

Application of biosensors in cancer research

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Abstract: Cancer research is based mainly on identifying many features of cells including mutations in DNA or RNA, changes in proteins and protein levels as well as on comparing cells' properties like morphology, adhesion or elasticity.

The most interesting techniques for biological applications, among different microscopies, are those using biosensors. As an analytical device, biosensor enables the binding of the selected analyte to the examined biological material (e.g. tissue, cells, proteins, RNA, DNA). They can be classified according to their transducer type or the biorecognition elements placed on the sensor. Most analysis performed on biosensors require the labeling of the analyte with a specific marker.

However, there are also many techniques that allow a direct detection of analytes without prior labeling. The common "label-free" biosensor technologies are the quartz crystal microbalance (QCM) and the surface plasmon resonance (SPR).

In this review we would like to focus on the currently described examples of biosensors used as diagnostic and prognostic tools for cancer development.

1. Introduction

The first person to use the term 'biosensor' was the Professor of Analytical Chemistry Karl Cammann in 1977. However, the definition of biosensor was not specified by the International Union of Pure and Applied Chemistry (IUPAC) until 1997. It was designed to combine three areas of science (chemistry, biology and engineering) in one device to detect bioanalytical molecules from samples [1].

For the construction of a biosensor two components are unavoidable: the transducer and the biorecognition element placed on the surface of the sensor. Transducer transforms the biochemical response appearing on the sensor surface to a measurable output signal. Some commonly used types of transducers are calorimetric (thermal), electrochemical (amperometric, impedimetric, potentiometric), magnetic (electromagnetic, electrodynamic and piezomagnetic), mass sensitive (acoustic, piezoelectric) and optical (colorimetric, photometric) based systems [2-4].

In table 1 different techniques using transducer types are gathered. On the other hand, the biorecognition element (bioreceptor) placed on the sensor ensures capturing the matching analyte from the solution sample. They may be classified as elements with biocatalytic properties (enzymes/substrate) or component with specific affinities (antibodies/antigens, nucleic acids, receptors/ligands, cells/tissues). The detection of some metabolic or biological components is also possible [1, 5, 6].

Table 1. A set of example methods used in bioanalytical techniques

Techniques	Calorimetric	thermal biosensors	enzyme thermistor (ET)-based sensors	[7]	
			metal oxide thermistors-based sensors		
			ceramic semiconductor thermopile-based sensors		
	Electrochemical	amperometric	first-/second-/third-generation biosensors	first-/second-/third-generation biosensors	[8]
				cyclic voltammetry (CV)	
		voltammetric	differential pulse voltammetry (DPV)	[5]	
			square-wave voltammetry (SWV)		
			potentiometric		ion-selective electrodes (ISE)
	ion-sensitive field				
	Magnetic	magnetic nanoparticles (MNPs); magnetic beads (MBs); semiconductor quantum dots (QDs)	magnetoresistance (MR)-based biosensors	[9]	
anisotropic magnetoresistance (AMR)-based biosensors					
giant magnetoresistance (GMR)-based biosensors					
Mass sensitive	bulk acoustic wave (BAW) devices	shear horizontal acoustic plate mode (SH-APM) sensor	[10]		
		quartz crystal microbalance (QCM)			
		quartz crystal microbalance in dissipation mode (QCM-D)			

Optical	surface acoustic wave sensors (SAW)	capacitive micromachined ultrasonic transducers (CMUTs)		
	microcantilevers	microelectronic mechanical systems (MEMs)	[11]	
	interferometric changes	resonant mirror (RM)		[12]
		resonant waveguide grating (RWG)		
		surface plasmons resonance (SPR)		
	fluorescence	surface plasmons resonance imaging (SPRi)		[4]
		fluorescence resonance energy transfer (FRET)		
chemiluminescence/ luminescence	luminescent semiconductor-based sensors			

Biosensor measurement methodology determines the type of detection: label-free or non-label free. Label-free detection is based on binding the original and unmodified analyte molecule directly to the biorecognition element, whereas in some methods (amperometric, voltammetric or fluorescent experiments) only analyte molecules tagged with a label may be recognized by the biorecognition element to obtain an electroactive signal [13]. Some commonly used labels are fluorophores (especially for fluorescent microscopy), enzymes (often for Western blot analysis) and several nanoparticles (essential for magnetic resonance imaging; MRI). For medical applications the usage of magnetic nanoparticles (MNPs), magnetic beads (MB), semiconductor quantum dots (QDs) with combined biosensor techniques is increasingly justified. Metal nanoparticles (mainly gold or silver) have a significant affinity for cancer cells, that is why they are frequently used in cancer research [6, 9]. Specific markers are often introduced into the tested compound using chemical synthesis or genetic engineering methods. However, the labeling process may require additional sample preparation or must be

followed by a second molecule binding. Unfortunately, the attachment of the label may significantly alter the properties of the tested molecule; substances used as markers may attach to other molecules than the target, and when using living cells, they may interfere with their metabolism. Considering all the above, label-free methods gain much more attention [14]. Nowadays, popular label-free methods are quartz crystal microbalance (QCM), surface plasmon resonance (SPR) and microelectronic mechanical (MEM) cantilevers, where cantilever sensors emerged from the atomic force microscopy (AFM) [13] (Fig. 1). These techniques allow tracking and determining the kinetic/thermodynamic analysis of the interaction process of two complementary molecules in real time, where one molecule is immobilized on the surface and the second one is in flow. Moreover, QCM and MEM-cantilever use changes in resonant frequency to observe the mass shifts on the sensor [15, 16], but SPR utilizes changes in the refractive index of thin metal layers (like gold surfaces) to quantify the binding process of biomolecule to the sensor surface [17].

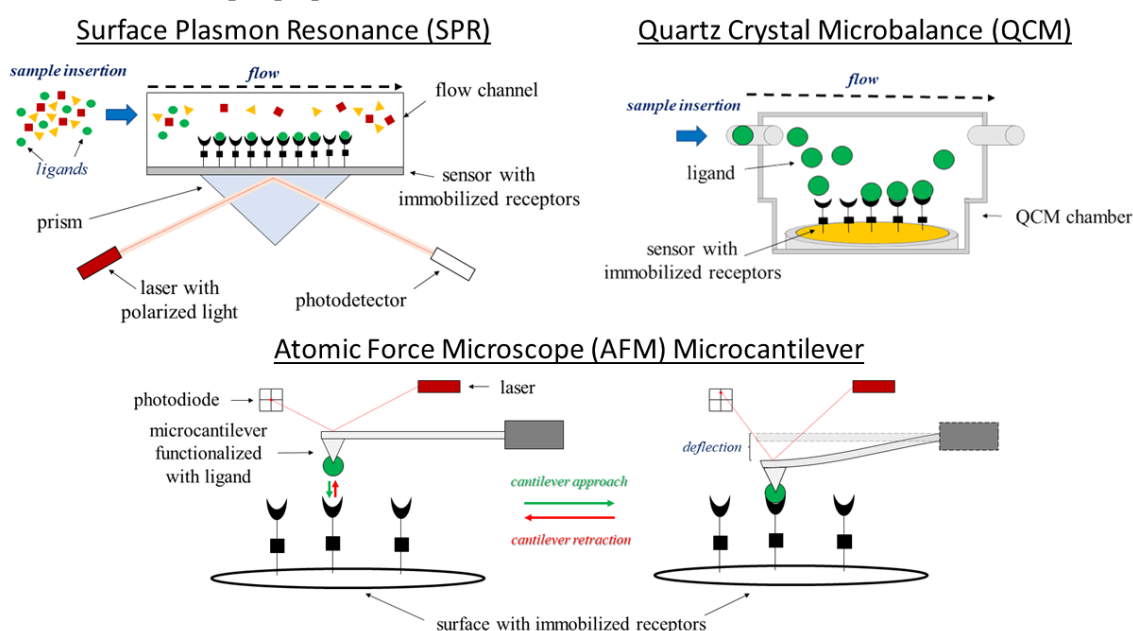


Figure 1. Schematic illustration of the three selected label-free methods: SPR, QCM and AFM microcantilever (own elaboration).

The development of reliable and sensitive molecular electronic devices is a matter of great importance in the field of biotechnology, medicine and pharmacy [5]. Especially in case of diagnostics, biosensors may significantly facilitate and accelerate early detection of several diseases like the Parkinson and Alzheimer disease, diabetes or various types of cancer. The monitoring of clinical treatment may also be performed via the usage of properly designed biosensors [18]. Body fluids such as urine, blood, saliva, tears or sweat may be treated also as samples full of specific disease biomarkers for micro/nanotechnology-based techniques (SPR, QCM, microcantilevers), even though their concentration in these samples is often very low [16, 19]. These methods are non-destructive for samples in comparison to others like Western blot, mass spectrometry (MS), matrix-assisted laser desorption ionization-time of flight mass spectrometry (MALDI-TOF MS) or electrospray ionization mass spectrometry (ESI-MS) [17]. On the other hand, isolation of the selected biomarkers from patient's tissue or cells (Fig. 2) may simplify their utilization as analytes on a biomolecular-based biosensor (DNA-, RNA-, aptamer-, protein-, antibody-based biosensors). Identifying mutations in DNA/RNA or uncovering changes in proteins and protein levels are common diagnostic strategies for cancers,

though they require expensive and time-consuming tests performed in well-equipped laboratories, as well as good samples quality and an appropriate sample size. The most popular microarrays for these analyses are the quantitative polymerase chain reaction (qPCR) and enzyme-linked immunosorbent assay (ELISA). Nonetheless, experiments executed on whole cells or even tissues are far more reliable because they reflect the real conditions prevailing in the human body [1]. Studying cancerous cell properties with advanced nanotechnological methods can bring many important biophysical information about these cells and may improve the knowledge about metastasis. Optical and fluorescent microscopies are often used for the examination of cell morphology, as well as the topography imaging with atomic force microscopy. What is more, AFM may be applied for studies concerning cell elasticity and adhesion, properties that change during the cancer progression, and even molecular interactions [15]. For the adhesion and molecular interaction QCM investigations can also be adjusted, not only with the molecular-based biosensors, but also with immobilized cells or even tissues on sensors. Yet, those cell-/tissue-based biosensors have to be properly prepared [7].

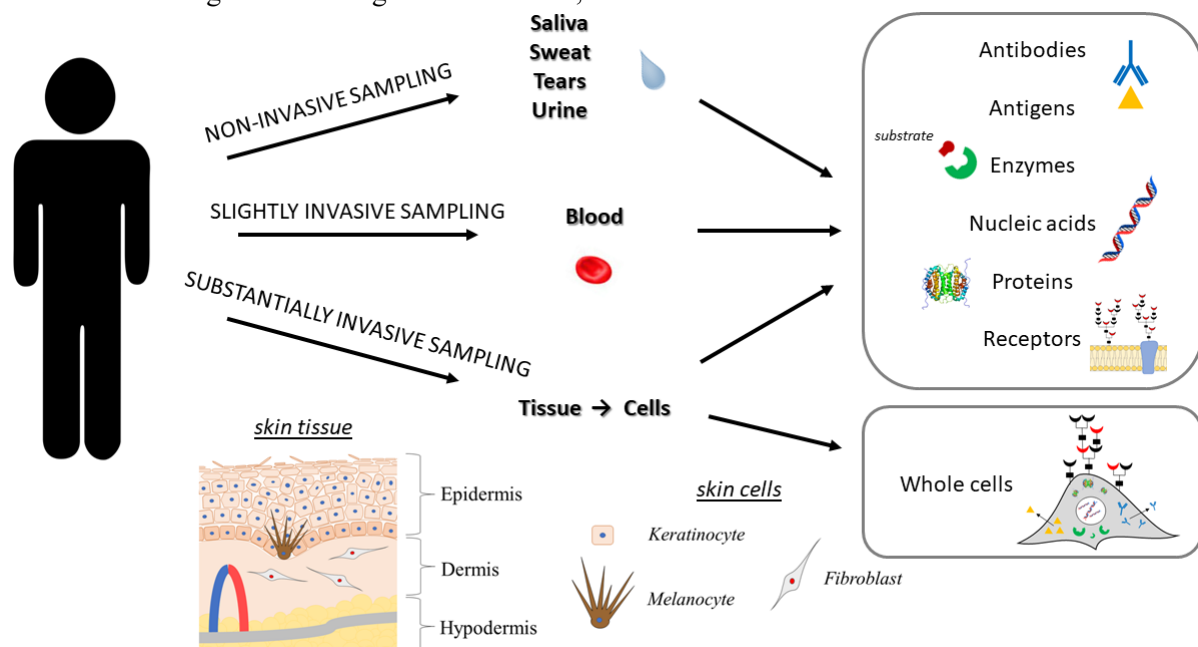


Figure 2. Schematic illustration of various biomarkers as targets for cancer detection with the biosensors methodology (own elaboration).

2. New insight

Biosensors may be applied successfully in the medical field, especially in cancer research. Due to the rising number of cancer cases each year all over the world, investigations concerning early cancer detection becomes a vital matter. Also, the possibility of cancer treatment monitoring with biosensor techniques gives hope for a personalized therapy. That is why providing a more

sensitive and simplified method at lower costs, which brings even more information about the basis of the disease, is still desired [20]. For these advanced studies some special molecular-based or cell-/tissue-based biosensors have already been developed and will be briefly characterized in the following sections.

2.1. Nucleic acid-based (NABs) biosensors

Some typical NABs are deoxyribonucleic acids (DNA), ribonucleic acids (RNA), peptide nucleic acids (PNA) and aptamers. During the investigation the nucleic acid should be immobilized on the surface of the sensor by adsorption, biotin-avidin interaction, covalent bonding, entrapment in a polymer matrix, ionic interaction or self-assembly [20]. The most frequently used immobilization method includes the application of thiolated-NABs for creating a self-assembly monolayer on a gold sensor surface. The single-stranded DNA and RNA sequences could anneal to the immobilized complementary sequences and the occurring interaction depends on the molecules. For DNA and RNA sequences binding, the Chargaff's rules of base pairing is fulfilled (DNA: A=T, C=G; RNA: A=U, C=G). On these bases, related to cancer mutations in DNA or RNA may be revealed [2]. Very interesting molecules for cell research are microRNAs (miRNAs), which are naturally existing small non-coding ribonucleic acids (RNA). They play a significant role in cell development (proliferation, cell cycle progression, apoptosis) and is related to a number of cancer cases. miRNA may be extracted from cells or tissues, however, their amount in the cancer cells differs from that of normal cells [21, 22]. PNAs are synthetic DNA or RNA analogues (sugar-phosphate backbone is replaced by pseudo-peptide backbone) that bind to their complementary strands with higher specificity and strength. Aptamers may be classified into two groups: DNA- or RNA-aptamers (short oligonucleotides) and peptide-aptamers (short peptide domains), but their detection is more similar to antigen-antibody or receptor-ligand interactions. They can be easily modified or integrated with a variety of nanomaterials [2, 23].

DNA-DNA binding: Breast cancer is associated with various gene mutations like breast cancer 1 (BRCA1). Its detection in the concentration range of 10 and 100 μM is possible due to the designed electrochemical biosensor. Short oligonucleotide DNA was immobilized onto zinc oxide nanowires that was chemically

synthesized onto gold electrode via the hydrothermal technique. The hybridization of ssDNA was studied by the differential pulse voltammetry (DPV) [24].

RNA-RNA binding: The detection of mir21 from the total RNA breast cancer samples was carried out on a selective and sensitive enzyme-based electrochemical biosensor. mir21 was covalently attached onto the pencil graphite electrode (PGE) by coupling agents and the hybridization was achieved with a biotinylated complementary target. Next an avidin labeled alkaline phosphatase was introduced to the system for obtaining the biotin-avidin interaction. Through the enzymatic conversion of the reaction substrate alpha naphthol phosphate to the reaction product alpha naphthol (α -NAP) the oxidation signal was detected by Differential Pulse Voltammetry (DPV) [21].

PNA-RNA binding: In order to detect let-7b in the total RNA extracts from HeLa cells (human epithelial cervical cancer) via base pairing, the silicon nanowire field-effect transistors (SiNW-FETs) with immobilized complementary PNA were used. With the optimized assay the detection limit of 1 fM could be obtained [22].

DNA aptamer-antigen binding: One of the well-known biomarkers for prostate cancer is the prostate-specific antigen (PSA) present in blood samples. The functionalization of a gold sensor with thiolated-DNA aptamer enabled the detection of PSA with the quartz crystal microbalance in dissipation mode (QCM-D) with the affinity constant equal 37 nM. These experiments provided not only information about the amount of PSA bound to the sensor, but also information about the aptamer conformation and layer hydration [25].

DNA aptamer-cells-nanoparticles binding: Blood cancer is a very aggressive type of cancer disease. Leukemia cells may be selectively captured by special DNA aptamers immobilized on the QCM sensor and the signal may be increased after the application of gold nanoparticles (AuNPs) on the already attached cells [26].

2.2. The biomolecule-based biosensors

The noncovalent, purely physicochemical binding forces are involved in many specific interactions like antigen (Ag)-antibody (Ab), enzyme-substrate, lectin-carbohydrate and ligand-receptor [27]. These interactions may be based on hydrogen bonds, van der Waals forces and hydrophobic interactions [28]. To be more precise, some conventional biochemical methods are the basis of these interactions, especially ELISA or immunofluorescence assay on Ag-Ab specific binding [29]. However, these methods require several steps of preparation (proper sample/antibody concentration, washing stages, sample labeling). Label-free techniques based on biomolecular biosensors (for example, antibody-/lectin-/protein-based) may not only accelerate the interaction measurement, but also provide full analysis of the interaction with the affinity calculation for the examined compound.

Antibody-antigen binding: Three liver cancer antigens: alpha-fetoprotein (AFP), hepatocyte growth factor (HGF) and gamma-glutamyl-transferase-2 (GGT-2); were detected with high specificity as well as good precision by the novel microcantilever with immobilized antibodies [16]. Also, the p53 antibody accumulates in human serum for many types of cancer including breast, lung, prostate, ovarian and melanoma. A quantitative detection of p53 antibody ranging from 20 ng/ml to 20 μ g/ml was obtained for human serum samples with p53 antigen-coated microcantilever [29]. Moreover, three independent SiNW-FET devices were designed, on which different antibodies were immobilized for the detection in pg/ml scale of PSA, carcino-embryonic antigen (CEA) and mucin-1 from blood samples [30].

Antibody-protein binding: Vimentin protein indicates the presence of Osteosarcoma, a common type of bone cancer. Immobilized anti-vimentin antibody on the surface of the MEM-cantilever was successfully used in the early detection of this cancer [31]. At the same time, collagen type IV (COLIV) occurs in serum samples of patients with colorectal, gastric, lung, liver and breast cancers. The immobilization of anti-COLIV antibody to the Surface Plasmon Resonance imaging (SPRi) gold sensor gave a dynamic

response in molecular binding in the range between 10 and 300 ng/ml for COLIV [32]. The laminin-5 protein functions as a motility factor or as an adhesive factor, depending on the proteolytic processing state. During the interactions with several cell-surface receptors it may promote tumor invasion. An antibody-based SPRi biosensor was used to determine the laminin-5 concentration in blood plasma with the detection limit 4 pg/ml [33].

Lectin-carbohydrate binding: Binding kinetics of lectin-carbohydrate interactions gains attention, due to the fact that cancer cells during their progression change their glycosylation profile what could be a possible drug target. Two mannose specific lectins (*Lens culinaris* and Concanavalin A, Con A) were immobilized onto gold QCM-D sensors separately via thiol groups and next carboxypeptidase Y was introduced in the buffer solution. The analysis of the lectin to carbohydrate affinity may serve as a quick biomarker classification assay in cancer research [34]. Furthermore, an interesting application of lectin-based sensors was achieved for cells in suspension. The modification of QCM sensor with Con A induced the binding of human leukemia cell line, which then was followed by the attachment of the second lectin on top of the cells. This approach may lead to the development of a novel label-free suspension cell-based biosensor [35].

Protein-DNA binding: With the SPR method it was also possible to semi-quantitatively detect the UV-irradiated DNA sequence obtained from human cell extracts. The DNA sequence was biotinylated and captured onto a streptavidin-coated sensor chip [17]. Protein-DNA binding can also be investigated by the DNA functionalized (SiNW-FET) biosensor. The estrogen receptor alpha (ER α , protein) regulates gene expression by the direct attachment to estrogen receptor sequences (ERE, dsDNA) immobilized on the sensor, which may be used for detecting these protein-DNA interactions in nuclear extracts from breast cancer cells. The designed biosensor was capable of detecting ER α in the 10 fM concentration [36].

2.3. Cell-/tissue-based biosensors

Methods in which you could apply whole cells deliver information which is more congenial than the techniques using samples isolated from cells or tissues (for example, DNA/RNA, proteins). This is due to the isolation process, which might damage or change the conformation of the biomolecules, influence the concentration of the required analyte or the stability of the sample. What is more, measurements performed on whole cell-based biosensors may simulate processes taking place in physiological conditions. Cells seeded onto sensors may be assigned for experiments with living cells (for compound absorption tests) or fixed cells (like compound binding ability tests) [37]. Cells *in vitro* are typically cultured on polystyrene or glass surfaces, often on precoated surfaces with proteins from the extracellular matrix (ECM) like fibronectin, collagen, laminin, vitronectin; but rarely on metals such as titanium, zirconium or gold [38, 39]. Bare gold sensors, common for QCM and SPR methods, are not quite desirable surfaces for cell culture experiments due to non-specific adsorption of many biological molecules like DNA and proteins that may be used as analytes [40]. That is why gold sensors are frequently coated with polystyrene or silicon dioxide. Nevertheless, surface modification plays an important role in the up or down regulation of physiological processes of cells such as adhesion, proliferation and differentiation [41]. Monitoring of the cell attachment and spreading may be crucial for designing devices that control the behavior of living cells. Simultaneously, it could bring a more detail knowledge about the metastasis process. Adhesion of cells is a complex process, which begins with cell sedimentation followed by the formation of nonspecific cell–substrate interactions and next the establishment of specific molecular binding of the receptor–ligand type. With time cell adhesion to the surface alters due to the ongoing physiological processes. To measure the cell-type-specific interactions, QCM-D technique can be applied [42, 43]. Some examples of cell-/tissue-based biosensor applications are:

Compound absorption tests on cells: The combination of whole cell sensing and real-time label-free monitoring of nanoparticle uptake by cells can be obtained by means of the SPR technique. The uptake kinetic of selected nanoparticles has already been tested on HeLa cells in the $\mu\text{g}/\text{mL}$ concentrations. However, this process is temperature-dependent: for about

20°C the uptake is higher, whereas for 37°C it is lower [44].

Compound binding ability tests on cells: Two stages of human colorectal cancer cells were derived from the same patient (primary and metastases), seeded onto a gold QCM sensor coated with polystyrene and lectin–carbohydrate interaction was measured with lectin *Helix pomatia agglutinin* (HPA). At the end a higher affinity of HPA to metastatic cells was obtained [45]. Also, the glycosylation level of melanocytes and melanoma cells (cultured on QCM-D gold sensors coated with polystyrene) was investigated by lectin Con A. The study revealed that mannose and glucose types of oligosaccharides present on metastatic melanoma cells consists of long and branched structures, whereas primary tumor cells and normal cells have short and less ramified oligosaccharides. Furthermore, the affinity of Con A to oligosaccharides on metastatic melanoma cells was ten times higher than for primary tumor cells and melanocytes [15]. Cell-based biosensors may be also used for cancer drug tests. Antibody-conjugated drug Herceptin detects the human epidermal growth factor receptor 2 (HER2) protein, which is over-expressed in 25–30% of breast cancers. It induces a cytostatic effect associated with the G1 phase cell cycle arrest, as well as antibody dependent cell-mediated cytotoxicity [46]. On the other hand, the G protein-coupled receptors (GPCRs) are crucial drug targets that may be activated by histamine, respectively. The SPR study has shown a triphasic response of HeLa cells to histamine interaction: 1-GPCRs triggered calcium release, 2-alternations of cell-matrix adhesion after the activation of Protein Kinase C, 3-dynamic mass redistribution in cells [47]. Surprisingly, up till now only few tissue-based biosensors have been described. Tonsil, prostate and breast tumor specimens were obtained and immobilized on the surface of gold QCM sensor. Next the interaction between the rVAR2 protein and placental-like chondroitin sulfate present on most cancer cells was analyzed and the calculated affinity was in the nanomolar range [48].

Cell adhesion tests: These strategies may be applied for the characterization of cell membrane receptors activity in cancer cells and for the search of other cell-specific ligands. For example, cell attachment to the surface is controlled mainly by the cell transmembrane integrin receptor that binds to Arg-Gly-Asp (RDG) sequence. The QCM-D sensor was modified with a photo-

activatable RGD peptide for determining the time point of presentation of adhesive ligand from the human umbilical vein endothelial cells (HUVEC) [42]. Also, with the use of a novel high-throughput label-free resonant waveguide grating (RWG) imager the HeLa cells spreading kinetics on the

ligand RGD tripeptide was determined [49]. On the other hand, vitronectin protein – as well as antibody (CA-125)-based QCM biosensors were used for the binding of the suspended melanoma, cervix and ovarian cancer cells [50].

3. Review and discussion

Cancer could develop at a very rapid pace, therefore the simplicity of measurements, quickness of the test and low costs are in request from the potential new methods that are to be applied. For this reason, biosensor techniques, especially those with label-free detection, have gained massive attention recently. Their main assumption is the specific interaction occurring between the biorecognition element and the selected analyte. What is important, some of these measurements (like those performed on QCM-D

device) can be made on living or fixed cells and may deliver kinetic and thermodynamic analysis of the obtained interaction, as well as the information about the affinity, conformation of the created complex and even viscoelastic properties of the new appearing biomolecular surface. A wide variety of biosensors is available among the transducer type and the biorecognition element alike. This is why biosensors may have a versatile application.

Acknowledgements

This work was supported by the Faculty of Chemistry, Warsaw University of Technology and by the National Science Centre (Poland), project no 2017/27/N/ST4/01389.

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