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REVIEW

Biosensors with label-free detection designed for diagnostic applications

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Abstract Since the first biosensor was introduced in 1962 by Clark and Lyons, there has been increasing demand for such analytical devices in diagnostic applications. Research initially focussed mainly on detector principles and recognition elements, whereas the packaging of the biosensors and the microfluidic integration has been discussed only more recently. However, to obtain a user-friendly and well-performing analytical device, those components have to be considered all together. This review outlines the requirements and the solutions suggested for the integration of suitable biosensors in packaging and the integration of those encapsulated biosensors into a microfluidic surrounding resulting in a complete and efficient analytical device for diagnostic applications. The components required for a complete biosensor instrument are described and the latest developments which meet the requirements for diagnostic applications, such as single-use components and arrays for multiparameter detection, are discussed. The current state and the future of biosensors in the field of clinical diagnostics are outlined, particularly on the basis of label-free assay formats and the detection of prominent biomarkers for cancer and autoimmune disorders.

Keywords Biosensors · Label-free · Single-use · Packaging · Microfluidics/microfabrication · Diagnostics

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Introduction

A biosensor is an integrated receptor–transducer device combining a biochemical recognition system (receptor) and a transducer (detector) which transforms the biochemical (biological) response into a measurable output signal [1]. The first biosensor generally accepted as meeting this definition was an amperometric biosensor for glucose monitoring called an "enzyme electrode" which was introduced in 1962 by Clark and Lyons [2]. Since then, biosensors have been developed for a multitude of applications, ranging from medical applications, including clinical diagnostics and monitoring [3] as well as drug discovery [4], to food analysis [5], environmental monitoring [6], and process control [7], to name but a few. However, the most common applications for biosensors are clinical applications, which are still the major driving force behind research into, and development of, biosensors [8, 9].

The necessity, as well as the possibility, to find means to objectively assess the state of health of a patient as well as increasing knowledge about patterns of biomarkers being characteristic for certain diseases (or the progress of diseases) have caused a major increase in the average number of tests performed on patients in recent years. Currently, testing for established biomarkers is typically performed at centralized laboratories using large automated clinical analyzers. This may leave the patient having to wait a considerably long time, sometimes under enormous psychological stress, for the outcome of the test [3]. In contrast, biosensors have the potential to provide rapid, real-time, and accurate results at the physician's office, in accident and emergency departments, or even at the patient's bedside [10]. A widely known example for the latter is the diabetic patients' self-monitoring of capillary

glucose. However, there is still demand for advanced and more complex biosensor devices, particularly in the field of multiparameter diagnostics [3, 10, 11].

This review considers the components required for a market-compatible biosensor system for clinical applications, focussing on systems with label-free detection. Selected applications are discussed to demonstrate the current needs of diagnostic systems. State-of-the art biosensor setups available in these applications are discussed as well as the chances and current limitations of future biosensor systems in this field.

Biosensor system components

General considerations

Considering the development of complete analytical systems based on biosensors, it is more than the biosensor itself which has to be taken into account to realize user-friendly, reliable, and market-compatible devices. Figure 1 shows an illustration of the main components required for a complete stand-alone biosensor system.

The biorecognition element (e.g., an antibody; for details see "Immobilization of biorecognition elements") is immobilized on the transducer surface (for details see "Transducers for label-free test formats"), the combination of both forming the actual biosensor component [1]. The biorecognition element determines the degree of selectivity or specificity of the biosensor, whereas the biosensor's sensitivity (here the slope of the calibration curve, implying the ability to detect low concentrations) is mainly influenced by the detector element, as it transforms the biochemical (biological) response into a quantifiable output signal [12].

A fundamental question which arises when considering biosensor systems for diagnostic applications is whether single-use or multiuse biosensors are to be preferred. A crucial aspect in performing measurements in any analytical task is the avoidance of carryover effects, so that the result obtained with one sample is not altered by the preceding one. The majority of biorecognition elements bind with high affinity to the corresponding analyte molecules. In consequence, a regeneration step resulting in the dissociation of the binding between the biorecognition element and the analyte is often necessary to regain the sensing capability of the biosensor. Regeneration procedures may be quite laborious, so savings provided by the use of multiuse sensors could easily be exceeded by additional expenses for cleaning and maintenance. Reusable devices might be more advantageous when working with bench instruments in centralized or clinical laboratories; however, when the development of diagnostic instruments in a physician's office or at the patient's bedside is considered, single-use disposable devices are typically preferred [3]. Needless to say, these considerations about single use or multiuse are valid not only for the biosensor component but are also valid for all system elements coming into contact with the sample, such as the biosensor housing and the microfluidic surrounding.

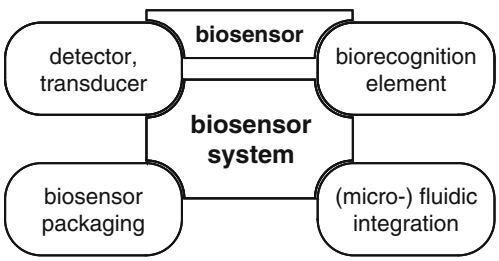

Fig. 1 Components to be considered in an efficient biosensor system

Developing operational stand-alone biosensor components is a task commonly performed within the scientific community and it is suitable for proof-of-principle evaluation or preliminary sensor characterization. However, this stage is not sufficient to fulfill the need for a biosensor system as requested once such a system is to be commercialized. With respect to system integration, the first step is a suitable type of biosensor packaging to protect the biosensor element from unwanted external influences and to ensure its electrical, optical, mechanical, and biological integrity [13]. Furthermore, the biosensor has to be interfaced with a suitable fluidic system to supply the biosensor with the desired analyte and at the same time to provide an easy interface for the user to interact with the biosensor [14]. In diagnostic applications an additional aspect to consider is that the sample volume usually is limited. Ideally, volumes of less than 1 μl are desirable to keep the patient's pain at a minimum during sampling [3].

In the following sections, the requirements for the single components will be discussed in detail as well as the latest developments and limitations.

Assay formats with and without labels

Depending on whether the output signal caused by analyte binding is obtained using labeled compounds or not, biosensors and biosensor test formats, respectively, can be classified into labeled and label-free types. The term "label-free detection (method)" is also used [15, 16].

Widely used labels involve enzymes, radionuclides, nanoparticles, and fluorescent or electrochemiluminescent probes. Available test formats are sandwich assays, competitive assays, and indirect assays (binding inhibition tests), which are described elsewhere [12, 15, 16]. So far, the main advantage of labeled test formats is the higher potential for the detection of lower concentrations; however, the need for labeled compounds usually implies higher operational costs, including longer assay times, compared

with most label-free test formats. Real-time monitoring of the analyte binding to the surface is not possible and, furthermore, the labels can interfere with the analyte binding, leading to distorted results.

Label-free test formats are performed without any labeled compound, and available test formats are direct assays and indirect assays (binding inhibition tests), which are described elsewhere [15, 17]. In the direct test format, the signal response changes at the moment when analyte molecules bind to the transducer surface, i.e., it is a one-step process without any other reagents. The simplicity and hence the inexpensiveness of this approach is the major advantage of this procedure. However, when working with complex sample matrices, such as serum, the simplicity of the direct test format might turn out to be a considerable disadvantage, because nonanalyte components in the sample matrix could also bind on the sensor surface, leading to false-positive results. With use of labeled test formats, unwanted effects resulting from the sample matrix are reduced, because the final biosensor signal response is usually determined by the labeled compound, whose binding behavior is largely independent of the matrix components. Hence, when using direct test formats with label-free detection, one has to ensure that only the analyte binding to the corresponding biomolecular recognition element immobilized on the biosensor surface will lead to a significant change in the biosensor signal response, not any unspecific interaction with the sample matrix. This can be achieved by suitable immobilization procedures; for details see "Immobilization of biorecognition elements." Still, with regard to rapid and inexpensive biosensor systems and testing procedures, label-free detection methods using direct test formats should be preferred, even though additional research and development work might be necessary to obtain the limit of detection needed for a specific application.

Transducers for label-free test formats

Label-free test formats take advantage of electrochemical, optical, acoustic, and, more rarely, thermal transduction mechanisms. Electrochemical biosensors make use of amperometric, potentiometric, or conductometric/impedimetric transduction principles [18]. Label-free optical detection methods are mainly based on refractometry or reflectometry [19]. The best-known acoustic biosensors are quartz crystal microbalances (QCM), surface acoustic wave biosensors, and cantilever biosensors [20]. Thermal transduction is based on the principles of calorimetry, which implies measurement of temperature changes [21]. So far, electrochemical transducers (including both labeled and label-free test formats) are the most widely used in sensor technology; however, the use of optical and acoustic biosensors is increasing. The reason for this is that up to now, only a few label-free electrochemical biosensors have been reported [12, 18]. Most of the electrochemical assays still require labels, such as enzymes or nanoparticles, leading to increased expense. Optical and acoustic transduction formats can be provided more easily for label-free test formats [22].

Besides the assay format and the detection limit, another criterion to be met when developing an application-oriented system for medical diagnostics is that the detector should be easily and inexpensively parallelizable as recent trends demand multiparameter diagnostic systems to determine patterns of biomarkers which are characteristic for diagnosis or prognosis of certain diseases, such as cancer and autoimmune disorders [3, 23, 24]. Promising and partly commercialized label-free multiparameter biosensor-based systems have been summarized previously [11].

Immobilization of biorecognition elements

To turn the detector element into a biosensor, it has to be coated with biorecognition elements. These are typically biomolecules, such as proteins (e.g., antibodies, enzymes), peptides, and oligonucleotides (e.g., DNA), or artificial receptors such as aptamers [25]. The coating procedures have to ensure the integrity of the transducers and of the biomolecules as well as the formation of stable and robust sensing layers. Moreover, in a commercialized biosensor system setup, a certain lifetime of the sensing layer has to be guaranteed. The immobilization of the biorecognition elements on the transducer surface is largely independent of the transduction mechanism; however, the immobilization chemistry strongly depends on the underlying device material and hence on the chemical environment available [26].

Transducer surfaces are typically metals (e.g., gold), oxidic layers (e.g., metal oxide or quartz/glass), or polymers. Direct adsorption of the biorecognition elements would be the easiest way; however, owing to unavoidable denaturation of the biomolecules and insufficient shielding against unspecific binding of sample matrix components to the substrate, this method is generally not recommended. Electrostatic binding of, e.g., oligonucleotides might not be stable enough for use in biosensors; hence, other immobilization protocols based on covalent coupling procedures are preferred [25].

As a first step, especially if the transducer surface is inorganic, which is the case for metals or oxidic layers, functional groups have to be introduced. In the case of metal layers, suitably substituted thiols forming self-assembled monolayers (SAM) are used as adhesion linking layers for further coupling. A chain length of more than ten carbon atoms is recommended to obtain well-defined and

stable SAM. Oligonucleotides with thiol substituents might be coupled directly to gold surfaces. On oxidic layers, SAM can be formed by treatment with silanes. Frequently used silanes are aminopropyltriethoxysilane and glycidyloxypropyltrimethoxysilane, providing amino groups and epoxy groups, respectively, for further coupling procedures [25]. If the transducer has polymer surfaces, these can be activated by oxidative treatment via wet etching or plasma treatment and processed directly [25] or silanized similarly to the oxidic layers [27] prior to the next modification step. Subsequently, biomolecules can be coupled directly on the transducer surface via these adhesion linking layers.

It was shown that sensing layers consisting of antibodies coupled on gold surfaces by means of 16-mercaptohexadecanoic acid could be used for analyte detection in serum samples, because unspecific binding was effectively reduced [28]. If gold surfaces are not at hand, it is often advantageous to couple the biorecognition elements via an additional hydrogel layer, because these layers effectively prevent unspecific binding on sensor surfaces. This aspect is particularly important for label-free biosensors in diagnostic applications where complex sample matrices, such as serum, have to be investigated (for details see "Assay formats with and without labels"). Furthermore, hydrogels enable mild reaction conditions so that biomolecules will retain their physiological structure and hence their functionality in the subsequent modification step [25, 29]. The most commonly used hydrogels featuring the above-mentioned properties are functionalized dextran [30], particularly carboxymethyl dextran [29], and poly(ethylene glycol) [31].

In the case of protein-based biorecognition elements, hydrogel layers bearing carboxyl groups are preferred, because proteins can be immobilized by amine coupling via reactive esters. For this, the carboxyl groups are activated by a carbodiimide, e.g., 1-ethyl-3-(3-dimethylaminopropyl) carbodiimide (EDC), and converted into an active ester intermediate, e.g., by reaction with *N*-hydroxysuccinimide (NHS), which can easily react with the amino groups of any protein. Owing to its flexibility, relative ease of use, high coupling yields, and robustness, the EDC/NHS coupling procedure is the most frequently employed immobilization method. Therefore, if hydrogel layers bear amino groups instead of carboxyl groups, it might be useful to convert the amino groups to carboxyl groups, e.g., by means of dicarboxylic acid anhydrides, prior to protein coupling to obtain a higher coupling yield [25, 32].

One drawback of the EDC/NHS coupling procedure is that a protein's amino groups are distributed all over the molecule; hence, the protein is coupled to the surface in a randomly oriented manner, which might result in blocking of the protein's binding sites. A way to overcome that problem is the affinity binding of the biorecognition molecules via corresponding capture molecules. This may be performed, e.g., by binding of antibodies via protein A or protein G or by binding of biorecognition elements functionalized with biotin groups via streptavidin [25]. However, these capture molecules might increase the degree of unspecific binding on the sensing layer. In addition, if the sensing layer is too thick, this might be disadvantageous for detection methods which imply a transduction mechanism near the surface, such as in evanescent field techniques [25] or surface acoustic wave biosensors [33]. Here, the further away from the sensing surface the binding effect occurs, the less it will be detectable by this kind of transducer.

Biosensor housings and packaging

Packaging requirements and development considerations

When designing a single-use, disposable biosensor component for medical applications, the housing design or packaging of the biosensor is of crucial importance. This is especially true if a sensor system is to be market-compatible. Proper device packaging is supposed to fulfill a number of requirements, such as mechanical protection of the mostly brittle and fragile sensor devices while still allowing the component to interact with its periphery in an adequate way. Therefore, the housing must provide a suitable means of microfluidic integration and interconnections to the surrounding fluidics. These are required for preexperimental treatment steps of the sensor surfaces such as the introduction of functional groups and the application of the biospecific sensing layer as well as for the application itself.

In biochemical or bioanalytical applications, the detection of one analyte at a time is mostly not sufficient. An important consideration when designing the sensor packaging is the question of whether or not the housing supports the creation of sensor arrays. As of today, numerous papers can be found within the scientific literature which discuss this question, but there are only a very limited number of papers reporting the actual implementation of such array-compatible sensor housings. Examples were developed by Blaess [34] and Rapp [35]. In both cases the authors described the integration of an array-compatible sensor housing for a surface acoustic wave biosensor [36]. The housing allows the integration of single detector elements which can subsequently be provided with a biospecific binding layer and assembled into flexible arrays afterward.

Even though designing a suitable sensor housing is a challenging task, it is seldom performed adequately [13]. This can be ascribed partially to the fact that the development of biosensor packaging is mostly considered at the end of the development chain. Within the scope of integrated scientific projects, funding is seldom accounted for and for the development of the sensor packaging, the scientific benefits

are accredited mostly to the development of the component itself. This makes it difficult to convince funding agencies to properly fund the project also at this stage [37]. A more detailed explanation was given by Hsu [38], who stated, among others, the lack of information concerning available materials, processes, and standards in industry when biosensor housings are designed within the scientific community. This problem is further aggravated by the fact that in high-risk markets, where investments in and payoffs from a single product can often decide the fate of a whole company, the will to share information about clever and robust packaging aspects is understandably not very high.

Production technology

Biosensors are not necessarily microelectromechanical systems (MEMS) or microoptoelectromechanical systems (MOEMS) by definition; however, they also tend to be delicate components that are to be protected and shielded from ambient influences in an adequate way. For the development for MEMS or MOEMS, packaging has been a critical issue since the first description of such systems. Still, for many years it was merely considered an evolutionary step from classical packaging concepts for integrated circuits (IC), and the requirements for MEMS or MOEMS packaging were estimated to be equivalent to those of classical IC packaging. Today, it is well understood that these two technologies may seem to have a lot in common but they are quite different when looked at in detail [37]. One very important aspect is the fact that IC are mostly packaged to be noninteractive with the environment; thus, IC packaging can be constructed to be robust and static, provided with mechanical and chemical sealing. In contrast, MEMS and MOEMS components and foremost sensors such as biosensors are designed to allow the impingement of an external stress, e.g., for pressure measurement [39], or the presence or absence of chemicals in the surroundings, the latter being the case for biosensors. This is why packaging for biosensors has to be designed in a way that it is only sealed partially. Of significant importance in this context are processes that can be applied on the wafer level, which would allow for significant cost reduction owing to the parallel processing aspect. Among others, wafer-level bonding [40] as well as wafer-level packaging [41] are techniques that are available today.

Depending on the targeted application and the range of allowed process cost, the choice of material and, therefore, the choice of production technology includes, among others, polymers, ceramics, and glass. In rare cases, metals, silicone, diamond [42], liquid crystal polymers [43], and even hydrogels may be considered a choice as well [44]. Among the most commonly used materials are polymers, which can be replicated cheaply via polymer replication. Today, polymer replication is a standard industrial process and is widely considered as the best choice for easy and cost-effective packaging solutions [45]. Among the most common processes for the production of housings are injection molding [46] and hot embossing [47].

Injection molding, which is the most widely available industrial production standard, is used if a short cycle time and thus a high production yield for polymer components is required. The main disadvantage of injection molding is the induced high internal stress in the molded component due to the short cooling time which does not allow relaxation of the material and thus the reduction of internal stresses [48]. This is problematic if the detector package is exposed to higher temperature during production or application, as this could lead to the deformation of the housing and thus the impingement of stress onto the detector.

As an alternative to injection molding, hot embossing allows the production of components with very low internal stresses owing to the extended cooling time [47]. This, however, raises the costs for the production owing to longer cycle times. Recently, large-scale hot embossing has been suggested as a means to reduce the cycle time per piece by significantly increasing the number of parts per replication [47, 49]. In addition to the classic hot embossing process, a process modification called “hot punching” has been described lately that allows the production of holes that penetrate the complete substrate height. This feature is usually not possible via classic hot embossing [50]. Subsequent to the polymer replication of the housings, the detector element itself is mounted, e.g., via flip chip [51] or adhesives [52].

(Micro-) fluidic integration

Microfluidics is usually considered to be the link between the sample, which is typically provided by the user, and the biosensor itself. Cass and Toumazou [14] pointed out that if this link is missing, a biosensor could never be commercialized.

Since the early 1990s, the scientific community has been developing microfluidic systems or systems incorporating microfluidic components. However, the first scientific contribution describing a complete analytical system on the microscale was published in 1979 by Terry et al. [53]. It described a fully integrated miniaturized gas analysis system on a silicone wafer. The paper went largely unnoticed, as it seemed to be way ahead of its time and was only credited with its true scientific value retrospectively. During the course of the 1990s, Manz et al. began developing the concept of “miniaturized total analysis systems,” i.e., systems that are targeted to a specific application replacing a laboratory setup or protocol by a small fully integrated biosensor chip [54].

In recent years, the scientific community has worked on adding active components, such as micropumps [55] or microvalves [56], to these mostly passive microfluidic biosensor chips. There are a countless number of microfluidic devices intended or targeted for biomedical applications that incorporate a large number of such active structures [57, 58]. However, the commercialization of such systems suffers from severe restrictions, mainly due to the fact that producing these systems on a large scale has proven to be difficult, mainly due to high costs and the high complexity of such systems. This has recently been discussed in a series of papers [59–61].

There have been other suggestions from the scientific community that try to overcome the problem of how to set up complex systems and still keep the complexity low. Very commonly used are monolithic systems based on polydimethylsiloxane (PDMS). These systems rely on a very simple and robust variation of a classic microvalve. Instead of using an actor which actively opens and closes a microfluidic channel, the soft PDMS channels are compressed by an externally applied pressure, e.g., via pressurized air [62]. The pressure compresses and seals off the channels locally, thus providing a switchable valve without any active structures. This allows the production of monolithic microfluidic systems with an arbitrary number of microfluidic valves (up to several tens of thousands of valves per chip), which resulted in the term "microfluidic large scale integration," first formulated by Quake and coworkers [63, 64]. The main drawback of this concept is the fact that there are restrictions on the use of PDMS owing to its chemical resistance and solvent resistance [65]. If fluids are to be used that dissolve PDMS, these monolithic systems cannot be used, which limits the range of commercial applications.

Recently, Rapp et al. [66] described another approach to overcome the question of how to separate a microfluidic system into disposable and reusable components. This can be performed by means of a so-called indirect microfluidic system, where the fluids in question are not handled by active microfluidic components such as microvalves and micropumps directly but rather via an intermediary liquid. This intermediary liquid is chosen to be nonmixable with the fluids to be provided in the microfluidic system. By this means, it is possible to transfer the function of a classic active microfluidic system to a passive microfluidic chip which is connected to a fluidic setup consisting of valves and pumps that do not necessarily need to be microscopic in scale. The switching of the liquids is performed in these external components via the intermediary liquid, which is exchanged against the liquids of interest in so-called fluid changers. The resulting microfluidic systems operate just as any microfluidic system but strictly divide the system into a disposable system part consisting only of cheap passive structures and a reusable system part consisting of active structures such as pumps and valves.

Some biosensor tests may not require continuous purging with a liquid. For such systems, setting up a complete microfluidic system can be considered overdone. The detector can also be supplied with a passive microfluidic system relying exclusively on capillary forces. These systems are commonly used in already commercialized home test systems such as pregnancy tests or blood glucose meters. By careful design and layout of the microfluidic channel arrangements, even complicated flow profiles and channel filling sequences can be implemented by capillary forces. Such layouts can be used to implement even valvelike functionality onto a chip [67]. Examples of using such systems with a combination of a PDMS and hot-embossed polymethylmethacrylate substrate have been shown [68, 69].

Biosensors used for diagnostic applications

Between centralized laboratories and patient self-testing

Diagnostic applications include detection of biomarkers, e.g., proteins, for diagnosis, therapy monitoring, and the prediction of disease progress. Owing to the growing field of biomarker identification, the number of clinical tests is rising. Currently, the diagnostic market is dominated by well-established clinical analyzers based on DNA or protein microarrays in centralized laboratories [70]. On the other hand, portable analytical instruments for patient self-testing and self-management are available, such as glucose meters for determining blood glucose levels (and hence enabling their adjustment) [71, 72] and instruments to monitor blood coagulation (and hence enabling the adjustment of the dosage of anticoagulants) [73]. Biosensors based on electrochemical detection using mediator enzymes have found their way into instruments for both types of applications. All these instruments use capillary forces to draw a drop of blood into the measurement chamber. One thing that all these biosensor-based instruments for patient self-testing have in common is that they determine a single analyte only, which is sufficient for the application for which they were designed.

However, there is a huge gap between the multianalyte high-throughput arrays used in centralized laboratories requiring trained staff and increased waiting time and the single-analyte devices delivering fast results being used by the patients themselves. User-friendly instruments for decentralized testing, e.g., at a physician's office, which are suitable for the rapid detection and quantification of several analytes without the need for specialized staff are still required. For this testing scheme outside the centralized

laboratories, i.e., near the patient, the term "point-of-care testing" (POCT) has been adopted. POCT devices are not necessarily restricted to biosensor instruments, but according to the definition (see "Introduction") these integrated receptor–transducer devices should not require additional processing steps or reagents between sampling and signal output and hence are most promising when it comes to quantitative, fast, and economic measurements [3, 10, 22, 23, 74, 75].

In recent years, a tendency toward the development of biosensor systems intended for clinical use could be observed within the scientific community [8]. However, in many cases only individual aspects of biosensor systems were thoroughly investigated, resulting in the development or adaptation of only single components of a complete system (see "Biosensor system components"). This was shown in detail by Rich and Myszka [76] using the example of commercial optical biosensors. Another reason for biosensors not being established in the diagnostic market yet is that marker profiles characterizing a disease or state of disease are also still under investigation. In the following sections, this will be discussed using the example of two applications, cancer and autoimmune disorders. We will show what is required in these specific cases, what has been achieved by now, and how biosensor instruments are to be developed to meet the requirements in the future.

Biosensors in cancer diagnostics

Worldwide, cancer is a major cause of death [77]; in developed countries, it is the second most common cause of mortality and morbidity [3]. There are more than 200 cancer-related diseases and it is crucial for a positive outcome for the patients that the cancer is detected and classified early, so that a specific treatment may be applied as soon as possible [22, 75, 78]. Given this background, an excess of molecular markers, both on the genomic and on the proteomic level, are being investigated to identify molecular patterns characteristic for a certain disease state [22, 75]. In addition to traditional methods based on histology and morphology, these biomarkers are regarded as a promising tool in the diagnosis and prognosis of the occurrence of cancer as well as a predictive tool for the success of a therapy. Up to now, only a few novel technologies for testing biomarkers have been transferred into the clinical area [77]. This will be discussed in the following on the basis of two prominent biomarkers: prostate-specific antigen (PSA) and human epidermal growth factor receptor 2 (HER-2/neu; also known as erbB2).

The single biomarker which is currently used as an individual element for screening purposes is PSA [79]; it is used for the detection of prostate cancer. Prostate cancer is the sixth most common cancer among men worldwide [77]. Critical values for PSA are determined to be above 4.0 ng/ml [80]. On one hand, this biomarker is strictly associated with one kind of cancer, namely, prostate cancer; however, it is actually not a cancer-specific marker. Other parameters can also increase PSA levels and the PSA concentration is not increased in every prostate cancer patient. Hence, the use of PSA levels in combination with other marker concentrations is discussed [22]. PSA levels are specific only after prostatectomy, because in this case no PSA should be present; hence, a detectable PSA concentration is a sign of disease recurrence [80]. Biosensors for PSA detection, including electrochemical, optical, and mass-sensitive detection methods, were recently reviewed [3, 22, 80] and are under development, demonstrating the need for this kind of POCT device. However, up to now these approaches are more in an experimental stage, i.e., development has been focused on signal transduction and surface functionalization, and less on complete instrumental setups. Therefore, biosensor-based POCT devices for PSA detection including both assay formats with and without labels have not yet been established, and currently the majority of PSA tests are still performed as immunoassays at centralized laboratories using large automated analysis [80], implying all the advantages and disadvantages depicted in the previous section.

HER-2/neu is mainly regarded as prognostic or as a therapy-predictive marker for breast cancer [81, 82]. Breast cancer is the most common cancer among women worldwide [77]. Critical values for HER-2/neu are determined to be above 15 ng/ml [83]. For the detection of HER-2/neu, an automated immunoassay is commercially available: Bayer Immuno 1 assay. It is based on a magnetic particle separation immunoassay combined with a colorimetric detection principle and features a linear detection range from 0.1 to 250 ng/ml. However, it needs labeled compounds and the assay time is more than 1 h [84]. Hence, a biosensor allowing label-free and direct detection of HER-2/neu with no or only a few preparation steps would be preferred. Martin et al. [85] introduced a biosensor based on surface plasmon resonance for direct and label-free detection of HER-2/neu. Surface acoustic wave biosensors allowing the integration in disposable, array-compatible housings [34, 35] are under consideration for detection of HER-2/neu [86]. Both approaches have not reached commercial status yet.

Usually, cancer diseases (or stages of) cannot be diagnosed using a single biomarker only; instead, a complete marker pattern ("signature") has to be determined [22, 23, 75]. According to Sadik et al. [87], for diagnostic purposes typically 30–100 sensors are required, e.g., 20–30 susceptibility genes plus positive and negative controls for breast cancer screening. Most efforts have been made in the field of electrochemical biosensors for

POCT cancer diagnostics as reviewed previously [78, 87], envisioning single-use electrode strips integrated in disposable cartridges, comparable to handheld instruments for glucose detection. However, those devices are still in the stage of research and development [78]. A recent work demonstrated the use of an antibody-functionalized silicon nanowire sensor array for multiplexed label-free real-time monitoring of cancer markers in undiluted serum samples [88]. One-dimensional nanostructures, such as semiconductor nanowires or conducting-polymer nanowires, are extremely attractive for designing high-density protein arrays. Because of their high surface-to-volume ratio and novel electron transport properties, their electronic conductance is strongly influenced by minor surface perturbations (e.g., binding of biomolecules). Hence, they seem to be very promising for label-free real-time protein detection. Electrode arrays having multiple individually addressable electrochemical transducers can be readily fabricated to meet the requirements of multianalyte protein arrays. But those arrays also require suitable and cost-effective packaging.

Biosensors in diagnostics of autoimmune diseases

Patients with autoimmune disorders have their immune response directed against their own cells and tissues. Up to now, more than 80 autoimmune diseases have been described [89]. These includes many rare diseases [90], but also common diseases, such as type 1 diabetes [91], rheumatoid arthritis [92], and multiple sclerosis [93]. All of them have in common that an early diagnosis is beneficial to allow early treatment and hence reduce the risk of severe symptoms. Therefore, finding disease-related molecular patterns for use in diagnostics to identify disease type, disease state, and response to therapy is currently being attempted [93–95].

In contrast to cancer diseases, where a great variety of DNA and protein markers exists, autoimmune diseases are mainly characterized by one group of biomolecules, the autoantibodies. These are high-affinity antibodies directed against self-proteins leading to cell and tissue damage [96]. The large size of antibodies (IgG, M_r 146,000; IgM, M_r 970,000 [97]) is beneficial for the detection of autoantibodies with label-free biosensor principles. Epitope specificity of autoantibodies and their connection to certain autoimmune diseases have been investigated widely [94, 98].

In rheumatoid arthritis patients, autoantibodies of various types are known, the most established being the so-called rheumatoid factor which is directed against IgG [95]. However, comparable to PSA occurrence in prostate cancer patients (see “Biosensors in cancer diagnostics”), it cannot be used as a single marker, because it is only present in 75% of all rheumatoid arthritis patients as well as being present in patients with other diseases, autoimmune or infectious, and even in healthy persons (3–5%) [92]. A better specificity for the occurrence of rheumatoid arthritis is provided by autoantibodies directed against citrulline amino acid moieties (i.e., anti-citrullinated protein antibodies, ACPA) [99]. They are frequently detected by means of immobilized cyclic citrullinated peptides in ELISA tests [96], revealing a specificity for rheumatoid arthritis between 95% and 99% [100].

For the detection of autoantibodies, several label-free biosensor test formats were reported. They used biosensors based on surface plasmon resonance [24, 92], QCM [101], or both [102]. Label-free detection of ACPA was also demonstrated using surface plasmon resonance microarray imaging, a method which allows multianalyte detection. In this study, the smallest detectable concentrations were reported to be slightly higher than those obtained with ELISA tests [96]. Another method was based on functionalized single-walled carbon nanotubes deposited on a QCM sensing crystal for the direct assay of ACPA in human serum [101]. The sensitivity (here the proportion of patients with rheumatoid arthritis who have positive ACPA test results) of this label-free biosensor was reported to be higher than that of the established ELISA; therefore, it might be promising to incorporate this type of biosensor as part of a complete biosensor instrument for diagnostic applications.

Conclusion

Since Clark and Lyons introduced the first biosensor for glucose monitoring in 1962, clinical applications have been the main focus of biosensor development. Even though biosensors have found their way into patient self-testing single-analyte POCT devices, such as glucose meters, they are far from being applied in centralized clinical laboratories, which are still dominated by clinical analyzers based on microarray methods. It is undeniable that there is growing need for rapid multianalyte detection systems suitable for decentralized testing in the physician’s office or maybe at the patient’s bedside and that biosensors, particularly those allowing label-free test formats, meet the requirements of such setups to a large extent. However, it is not only the biosensor itself but also the integration of the biosensor in an efficient, user-friendly instrumental surrounding which makes a powerful analytical device.

Plenty of research is being conducted regarding the single components of biosensor instruments, i.e., the biosensor (consisting of a transducer and a biorecognition element), the biosensor housing, and the microfluidics; however, only a few approaches have dealt with all aspects involved. Furthermore, biosensors have to be adapted to

specific applications. As the marker profiles specific for certain diseases (or disease states) are still under investigation, specifications regarding a suitable analytical (biosensor) instrument (or group of instruments) can hardly be given at the moment.

Hence, biosensor instruments will probably have to be designed more or less for specific applications requiring a market still large enough to be commercially attractive. Regarding multiparameter analyses, a loophole might be to reconsider the philosophy of the current packaging of biosensors, which usually involves integration of several biosensor elements in one housing. The integration of each biosensor element in a single, array-compatible housing would make the arrays more flexible and hence the application of the biosensor instrument more versatile. Such an instrument could be of interest to medical specialists medicating patients with chronic diseases. In this case, the number of biomarkers to be detected is more limited than in screening tests, and a flexible array could be adapted more easily to a patient or at least to a group of patients. Still, a lot of tasks remain, not only regarding the instrument (storage of biologically active components, accurate handling by nonspecialized staff, etc.), but also regarding acceptance by potential users.

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