surface plasmon resonance biosensor for detection of microRNA based on gold nanoparticles-decorated molybdenum sulfide, Anal. Chim. Acta. 993(2017)55-62. https://doi.org/10.1016/j.aca.2017.09.015

[64] J.Li, P.Lei, S.Ding, Y.Zhang, J.Yang, Q.Cheng, Y.Yan, An enzyme-free surface plasmon resonance biosensor for real-time detecting microRNA based on allosteric effect of mismatched catalytic hairpin assembly, Biosens. Bioelectron. 77(2016)435–441.

[65] L.Zhu, K.Wang, J.Cui, H.Liu, X.Bu, H.Ma, W.Wang, H.Gong, C.Lausted, L.Hood, G.Yang, Z.Hu, Label-Free Quantitative Detection of Tumor-Derived Exosomes through Surface Plasmon Resonance Imaging, Anal. Chem. 86(2014)8857−8864.

[66] J.Park, H.Im, S.Hong, C.M.Castro, R.Weissleder, H.Lee, Analyses of intravesicular exosomal proteins using a nano-plasmonic system, ACS Photonics, 5(2018)487−494.

[67] A.A.Ibn Sina, R.Vaidyanathan, S.Dey, L.G.Carrascosa, M.J.A.Shiddiky, M.Trau, Real time and label free profiling of clinically relevant exosomes, Scient. Rep. 6(2016)30460.

[68] G.Liao, X.Liu, X.Yang, Q.Wang, X.Geng, L.Zou, Y.Liu, S.Li, Y.Zheng, K.Wang, Surface plasmon resonance assay for exosomes based on aptamer recognition and polydopamine-functionalized gold nanonparticles for signal amplification, Microchim. Acta, 187(2020)251.
[69] A.T.Reiner, N.-G.Ferrer, P.Venugopalan, R.C.Lai, S.K.Lim, J.Dostálek, Magnetic nanoparticle-enhanced surface plasmon resonance biosensor for extracellular vesicle analysis, Analyst, 142(2017)3913-3921.
[70] M.Z.Mousavi, H.-Y.Chen, H.-S.Hou, C.-Y.-Y.Chang, S.Roffler, P.-K.Wei, J.-Y.Cheng, Label-Free Detection of Rare Cell in Human Blood Using Gold Nano Slit Surface Plasmon Resonance. Biosensors *5*(2015) 98-117.
[71] S.Jia, P.Li, K.Koh, H.Chen, A cytosensor based on NiO nanoparticle-enhanced surface plasmon resonance for detection of the breast cancer cell line MCF-7, Microchim. Acta, 183(2016)683–688.