Point-of-Care and Implantable Biosensors in Cancer Research and Diagnosis

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5.1 Introduction

Biosensors are compact analytical devices that mimic natural chemoreception schemes: biological components react with the analyte of concern to produce biochemical information, readily translated into an electric signal by a chemical transducer (Fig. 5.1). In context, the analytical characteristics of any device depend upon the intra-component properties and inter-component correlations: specificity is assigned by the biological system used, response times are determined by the transducer, miniaturization comes mostly inherent by the nanosize of the biological moieties, and intrinsic signal amplification capabilities are determined by the bioelement-transducer interface (Palchetti and Mascini 2010; Shruthi et al. 2014). Since 1960s, when Leland C. Clark, Jr. in 1960s used an oxygen probe as a glucose meter (Clark and Lyons 1962), the realization of the biosensor concept has been almost explicitly linked with the biomedical sector, where prospects, expectations, and deliverables could be readily translated into a worthwhile market-based rate of return in the portfolio of products.

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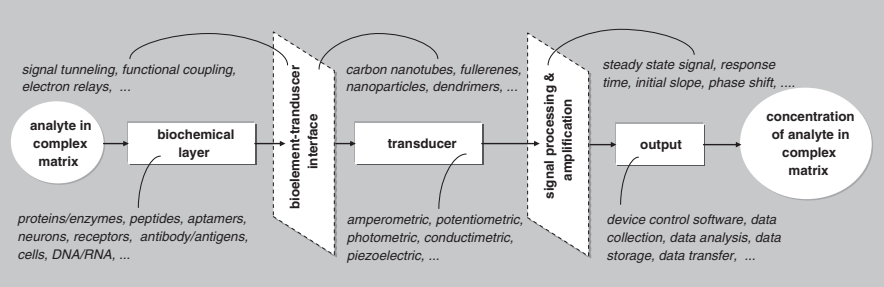

Fig. 5.1 Overview of biosensor architecture, general device assembly concepts, and basic mechanisms employed in the transduction of the biochemical information into a measurable signal

As diagnosis, monitoring, and therapy of diseases, especially cancer, shifts nowadays to a more molecular-based approach, biosensor technology may provide a suitable platform for real-time and personalized health monitoring. Fast responses, miniaturized sensor size, biocompatibility, rapid label-free detection, easy device tailoring, ultra-low detection limits, high reliability of measurements, and low development costs are appealing to patients, physicians, and the medical industry alike. The versatility of biosensor platforms offers a significant advantage in personalized and/or targeted monitoring: in concept, mush verified in practice, as well, any analyte can be correlated with a variety of suitable bioelements, which, in turn, can be paired to any transducer (and vice versa) and packaged according to any needs to yield a variety of devices with a larger variety of device characteristics. There exists the feasibility of engineering wearable or implantable point-of-care biosensors for monitoring clinical parameters, such as protein changes, biomarker concentrations, and drug targeting (Pantelopoulos and Bourbakis 2010; Jin et al. 2017). The development of unobtrusive, recurrent, and long-term nanomonitors can serve adequately early diagnosis of alarming health trends, while operating under strict medical specifications, several ergonomic constraints, and significant hardware resource limitations (Vasan et al. 2013).

The state-of-the-art in emerging concepts is presented herein, strategies and techniques in developing biosensor systems for cancer research and diagnosis. Critical issues, technology bottlenecks, and challenges are, also, discussed.

5.2 Construction of Biosensor Platforms

Most methods used for biosensor fabrication derive from the vast experience acquired in semiconductors and microelectromechanical systems. Briefly, bottom-up and top-down approaches are used (Prakash et al. 2017). The former involves the management of basic building blocks or materials. For example, self-assembly techniques use thermodynamic energy minimization processes to induce phase segregation and yield polymer structures (Ma et al. 2016; Prakash et al. 2009); more advanced tools such as optical tweezers (Song et al. 2010; Suei et al. 2015) or

atomic force microscopy (Ozkan et al. 2016) enable greater accuracy for pick-and-place approaches. Top-down approaches rely on the machining of advanced materials through lithography and etching (Prakash et al. 2017). The processes used for the immobilization of the biological system on the transducer surface depend strongly on surface-species interactions; thus, the ability to control and manipulate surface properties (charge, stress, etc.) is a critical parameter in biosensor design.

The target analyte determines the biological system to be used. Apart from affinity, other criteria that may apply in bioelement selection include, inter alia (Siontorou and Batzias 2013): (a) kinetic parameters for the analyte-bioelement interaction; fast kinetics could provide fast response times in the event that the speed at which the biochemical information is transduced is equally fast (otherwise, the signal might be missed); (b) non-toxicity of interaction products, in order to avoid detector biofouling or patient intoxication; (c) reversible interaction in order to ensure the regeneration of the biochemical layer; (d) tight ligation of the bioelement onto the transducer surface to avoid leaching; (e) sufficient bioelement ruggedness to avoid denaturation. Evidently, matching the target analyte to a bioelement is not of critical concern; matching the bioelement to the conditions under which the biosensor will operate and to the aims and scopes of detection (i.e., the sensitivity and selectivity requisites) may prove problematic. These parameters should be taken into consideration when designing the diagnostic system, since any optimizations that will be applied at device testing might prove unsuccessful (Siontorou et al. 2010).

Nanotools now available can offer several alternatives for engineering biological moieties to suit any need, analytical or regulatory. Their coupling to a transducer may come in many forms, mostly as electrochemical, optical, or mass-based, depending on the type of biological response (Fig. 5.2). Frequently, hybrid transduction (e.g., electrochemical and optical) schemes may be used for signal optimization purposes. Many strategies have been proposed for enhancing the performance of the detectors, both material-based and instrumental. Some examples are given here below.

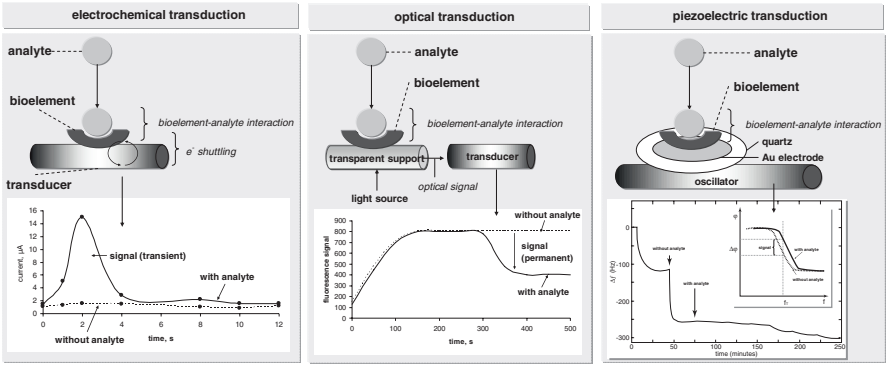

Fig. 5.2 Most commonly used transduction systems in biosensor for cancer diagnosis

Electrochemical sensors are compact devices of low resource settings; amenable to size reductions, they exhibit excellent linearity and repeatability and generally have a long life span, typically 1–3 years. Electrochemical sensors monitor changes in ion current, potential, conductance, or impedance (Bollella et al. 2017). Many devices have been suggested for targeting low molecular weight species, proteins, and cells. Recent advancements allow even for specific epitope targeting, such as the carbohydrate sites on cell surfaces; Cheng et al. (2009) developed a detection system for glycans on carcinoma cell surface. Signal enhancement may be achieved in a variety of ways. Strategies for accelerating the electron transfer with carbon nano-forms (nanotubes, nanofibers, nanosheets) have been proposed (Siontorou et al. 2016). Nanomaterials can be, also, used as tag molecules in hybrid transducers (Ding et al. 2008; Lai et al. 2011). Especially in electrochemiluminescent, these molecular tags behave as quantum dots and signals can be further amplified using ordered assembly or click chemistry, whereas the tags can be directly synthesized as dendrimers or polymers. If electrochemical transduction is preferred, gold nanoparticles can be added to produce conductive domains. Velev and Kaler (1999) have introduced this strategy when working on a conductivity immunoassay of proteins using antibody-functionalized latex spheres positioned between two interdigitated microelectrodes; the device could be miniaturized further to structure on-chip protein arrays with a picomolar detectablity. Silver-enhanced labelling may, also, prove quite useful. For example, Liu et al. (2010) used the silver enhancement technique in a conductimetric biochip, with a dual response: at a sub-threshold region, using electron hopping between silver islands and the electrolyte for conduction, and at an above-threshold region that employed direct flow of electrons. As the two regions use different conduction mechanisms and produce different slopes, the dynamic range of >40 dB produced gave a detection limit of 240 pg/mL. Single-walled carbon nanotubes can be easily fit into electrochemical systems to provide increased sensitivity to enzymatic reactions.

Optical transducers utilize light absorption, fluorescence, luminescence, total internal reflection, or surface plasmon resonance (SPR) for simple (Jeronimo et al. 2007) or multiplexed detection (Fan et al. 2008). Optical fibers and waveguide devices are used to improve sensitivity of the sensors by enhancing the interaction between the guided light and the sensor surface. Pu et al. (2010) proposed a new amplification strategy using hybrid nanomaterials (oligomeric silsesquioxane-based fluorescent nanoparticles) as signal amplifiers for biological imaging. These materials have fluorescent arms that can be chemically modified to adjust their emission wavelength, charge, and diameter according to needs; their signal amplification capabilities allow for the use of small quantities of indicator dyes for high-quality biological imaging. Similarly, semiconductor nanoparticles exhibit easily tunable absorbance and fluorescence. Jokerst et al. (2009) developed a microfluidic device for the multiplexed detection of cancer antigen 125 (CA125), carcinoembryonic antigen (CEA), and Her-2/Neu (C-erbB-2); the biosensor was based on fluorescence transduction of a quantum dot antibody probe immobilized on a microporous agarose bead array supported within a microfluidic system. The integration of semiconductor nanoparticles surpassed the response of standard molecular fluorophores by

30-fold. On the other hand, magnetic nanoparticles may offer certain advantages, especially in DNA-based platforms. Bi et al. (2009) used bio-barcode-functionalized magnetic nanoparticles as DNA hybridization platform to avoid cross-reactivity and lower detection limits; a femtomolar detection limit was achieved without any pre-concentration process.

Mass-based transducers detect the mass changes induced by the biochemical interaction. They consist of a piezoelectric crystal which oscillates at a particular frequency under an electric field. The mass of the crystal and the electrical frequency applied influence the frequency of oscillation of the crystal. Applications in cellular biology research involve mostly cell-surface interactions and morphological changes (Saitakis and Gizeli 2012). The process of transforming healthy cells to cancerous usually brings about changes in the morphology of cells and the arrangement of the cytoskeleton; these changes are expressed in dynamic cell adhesion processes and viscoelasticity modifications that can be monitoring in real time with a piezoelectric system (Zhou et al. 2011). The resistance vs. frequency changes provided a cell viscoelastic index that could be used to distinguish normal (HMEC) from malignant (MCF-7) mammary epithelial cells; during cell adhesion, malignant cells became softer, expressing a lower index compared to that of the healthy cells. The mechanical properties of cells were studied by applying centrifugal force during the interaction of cells on the surface of a quartz crystal microbalance, embedded in the rotor of centrifuge together with its driver (Webster et al. 2014). Apart from improving sensitivity, the viscoelastic properties of the cellular surfaces could be also measured. Su et al. (2013) developed a piezoelectric system for the direct detection of cancer biomarkers based on a lead titanate zirconate ceramic resonator as transducer. The dual sensing device had two resonators connected in parallel, one as the sensing unit and the other as the control unit; thus, they managed to minimize environment interference and compensate for temperature fluctuations. The device exhibited high sensitivity (0.25 ng/mL for prostate-specific antigen and α-fetoprotein) and fast analysis time (<30 min) of 1 μL samples. This ceramic resonator-based platform can be readily coupled to different chemical interfaces, for simple or multiplex detection.

Calorimetric biosensors are less common in cancer diagnostics, but nanotechnology-based modifications have broadened their range of applications. These systems measure enthalpy changes to monitor exothermic reactions, providing, indirectly, information about the concentration of the substrate (Bohunicky and Mousa 2011). Medley et al. (2008) developed a calorimetric biosensor based on aptamer-linked gold nanoparticles that could differentiate between acute leukemia cells and Burkitt's lymphoma cells. Their work demonstrated the feasibility of developing calorimetric platforms with aptamer-based recognition elements with the ability to discriminate between normal and cancer cells.

Microfluidic laboratory on-chip sensors may improve substantially patient care. Lab-on-chip technology integrates multiple steps of different analytical procedures, large variety of applications, sub-microliter consumption of reagents and samples, and portability (Gambari et al. 2003). Electrochemical detection based on paper-based microfluidic devices is also promising. Such devices could be developed as

portable, easy-to-use, and low-cost point-of-care testing systems (Lu et al. 2012). Photolithography arranges microfluidic channels on cellulose fiber-based paper, while screen-printing fabricates electrodes on paper (Pires et al. 2014). The surface of the screen-printed electrodes can be functionalized with enzymes or DNA strands that serve as capture probes for the target analytes.

The use of luminescent nanocrystals (quantum dots) as molecular labels opened new horizons in cellular labeling and visualization (Tothill 2009). The nanocrystals can be attached to molecules for tracking intracellular components or used for antibody labeling. Their narrow emission peaks and spectroscopic properties support multiplexed analysis. Moreover, they exhibit high emission quantum yields that improve signal/noise ratios and increase the reliability of measurements.

Biosensor technology has indeed reached a level where state-of-the-art processes can offer amply a huge variety of engineering solutions for the manufacture of advanced micro- and nanosensors. Some examples are presented in the following sections. Still, physics present certain insurmountable constraints. The critical dimensions of micro- and nanofluidic-based systems are comparable to the scales of physical processes engaging small molecules. The minimization of detectable concentration levels and detection times are only limited by mass transport phenomena and reaction kinetics (Prakash et al. 2017; Siontorou and Batzias 2013). Reliability of detection is further reduced by nonspecific adsorption, matrix effects, Debye length, and streaming potential (Siontorou et al. 2010). Nanosensors exhibit ultra-low detection limits because the screening of ions is reduced in packed spaces that are largely inaccessible by proteins, such as the corners between the nanowire and the substrate (Shoorideha and Chuia 2014). This corner effect exists in most biosensing structures, regardless of their scale; but at the nanoscale the effect becomes more important.

5.3 Biosensor Systems for Cancer

Using biosensors to monitor the levels of individual proteins secreted and/or expressed by cancerous cells may provide useful information to the health practitioner regarding cellular states. More than 160 types of biomarkers may be proven effective in diagnosing, staging, and treating early-stage cancer. For example, monitoring the levels of carcinoembryonic antigen (CEA) before and after treatment can be used to identify early recurrences or previously metastases (Kobayashi et al. 2012). Biosensor-based point-of-care monitoring could aid cancer management and facilitate earlier diagnosis. The systems developed are numerous, mostly on simple detection, although there are few platforms for multiplex analysis (Table 5.1). The detection limits achieved range between femto- and nano-scales, depending on the biosensor components used, such as carbon nanotubes, gold nanoparticles, quantum dots, and magnetic particles.

Antigen- and antibody-based biological systems are generally preferred due to the inherent specificity of antibody-antigen interactions. Kojima et al. (2003) developed an arrayed immunosensor with antibodies against α-fetoprotein immobilized

Table 5.1 Detection of tumor biomarkers with various biosensor platforms

Biomarker	Detection method	Biosensor principle	Detection limit	References
α-Fetoprotein (AFP)	Electrochemical	Arrayed immunosensor with antibodies immobilized in a plasma-polymerized film		Kojima et al. (2003)
		Prussian blue with screen-printed amperometric sensor	5 ng/mL	Guan et al. (2004)
α-Fetoprotein (AFP) and carcinoembryonic antigen (CEA)	Electrochemical	Dual immunosensor	1 ng/mL	Wilson (2005)
		Streptavidin-functionalized silver-nanoparticle-enriched carbon nanotube tag	0.093 pg/mL (AFP), 0.061 pg/mL (CEA)	Lai et al. (2011)
Breast cancer susceptibility gene (BRCA1)	Electrochemical	cDNA immobilized chitosan-co-polyaniline functionalized matrix	0.05 fM	Tiwari and Gong (2009)
		Mesoporous carbon nanospheres–toluidine blue nanocomposite	3.97 ng/mL	Fan et al. (2013)
Cancer antigen 125 (CA-125)	Electrochemical	Direct electrochemistry of horseradish peroxidase on titania sol-gel immunosensor	1.29 units/mL	Dai et al. (2003)
Cancer antigen 15–3 (CA15–3)	Optical	Gold nanorod -based plasmonic sensor	0.0249 units/mL	Chen et al. (2015)
Carcinoembryonic antigen (CEA)	Electrochemical	Direct electrochemistry of horseradish peroxidase on modified silica gel immunosensor	0.4 ng/mL	Tan et al. (2006)
		Thionine-doped magnetic gold nanospheres as labels and horseradish peroxidase as enhancer	0.01 ng/mL	Tang et al. (2008)
	Electrochemi-luminescence	$Ru(bpy)_3^{2+}$-graphene-Nafion composite	0.002 pg/mL	Hao et al. (2012)
Ferritin	Piezoelectric	Gold chip immunosensor	0.1 ng/mL	Chou et al. (2002)

(continued)

Table 5.1 (continued)

Biomarker	Detection method	Biosensor principle	Detection limit	References
Human chorionic gonadotrophin (hCG)	Optical	Fluorescence immunosensor	25 units/mL	Nakamura et al. (2001)
Human epidermal growth factor receptor 2 (HER2)	Electrochemical	Label-free capacitive aptasensor coupled to non-Faradaic Impedance Spectroscopy	0.2 ng/mL	Qureshi et al. (2015)
Human prolactine biomarker (hPRL-3)	Electrochemical	Phage-modified light-addressable potentiometric sensor	0.04 nM	Jia et al. (2007)
Interleukin 6 (IL-6)	Electrochemical	Direct electrochemistry of horseradish peroxidase on carbon nanotubes gold-modified surfaces	0.5 pg/mL	Malhotra et al. (2010)
Mucin 1 (MUC1)	Electrochemical	Magnetic beads coupling screen-printed array	0.07 nM	Florea et al. (2015)
Prostate-specific antigen (PSA)	Piezoelectric	Microcantilever immunosensor	0.2 μg/mL	Wu et al. (2001)
	Optical	SPR with colloidal gold nanoparticles	0.15 ng/mL	Besselink et al. (2004)
		Gold layered dielectric-metal nanoparticles immunosensor	0.1 ng/mL	Hirsch et al. (2003)
		Micromechanical silicon nitride cantilevers	0.2 ng/mL	Wu et al. (2001)
	Electrochemical	Direct electrochemistry of horseradish peroxidase on carbon nanotubes gold-modified surfaces	0.5 pg/mL	Mani et al. (2009)
		Amine-terminated DNA aptamers were coupled to sulfo-betaine gold electrodes	1 ng/mL	Jolly et al. (2015)
	Electrochemi-luminescence	Carbon nanotubes-chitosan/gold nanoparticles	0.6 pg/mL	Zhang et al. (2012)

Table 5.1 (continued)

Biomarker	Detection method	Biosensor principle	Detection limit	References
Vascular endothelial growth factor (VEGF165)	Electrochemical	A label-free electrochemical aptasensor based on ordered mesoporous carbon-gold nanocomposite-modified screen-printed electrode	1 pg/mL	Tabrizi et al. (2015)

in a plasma-polymerized film. Dual systems for α-fetoprotein and carcinoembryonic antigen have been, also, proposed with either conventional platforms (Wilson 2005) or functionalized nanoparticles (Lai et al. 2011). Prostate-specific antigen (PSA) can be reliably detected with an anti-PSA antibody. The most successful platforms developed involve microcantilever-based transducers (Wu et al. 2001) and surface plasmon resonance (SPR)-based sensors (Hirsch et al. 2003), in which PSA antigen binding to antibody changes the vibrational frequency in an extend analogous to antigen concentration. Jia et al. (2007) developed a light-addressable potentiometric sensor using a phage recognition element for human prolactine biomarker (hPRL-3) and human breast cancer cell line MDA-MB-231; the results showed that the biosensor developed was more applicable to cancer cells detection. The major constraints of immunosensor platforms include the reduced thermal and physical ruggedness of the biological moieties and the difficulty in regenerating the antibody-based systems (Mittal et al. 2017); both limit considerably the reliability of the sensors, especially towards the limits of detection.

Aptamers and nucleic acids have been also proposed for cancer biosensing, offering almost endless different sequences that can express high affinities for their targets. A combinatorial chemistry-based technology that uses exponential enrichment for the systematic evolution of ligands can be used to generate specific nucleic acid probes from a library of RNA and DNA oligonucleotides. Despite the low success rates and time-consuming attributes of this technology (Mittal et al. 2017), many relevant biosensors have been developed, focused on the discovery of new cancer biomarkers for early diagnosis, such as the breast-specific protein NY-BR-1, and the cancer testis antigens CAGE-1 and NY-ESO-1 (Bohunicky and Mousa 2011). The latter are either detected by the cytotoxic T-lymphocytes of cancer patients or induce a serological immune response in the autologous host; these markers could be used for the development of anti-cancer vaccines (Balafoutas et al. 2013).

Aptasensors usually employ sandwich type methods, where the aptamers are attached to the transducer surface and analytes are attracted from liquid samples to yield high efficiencies. A second antibody with a measurable label is then bound to the attracted analytes; this label is readily detected by electrochemistry or other methods. For example, Mucin1 has been detected in real serum samples using a screen-printed array biosensor with magnetic beads and alkaline phosphatase labeling (Florea et al. 2015); the detection limit achieved was 0.07 nM within a linear

range between 0 and 0.28 nM. Label-free schemes have been also reported. An aptasensor on carbon−gold nanocomposite-modified screen-printed electrode has been recently proposed for the detection of vascular endothelial growth factor VEGF165 in the serum of patients with lung cancer (Tabrizi et al. 2015). The sensor measures the changes in the interfacial charge transfer resistance of the electrode induced by the interaction of the immobilized anti-VEGF165 aptamer with the sample VEGF165 marker. In another study, a label-free capacitive aptasensor was developed for the human epidermal growth factor receptor 2 (HER2) protein using anti-HER2 DNA aptamers functionalized on interdigitated microelectrodes (Qureshi et al. 2015). The aptamer-protein complex induced concentration-dependent changes in the values of impedance/capacitance. Jolly et al. (2015) used impedimetric methods and an aptamer platform for detecting PSA in real blood samples. The authors compared two different methods in order to elucidate how the sensitivity and selectivity are impacted by surface chemistry. A thiolated DNA aptamer interacted with mercaptoethanol-modified gold electrodes; alternatively, amine-terminated DNA aptamers were coupled to anti-fouling sulfo-betaine gold electrodes. Although both fabrication processes were long and cumbersome, the detectability achieved was 1 ng/mL with sulfobetaine-probes and 10 μg/mL with mercaptoethanol-modified electrodes.

Light emission/absorption-based determination of biomarkers is a wide research field, mostly focused on nanoparticles, which involve photostable synthesis and provide noise-free fluorescence signals (Mittal et al. 2017). Chen et al. (2015) developed a combined detection assay for cancer antigen 15–3 (CA15–3) and copper level in serum using a gold nanorod-based plasmonic sensor. Manikandan et al. (2014) compared several surface-enhanced Raman spectroscopy substrates produced by in situ nucleation of gold nanohexagons on graphene nanosheets, gold nanoparticles, and gold-conjugated graphene nanomaterials; these nanomaterials enhanced Raman scattering to such a degree that human breast normal, cancer, and cancer stem cells could be discriminated. Cytotoxic studies indicated that graphene nanomaterials hardly enter cell; results on gold nanoparticles were inconclusive.

Some implantable electrochemical biosensors have been reported, designed to measure and transmit a specific response towards an analyte at the molecular level. A two or three electrode systems are commonly used, coupled to the appropriate enzyme. Apart from the use of nanomaterials to modify electrodes, some studies have been published on the development of devices with nanometric geometry (Goncalves et al. 2011), where one-dimensional structures serve as working electrodes for measuring femto- or pico-ampere activities. Various electrodes such as single-walled carbon nanotubes (Baughman et al. 2002) or boron-doped silicon nanowires (Goncalves et al. 2011) have been used in the construction of nanodevices. Hoeben et al. (2008) used a reduced scale of redox enzymes to electrochemically study a small amount of molecules. The measurements, made on lithographically fabricated 70 nm gold nanoelectrodes, showed successfully for the first time a distinct catalytic activity from less than 50 enzymes molecules. Cordeiro et al. (2015) developed an implantable biosensor for the continuous and simultaneous monitoring for glucose, lactate, and pyruvate. The sensor has been implanted in rats for evaluation; the brain levels of the carbohydrates could be monitored at the millimolar range. Zhang et al. (2016) developed a silicon-based 16-site implantable

25-mm long microelectrode array chip fabricated by standard lithography. The sensor was implanted in nonhuman primates for monitoring in real time the electrochemical activity of dopamine.

Flexible microelectrode arrays are expected to revolutionize point-of-care devices. Polyimide thin films have been proposed for implantable probe development. These films are deposited onto a carrier substrate; using anodic release, the carrier substrate discharges the polyimide structures in saline solution (Cheung and Renaud 2006). However, biocompatible interfaces between the implanted sensor and the surrounding tissue have not been demonstrated yet. The use of anti-inflammatory and biodegradable coating might interfere with analyte detection compromising the reliability of the measurements (Siontorou et al. 2010).

Optical platforms have been also proposed for in vivo sensing. Parameters such as fluorescence intensity and lifetime enhance sensitivity and offer long-term stability. Much work has been published on fluorescence resonant energy transfer-based biosensors for glucose, where the intensity of the signals is proportional to glucose levels (Khan et al. 2008). A transdermal system for continuous glucose monitoring has been reported by Ballerstadt et al. (2006). To further reduce invasiveness, transdermal glucose monitoring uses functionalized fluorescent microparticles injected in the patient (Shibata et al. 2010). Although promising, this approach is not suited for point-of-care continuous applications as a video camera and external light excitation are needed for image analysis. Valdastri et al. (2011) presented a miniaturized fluorescence biosensor suitable for long-term implantation. The device uses phototransistors as detectors and achieves fluorescence excitation and detection by driving a laser diode light source (Fig. 5.3). The signals are amplified and transmitted across the skin to a mobile device. Yet, the functionality of the sensor has been demonstrated only in vitro. Tong et al. (2016) studied the optical functionality, in vitro and in vivo, of a thermally hydrocarbonized porous silicon optical rugate filter, along with its stability and biocompatibility. The material proved to be cytotoxic, regardless of its surface chemistry, possibly due to the mitigation of reactive oxygen species levels during the pre-incubation of the film.

Magnetic resonance platforms have been also proposed. Harris et al. (2008) developed an in vivo sensor for measuring proteinase activity related to cancer. In

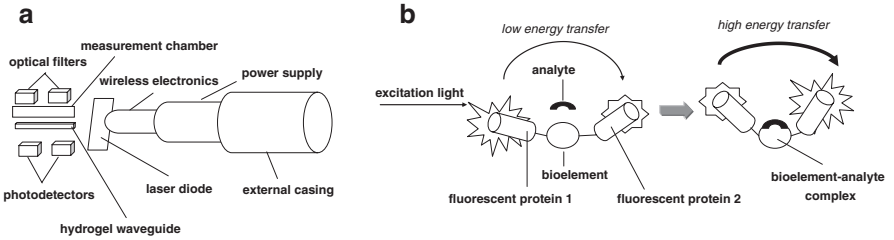

Fig. 5.3 (**a**) Architecture of the implantable fluorescent-based electrochemical biosensor. (**b**) The fluorescent resonant energy transfer concept: when two fluorescent proteins are covalently attached to the bioelement, a limited energy transfer from one protein to the other is recorded; the binding of the analyte to the bioelement induces conformation changes to the latter that result in bringing the two proteins closer and, thus, allowing for the transfer of a higher amount of energy (adopted from Valdastri et al. 2011)

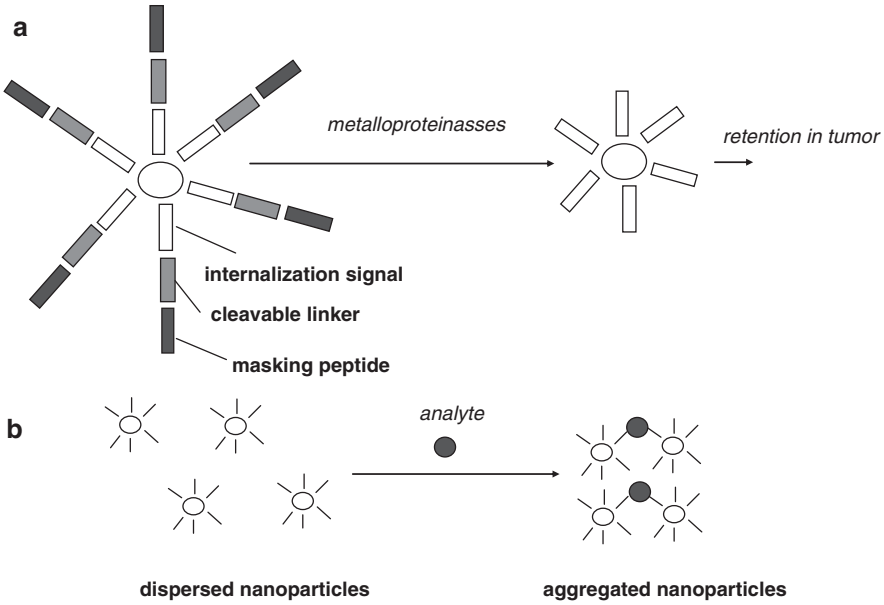

Fig. 5.4 Schematic representation of magnetic resonance platforms: (**a**) The magnetic nanoparticles are masked with protease-cleavable ligands to prevent internalization until the mask is removed by tumor-associated metalloproteins; the nanoparticles can then be efficiently internalized by the adjacent tumor cells. (**b**) Dispersed bifunctional particles exhibit a high relaxation time; when bound to the target analyte, they aggregate, quantitatively lowering the signal (adopted from Harris et al. 2008; Daniel et al. 2009)

this platform, protease-cleavable ligands coated a nanoparticle to mask a cell internalization signal embedded in the ligand (Fig. 5.4). When metalloproteinases are present in the matrix, the ligand is cleaved and the internalization signal is expressed. The presence of a tumor induces locally a high expression of proteases that drive the particles inside the tumor cells. Similar systems with other reporter proteases or with a combination of proteases and specific linkers could be developed for multiple cancer types. Daniel et al. (2009) developed an implantable biosensor that could sense the microenvironment. The sensor is built on a semi-permeable membrane containing nanoparticle magnetic relaxation switches. Ectopic tumors were produced in mice using a cell line that secreted a model cancer biomarker. After 1 day, tumor-bearing mice exhibited a transverse relaxation time that was 20 ± 10% lower than the healthy-control mice. The applicability of these devices in the verification of successful tumor resection may be realized quite soon.

5.4 Challenges in Implantable Sensor Development

In vivo monitoring has not been yet realized in a substantial extent. However, research in molecular therapy targets specific malfunctioning molecules and pathways in cancer. For example, kinase inhibitor imatinib proved promising for the management of

chronic myeloid leukemia and gastrointestinal stromal tumors whose growth is related to the expression of specific kinase mutants (Sawyers 2004). The efficiency of the inhibitor needs to be evaluated at the level of protein interactions. Biosensors based on fluorescence resonance energy transfer (FRET) may provide the technology for monitoring kinase inhibition in live cells, even for in vivo applications. Numerous FRET-based biosensors have been recently published for the detection of oncogene-related kinase activities (Wang et al. 2005; Zhang and Allen 2007), and for other molecules that indicate cancer migration and invasion (Wang et al. 2008).

One major challenge in in vivo systems is powering. Inductive links for powering remotely devices has already reached the market. Size reductions in inductors for in vivo applications remain an open topic. The use of micro-fabricated inductors demonstrates the greatest potential (Olivo et al. 2014). Less power consuming and autonomous platforms have been reported. For example, nanoparticle magnetic relaxation switches have been developed for in vivo sensing (Daniel et al. 2009). The sensor is covered by a semi-permeable membrane that allows the selective diffusion of cancer biomarkers or drug molecules into the surface of the sensor.

Further, biocompatibility issues have not been adequately addressed. Their role in device engineering is inevitably dual: to prevent foreign body reaction and sensor fouling. Many polymeric materials, such as polyallylamines, horseradish peroxidase, or polyethylene glycol derivatives, have been suggested as coating materials but proven unsuccessful (Norton et al. 2007). Wang et al. (2013) have recently proposed the use of hydrogels from poly(lactic-co-glycolic) acid microsphere dispersed in poly(vinyl alcohol); preliminary in vivo testing results were very promising but more research is required with different biosensor systems in order to evaluate its efficiency.

Notwithstanding, a very interesting field has been recently introduced: nanobiolectronics. In brief, nanomaterials are integrated with biology and electronics in order to overcome existing challenges in biosensors. The downsizing of electronic transducers affords them a more nature-relevant and biocompatible character that is expected to bring sensitivity to near-nature levels (Zhang and Lieber 2016). Nanobioelectronic devices are used to study neural circuits at the cellular and subcellular level. Nanowire-nanotube heterostructures can penetrate cell membranes for minimally invasive recordings; when coupled with phospholipid functionalization, these nano-probes can facilitate spontaneous membrane penetration and a tight membrane seal of high resistance (Duan and Lieber 2015). Intracellular sensing becomes possible, opening new avenues in cancer diagnostics.

5.5 Conclusions and Future Prospects

Clinical biosensors have undoubtedly much to offer in cancer diagnosis. Recent progress in the development of multiplexed platforms is promising, while the sensitivity and selectivity of nanosensors might prove quite advantageous for novel approaches in early diagnosis and therapy monitoring. Lab-on-chip platforms show a steady potential towards rapid commercialization of point-of-care and implantable systems. Nanomaterials, particularly quantum dots, can facilitate the tracking of cancer cells or drug molecules. Integrating nanomaterials and biosensors might

improve cancer imaging and drug delivery. Personalized health care systems might be a reality in the near future.

Biosensor technology presents the potential not only to serve the to-date cancer diagnostic strategy, but also to propose and support new, more efficient schemes. For example, cancer is usually expressed, at the molecular level, with a set of biomarkers; multiplexed platforms could be developed to provide reliable information for a wide dynamic range of many different biomarkers at ultra-low detectability. Further, the development of a diagnostic tool to inform on the borders of a tumor pre- or peri-operatively, could improve therapeutic success rates.

Notwithstanding, several issues need careful consideration when designing biosensor platforms. Despite progress in microfluidics, miniaturized transducers, and materials, the assembly of the biosensor components into a fully integrated device that could autonomously perform the analysis process has not been realized yet; possibly, the emerging nano-bioelectronics technology could support this goal. Also, as single-cell analysis is just started to post as requisite for early cancer diagnosis, nanoplatforms developed have not proved capabilities for detecting reliably just a limited number of biomolecules within a given cell. In addition, personalized medicine goes beyond disease diagnosis; more clinical information is required for a detailed molecular profiling, especially for the stage of tumorigenesis, the appropriate treatment regime, or in monitoring for disease recurrence. Thus, there exists the need for developing biosensors that could rapidly screen for DNA mutations and gene products.

Drug discovery and delivery may present another field where biosensing might prove beneficial and efficient. In vivo drug kinetics are affected by the properties of the active ingredient and how these properties are modified in vivo by transport, binding, or metabolism. This approach requires new strategies for reliably predicting drug delivery properties early in pharmaceutical development, so that the most efficient and suitable compounds move to clinical studies. This is especially true for the new therapeutic classes of gene-based drugs, although the proteomic information now available from gene expression data offers new prospects in both cancer management and biosensor development.

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