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Polymer Based Biosensors for Pathogen Diagnostics

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1. Introduction

Over the past three decades researchers have witnessed an enormous amount of activity in the area of biosensors. The major processes involved in any biosensor system are analyte recognition, signal transduction, and readout. Due to their specificity, speed, portability, and low cost, biosensors offer exciting opportunities for numerous decentralized clinical applications – point of care systems.

The ongoing trend in biomedicine is to go smaller. For almost a decade, the buzz word has been nano, and the analytical micro devices are now appearing in the clinic. The progress within microfluidic technologies has enabled miniaturization of biomedical systems and biosensors. The down-scaling has several advantages: refined control of fluidics, low sample consumption, applicability to point of care, and low cost.

Point of care is an emerging field within medical diagnostics and disease monitoring, and eventually disease control. Employing specially designed micro systems, a patient can be monitored continuously at bed side, and save precious time on commuting between home, doctor and hospital. The technological advancements in the biosensor technology within recent years have accelerated the R&D in point of care devices.

Cost benefit is always an important factor in development of novel medical devices. To reduce the expenses of biosensors, the use and cleanroom processing of noble metals should be kept at a minimum. Therefore, we predict a shift in the usage of gold and platinum to degradable polymer materials.

This chapter will look further into the advantages and applications of all-polymer microfluidic devices for biomedical diagnostics and compare with traditional systems. In many biosensor applications, only one analyte is of interest, and preferentially it should be isolated from an inhomogeneous patient sample. Section 2 provides the reader with an overview of the different novel microfluidic separation techniques in polymeric devices. Conductive polymers are the focus of section 3. They have many excellent properties and in fact, they can compete with gold in many applications. The focus of section 4 is sensitivity and specificity of biosensors. High sensitivity and specificity is crucial and can be achieved by functionalization with different molecules. The section will primarily center around the use of aptamers which is favourable above antibodies. Different detection methods are applied in biosensors, some of the promising techniques will be summarized in section 5. Finally, section 6 gives an overview of the current status in biosensor development while focusing on ongoing research.

2. Novel microfluidic separation techniques for sample preparation

The progress in microfabrication and lab-on-a-chip technologies is a major field for development of new approaches to bioanalytics and cell biology. Microfluidics has proven successful for cell and particle handling, and the interest in microdevices for separation of particles or cells has increased significantly (Giddings (1993); Nolan & Sklar (1998); Toner & Irimia (2005)).

Biological samples comprise a heterogeneous population of cells or particles, which is inconvenient for many biomedical applications, where the objective of study is often just one species. For example, the isolation of CD4+ T-lymphocytes from whole blood is essential to diagnose human immunodeficiency virus (HIV) (Kuntaegowdanahalli et al. (2009)), the isolation of leukocytes is important in drug screening assays, and the isolation of specific micro particles from blood plasma is critical for our understanding of inflammatory diseases. Thus, separation of cells or particles has a wide range of applications within different areas of medicine such as diagnostics, therapeutics, drug discovery, and personalized medicine (Gossett et al. (2010)).

Flow cytometry has remained the preferred method for cell sorting by many biologists because the technique is well established and has both high sensitivity and high throughput. Recently, fluorescence based sorting of cells and particles has also been implemented in microfluidic devices.

The microfluidic separation techniques are broadly classified as being either passive or active, depending on the operating principles (Table 1). Active separation of particles requires an external force (i.e. electrical power, mechanical pressure or magnetic force), whereas passive separation techniques rely on channel geometry and inherent hydrodynamic forces for functionality (i.e. pillars, pressure field gradient or hydrodynamic force). The following section will introduce a couple of novel separation principles with application in biomedical sensors. For further reading on continuous separation of particles, see review papers by Lenshof and Laurell (2010), Gossett et al. (2010), and Bhagat et al. (2010).

	Method	**Mechanism**
Active	Acoustophoresis	Acoustic waves
	Optical tweezers	Optical
	Dielectrophoresis	Electric field
Passive	Obstacles	Laminar flow
	Induced lift	Inertial force

Table 1. Active and passive separation technique with application in biomedical sensors

2.1 Active separation techniques

2.1.1 Acoustophoresis

Acoustophoresis is the separation of particles using high intensity sound waves. In a microfluidic system, particles with an induced acoustic standing wave will experience a force towards a node or anti node dependent on their physical properties (Lenshof & Laurell

(2010)). If two particles suspended in a fluid have opposite acoustic contrast, a separation will occur gathering one at node and the other at anti-node. Generally, rigid particles will have negative phase and move toward the node, whereas air bubbles and lipid vesicles gather at the anti-node (Lenshof & Laurell (2010)). After separation, the properties of the laminar flow in the microfluidic channel ensure that particles remain at their position in the channel, hence they can be collected separately with a flow splitter.

Both particles with opposite and similar acoustic contrast can be separated using this technique. The size of particles will influence the time scale. Large particles experience a higher force than small than the smaller ones, and thus gather at the node faster than the small particles. Peterson et al. (2007) described a microfluidic system with three inlets (Fig. 1), where a sample composed of different sized particles was introduced at the sides of a microfluidic channel with a sheath fluid in the middle to keep particles in close proximity to channel walls. The system is designed such that an ultrasonic transducer induces a force on the particles, which forces them towards the middle of the channel. Since the larger particles experience a higher force than small particles, they large particles immediately gather at the center of the channel. Particles are thus allocated proportional to their size. Making use of a flow splitter, particles are separated according to their size. Applying this technique, Peterson et al. (2007) demonstrated separation of a mixture of different sized particles.

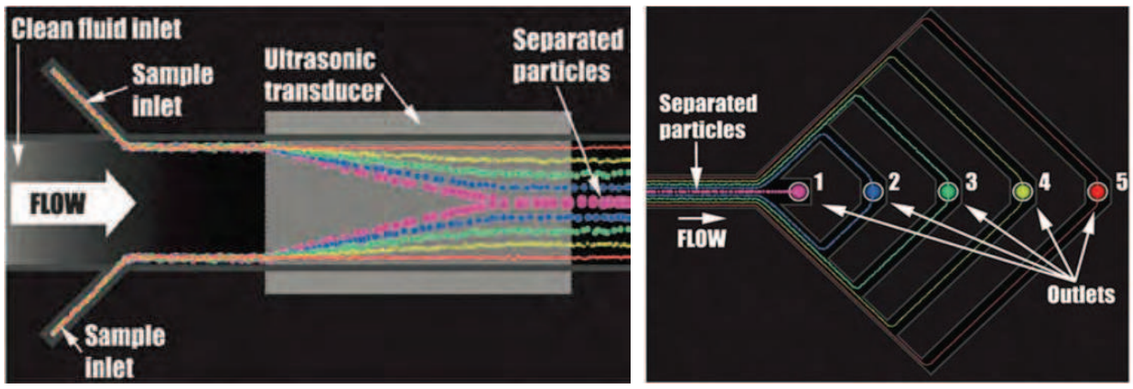


Fig. 1. Acoustophoresis. (a) Particles entering main channel from two side inlets. Particles are positioned near channel walls because clean sheat fluid is introduced at a third inlet. The flow of particles is controlled by the acoustic waves, which are introduced by an ultrasonic transducer. After this point, the particles distribute proportional to size. (b) Flow splitters are used for separation of different sized particles. Nine fractions of the flow can be gathered at five outlets (Adapted from Peterson et al. (2007)).

2.1.2 Ion depletion

Ion depletion is a microfluidic technique for separation and concentration of proteins. As the name indicates, the method is based on ion transfer in a nanofluidic channel (approximately 50 nm in depth). Counter-ions will migrate from the Debye layer through the nanochannel to a higher extent than co-ions, so that a net transfer of counter-ions is transferred from the anodic side to the cathodic side. Thus, the concentration of counter-ions decreases on the anodic side and an increase is achieved on the cathodic side. If a protein in solution is part of the co-ion population, this protein will be trapped in a plug on either side of the ion depletion region, and is hence separated from the bulk solution. The principle of ion depletion is illustrated on Fig. 2 and 3 (Wang et al. (2005)).

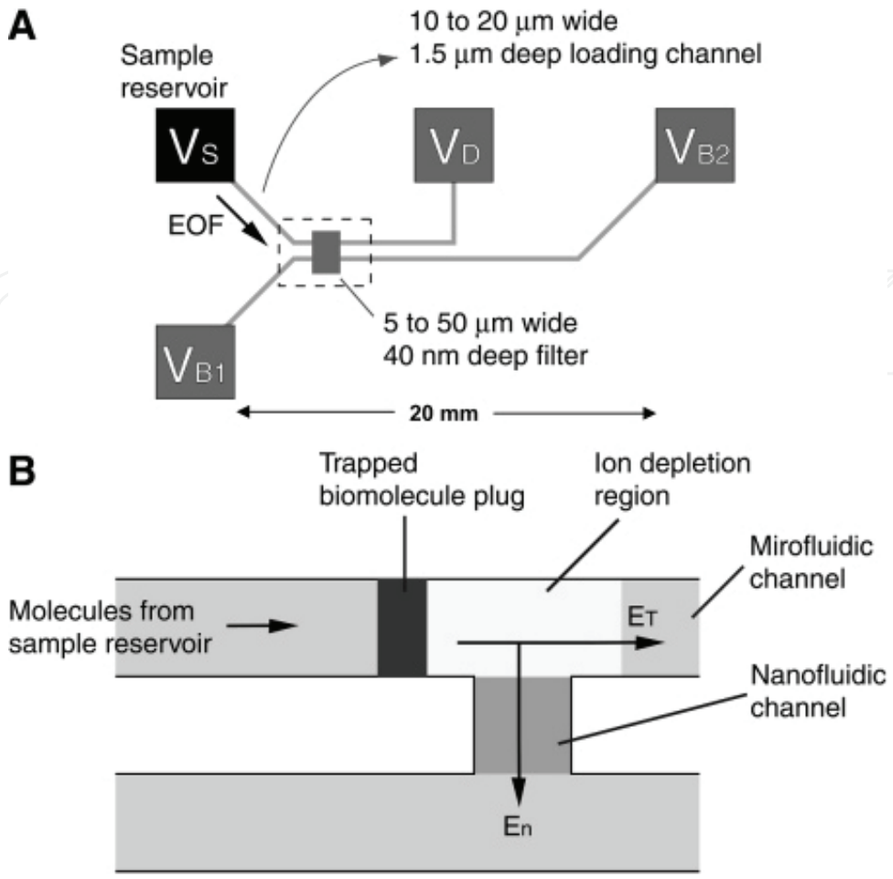


Fig. 2. Nanofluidic protein concentrating device by ion depletion: (A) Layout of the device. (B) Schematic diagram showing the concentration mechanism. Once proper voltages are applied, the trapping region and depletion region will be formed as indicated. The ET specifies the electrical field applied across the ion depletion region, while the En specifies the cross nanofilter electrical field (Adapted from Wang et al., (2005)).

2.2 Passive separation techniques

2.2.1 Obstacles

Obstacles arranged in microfluidic channels are commonly applied for preventing particles from entering certain areas or used to manipulate the flow of fluid in a microchannel. Deterministic lateral displacement is a method for size separation of particles or cells, accomplished by placing posts asymmetrically in a microchannel (Fig. 4) and thus forcing particles of different sizes to follow different flow paths.

2.2.2 Spiral microchannels

Separation of particles in a spiral microchannel was described by Kuntaegowdanahalli and colleagues (2009) (see Fig. 5).

It is a passive separation technique based on the centrifugal force. Centrifugal based techniques have been demonstrated using flows in curvilinear microchannels (Gregoratto et al. (2007); Seo et al. (2007)). In general, the flow of fluid through a curvilinear channel experiences a centrifugal acceleration, directed radially outward. The channel geometry

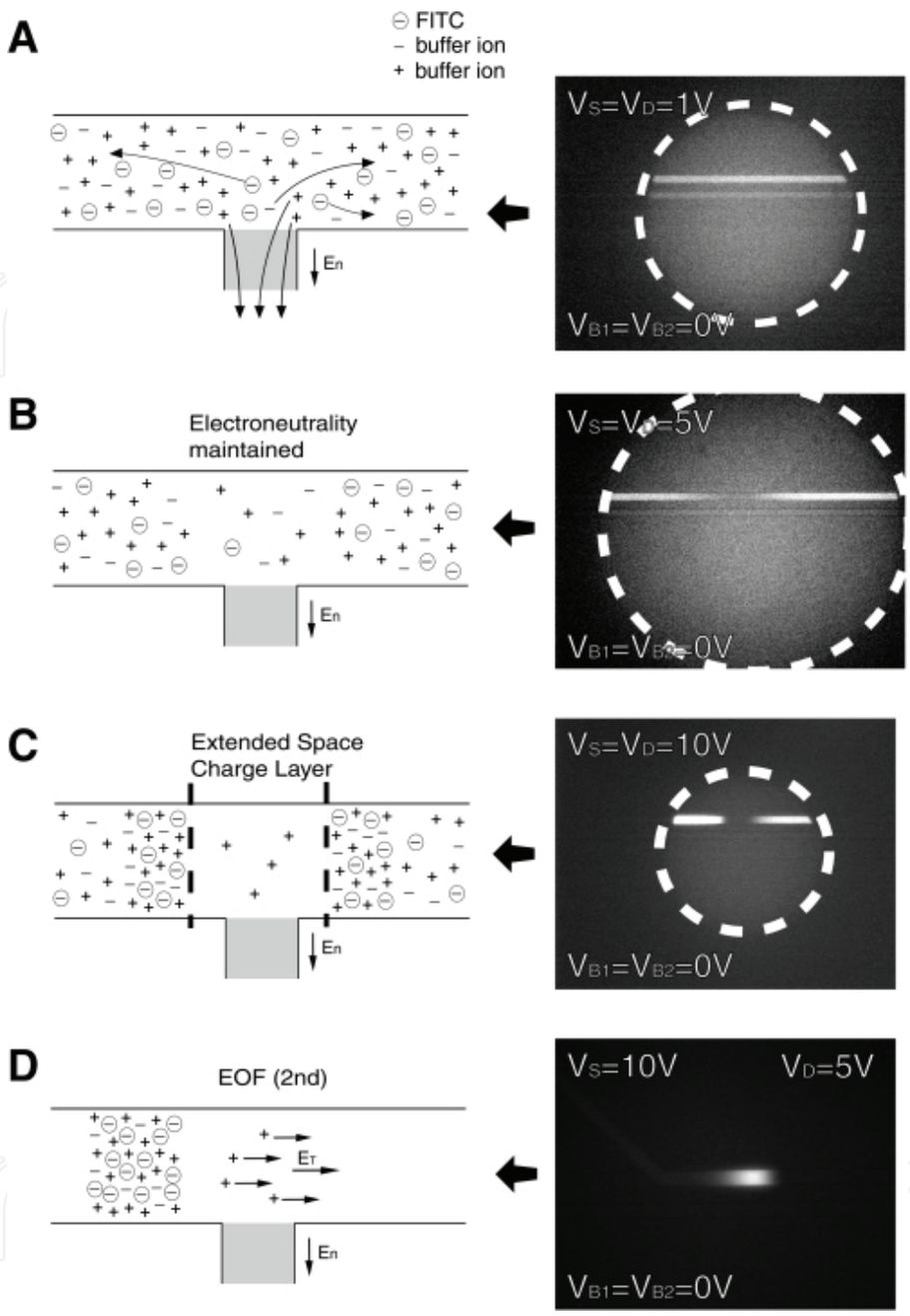


Fig. 3. Mechanism of preconcentration in the nanofilter device (A) No concentration polarization is observed when a small electrical field (En) is applied across the nanofilter. (B) As the En increases, the transport of ions becomes diffusion-limited and generates the ion depletion zone. However, the region maintains its electroneutrality. (C) Once a strong field (En) is applied, the nanochannel will develop an induced space charge layer, where electroneutrality is no longer maintained. (D) By applying an additional field (ET) along the microfluidic channel in the anodic side (from VS to VD), a nonlinear electrokinetic flow (called electroosmosis of the second kind) is induced, which results in fast accumulation of biomolecules in front of the induced space charge layer. (Adapted from Wang et al. (2005))

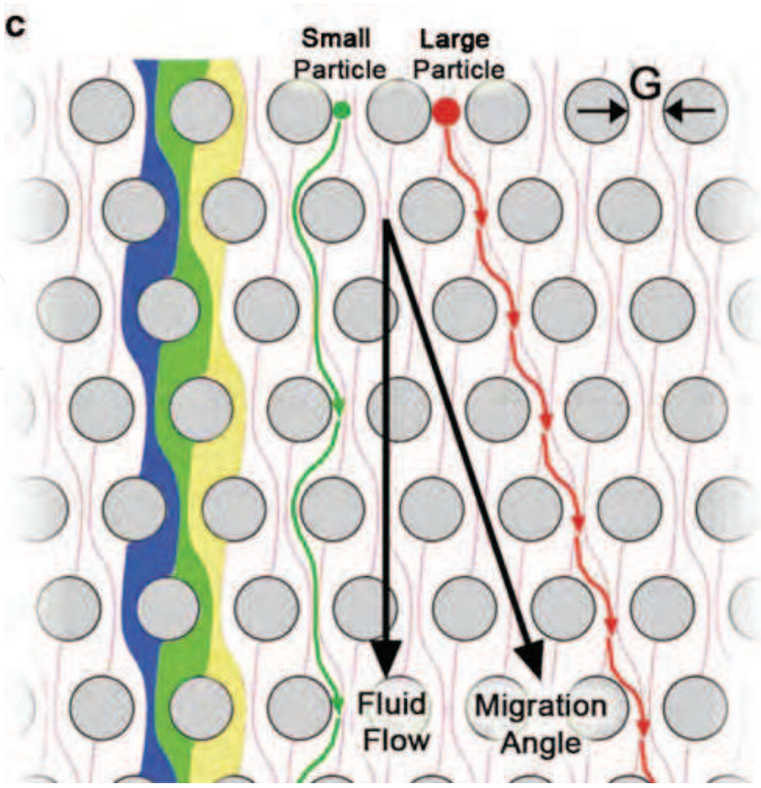
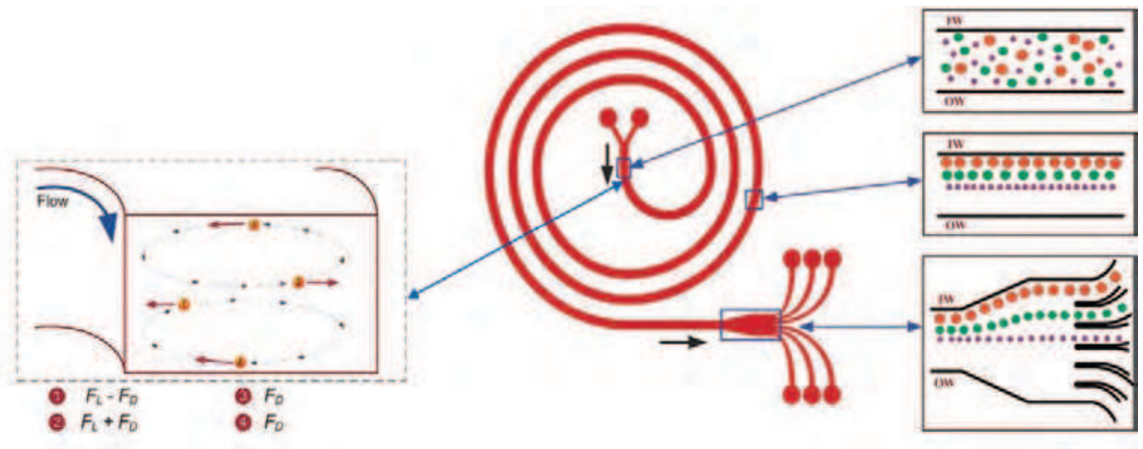


Fig. 4. Deterministic lateral displacement (Adapted from Gossett et al., (2010)).



(a) (b) (c)

Fig. 5. Spiral microschannels. (a) Neutral buoyant particles suspended in a medium in a spiral shaped channel experience forces and drag. Resultantly, particles redistribute within the microchannel. (b) Schematic representation of spiral channel for particle separation. (c) Different sized particles equilibrate at different positions in microchannel, and are collected at different outlets. (Adapted from Kuntaegowdanahalli et al. (2009) and Bhagat et al. (2008)).

gives rise to vortices, which are exploited for separation of different sized particles. Particles in the center of the channel will experience a drag away from the center, whereas particles in the proximity of the channel walls experience repulsion from the walls. Consequently, particles align at four equilibrium positions in the channel and different sized particles can thus be collected at different outlets (Bhagat et al. (2008); Di Carlo et al. (2007)).

3. Conductive polymers for sensing

Modern biosensors for medical diagnostics must be specific, quick, and producible at reasonable cost. A major cost factor is the electrode material - often a noble metal - demanding extensive production steps in cleanroom facilities. To cut down on these expenses there is a trend to utilize conductive polymers for sensing. This section will give an introduction to advantages of conductive polymers compared to noble metals, and guide through the considerations associated with selecting an appropriate polymer material for biosensor applications.

3.1 Polymers or metals?

The application of polymers as supporting materials in microfluidic systems is well established, however the electronic sensing units in most chips are fabricated from metallic conductors such as platinum or gold.

Biocompatibility, high sensitivity and specificity are a demand in modern medical biosensors. Biocompatibility is required because some biological applications involve living cells, bacteria or virus. High specificity and sensitivity is essential for detecting highly diluted analytes in biological samples, because the samples contain a cocktail of similar components, which can influence a measurement. All of these requirements can be fulfilled by the metal electrode materials such as solid platinum or gold (Prodromidis & Karayannis, 2002). Though, a major disadvantage of the noble metals is the high cost, which is continuously increasing.

Conjugated polymers are an alternative to the traditional electrode materials. The electronic structure of these compounds gives them properties similar to inorganic semiconductors. In 1977, Shirakawa et al. discovered that doping polyacetylene with halogens increased the conductivity by up to four orders of magnitude. The following research on this topic by Shirakawa, MacDiarmid and Heeger was awarded with the Nobel prize in chemistry in 2000.

Over the years, electronically conductive polymers have been proposed for many applications (Jagur-Grodzinski, 2002; Olson et al., 2010) - from biomedical sensors to nanowire integration in photovoltaic cells or printable RFID antennae - yet only few have made it to the market. Among those are electrochromic coatings for windows, antistatic coatings, organic light emitting diodes (OLEDs), corrosion protection for metals or surface finish for printed circuit boards (Groenendaal et al., 2000; Gustafsson et al., 1994; Wessling, 2001).

The usage of conductive polymer electrode in biosensor application is rising. The immediate advantage of conductive polymer electrodes is the much lower cost of the raw materials and the inexpensive production steps. Certain polymers offer high biocompatibility and options for modifying the properties by varying side groups. This can be useful for probe immobilization, which is a crucial procedure in biosensors. Conductive polymers allow a

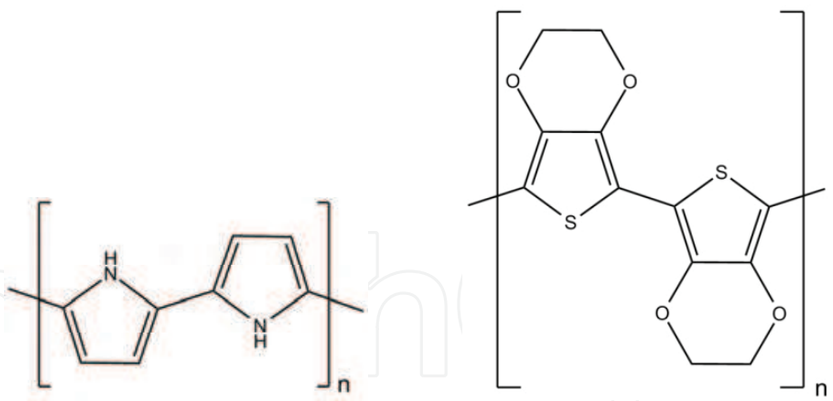
broad range of chemical modifications for covalent attachment of enzymes, antibodies, DNA or other bioprobes (Sarma et al., 2009; Teles & Fonseca, 2008).
In summary, replacing metals with polymers as electrode material does not only limit the cost on the materials themselves, but also allows for the inexpensive mass production by modern ink-jet printing methods (Loffredo et al., 2009; Mabrook et al., 2006) or agarose stamping (Hansen et al., 2007).

3.2 Polymer selection

As mentioned in section 3.1, biocompatibility is a very important factor in selecting an appropriate polymer. Biocompatibility is mainly influenced by the intrinsic toxicity of a material but also by hydrophilicity. Many conjugated polymers suffer from degradation because of irreversible oxidation processes, or they lose their conductive properties over time. A constant and reliable signal is crucial for sensor devices, and accordingly the polymer should be stable over a certain period of time.
In order to provide a good signal to noise ratio in electrochemical measurements, a low ohmic resistance (i. e. high conductivity) is preferred. Currently, these requirements are met by few polymers on the market.

3.2.1 Polypyrrole

The physical properties of Polypyrrole (PPy, figure 6(a)) makes it suitable for biosensor applications. PPy has high decomposition temperature (180–237 °C), glass transition temperature (Tg , 160–170 °C), and relatively high conductivity of up to 3 S·cm−1 (Biswas & Roy (1994)). Besides, PPy has a good environmental stability and different facile processing methods (Wang et al. (2001)).



(a) Ppy (b) PEDOT

Fig. 6. Monomer units of (a) polypyrrole (PPy) and (b) poly(3,4-ethylenedioxythiophene) (PEDOT).

In 2005, Dubois et al. developed a PPy based biosensor for label-free detection of peanut agglutinin. The lactosyl probe unit was immobilized on a biotinylated PPy film via avidin bridges. Their findings demonstrated that the bioprobe could be immobilized directly on the functionalized electrode surface, facilitating label-free detection by electrochemical methods. There are different strategies to functionalize the electrode surface, and another approach was described by Campbell et al. (1999). They incorporated human erythrocytes into the

PPy matrix, and upon capture and binding of Anti-Rhesus (D) antibody, a resistance change could be detected. Other techniques will be discussed in section 4.

3.2.2 PEDOT

Improved properties compared to PPy were found for poly(3,4-ethylenedioxythiophene) or PEDOT. It is either chemically or electrically polymerized from the commercially available monomer 3,4-ethylenedioxythiophene. As can be seen in figure 6(b), it has some structural similarities with PPy. PEDOT has exceptional high conductivity (up to 600 S·cm−1), high environmental stability and is biocompatible and transparent for visible light. The most common dopants used for PEDOT are poly(styrene sulfonate) (PSS) and tosylate (Rozlosnik (2009)).

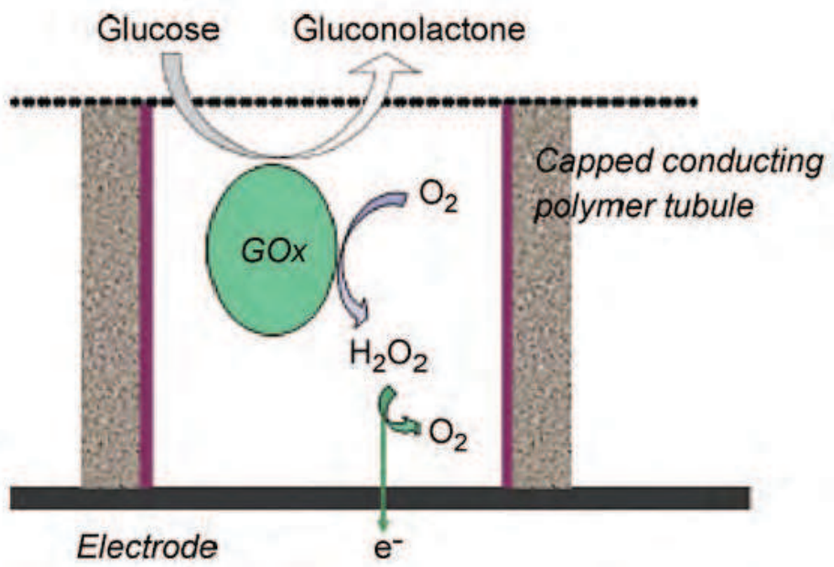


Fig. 7. Glucose oxidase is imprisoned inside a PEDOT microtube covered with a non-conductive polymer (Park et al., 2008).

The work by Balamurugan & Chen (2007) and Vasantha & Chen (2006) show the high potential and superior qualities of PEDOT, and this conductive polymer has been employed in a number of biosensor microdevices.

An interesting study was presented by Kumar et al. (2006). A biosensor was developed to determine the concentration of the important mammalian neurotransmitter, dopamine via an electrochemical process. Since the concentration of ascorbic acid is around a thousand times higher than dopamine in a biological sample, and the two analytes have similar electrochemical potentials, the challenge was to measure the concentration of dopamine in presence of ascorbic acid. Kumar et al. (2006) employed glassy carbon electrodes coated with PEDOT, and their findings demonstrated significant peak separation and improved anti-fouling properties compared to the more common electrode material glassy carbon, making PEDOT a good candidate for further applications in this field.

Glucose detection for blood sugar monitoring of diabetes patients is a huge and growing market for disposable biosensors. The established commercial systems make use of metal

electrodes (typically Pt) coated with a gel containing the enzyme glucose oxidase, and the effectively measured agent is thus the oxidation product, hydrogen peroxide (H_2O_2). In contrast to the direct oxidation of dopamine on the electrodes in the example above, this indirect detection of glucose is more complicated. Considering the current market price of platinum of about 41 €/g (http://platinumprice.org), replacing the electrode material with a low cost polymer such as PEDOT seems sensible. Park et al. (2008) imprisoned glucose oxidase in hollow PEDOT micro-tubules on an indium-tin-oxide (ITO) glass surface (figure 7). In this configuration, the enzymes are surrounded by the electrode, and therefore their activity is not constrained by immobilization on a surface or incorporation into a polymer. Although the performance of this biosensor cannot meet the requirements of a classic system, it can be refined by increasing the enzyme density or improving the conductivity.

Many biosensors for pathogen detection are based on antibodies as probes, and deliver an indirect signal. These immunosensors require a fluorescently tagged second antibody, which reacts with occupied immobilized antibodies in a so-called sandwich assay.

A different approach was tested by Kim et al. (2010), who worked on the development of a point of care system for prostate specific antigen/α1-antichymotropsin (PSA-ACT) complex detection. This cancer marker is associated with prostate tumors and important for preoperative diagnosis and screening. Instead of using the conventional optical methods, they constructed an organic electrochemical transistor (OECT) based on PEDOT. The antigen was captured by immobilized antibodies on the conductive polymer. For signal enhancement, a secondary antibody with a covalently tethered gold nanoparticle was used. The system provided a detection limit as low as 1 pg·mL−1 and is thus sensitive enough for reliable PSA-ACT analysis.

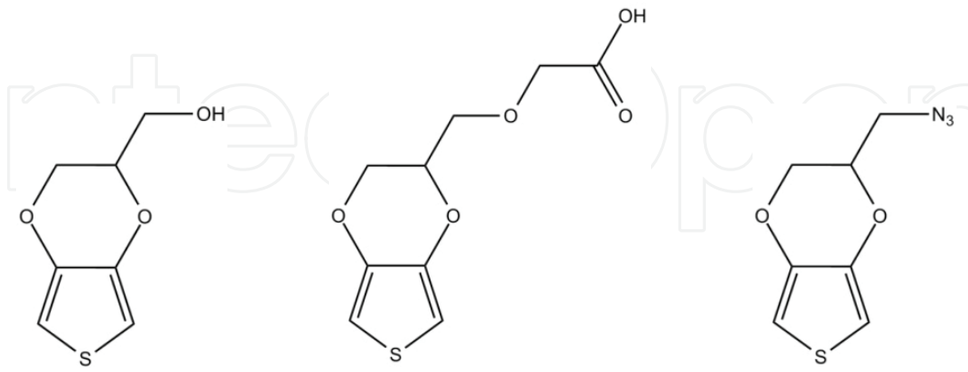
3.2.3 PEDOT derivatives

A field effect transistor (FET) based biosensor was demonstrated by Xie et al. (2009). The working principle is fundamentally different, considering it uses conductive polymer nanowires, which were electropolymerized between two gold electrodes. For minimizing the distance between polymer and binding event it was necessary to couple the probe (an aptamer, see also section 4.3) directly to the electrode material. Normal PEDOT offers no possibility for covalent bonding of other molecules, so a derivative bearing a carboxylic acid group was used. With this functional group the oligonucleotide for thrombin detection was attached with a simple 1-ethyl-3-(3-dimethylaminopropyl)carbodiimide/N-hydroxysuccinimide procedure (see section 3.3, (EDC/NHS)). Thrombin binds specifically to aptamers and becomes immobilized on the surface. The positively charged protein influences the transistor, so that the current flow changes. This type of biosensor has a broad dynamic range covering the physiologically interesting thrombin concentration range from a few nanomoles to several hundred nanomoles.

Other PEDOT derivatives have also been investigated (Akoudad & Roncali, 2000; Ali et al., 2007; Daugaard et al., 2008). The structural formulas of the most commonly used monomers are shown in figure 8; PEDOT-OH is more hydrophilic than normal PEDOT, and the azide modified PEDOT-N3 polymerizes slowly and has decreased conductivity. The only commercially available monomer is (2,3-dihydrothieno[3,4-b][1,4]dioxin-2-yl)methanol (commonly known as hydroxymethyl-EDOT or EDOT-OH) (8(a)), and it can be used as a basis for further modifications.

3.3 Coupling methods

There are different techniques for immobilization of biomolecules (e.g. DNA) on an electrode surface. The most popular methods are formation of a biotin-streptavidin complex, formation of different covalent bonds like esters or amides, or click chemistry.



(a) EDOT-OH (b) EDOT-COOH (c) EDOT-N3

Fig. 8. Different derivatives of 3,4-ethylenedioxythiophene (Ali et al., 2007; Daugaard et al., 2008).

3.3.1 Biotin-streptavidin complex

Streptavidin is a protein consisting of four identical subunits, each of which has an extremely high affinity for biotin. A biotinylated surface can be coated with streptavidin so it offers reactive sites for fixation of likewise biotin tagged (bio)molecules. The biotin-streptavidin interaction is one of the strongest non-covalent bonds in nature and it is very specific. Moreover, the system is easy to handle and very biocompatible.

Despite the many advantages of streptavidin, a major drawback is the instability at low or high pH values, and high temperature. For some detection methods the rather thick protein layer between electrode and probe can substantially decrease the sensitivity of the sensor.

3.3.2 Covalent bonding

Different activation methods have been used for a long time in chemistry, which requires the availability of certain functional groups on the surface. The activation of a carboxylic acid group with 1-ethyl-3-(3-dimethylaminopropyl)carbodiimide (EDC) and N-hydroxysuccinimide (NHS) is often applied for amide-bond formation under mild conditions and can be used for binding molecules bearing free amino groups (Balamurugan et al., 2008; Xie et al., 2009).

For hydroxyl functionalized polymers and target molecules, a technique from DNA synthesis can be employed. The alcohol groups are activated with phosphoramidites to form a phosphoester, which then reacts with another hydroxyl moiety and links the target molecules covalently to the surface (Pirrung, 2002).

3.3.3 Click chemistry

A very elegant approach for probe immobilisation is the usage of so called "click-chemistry". In the Cu-catalysed Huisgen-type 1,3-cycloaddition suggested by Daugaard et

al. (2008) an azide reacts in high yield with an alkyne to form a five-membered heterocycle. This bond is very stable and also the precursors have advantages such as stability toward hydrolysis and dimerization or ease of introduction (Kolb et al. 2001).
However, the azide functionalization of PEDOT downgraded its conductive properties significantly and remaining Cu catalyst could influence biological systems.

4. Electrode functionalization

Functionalization of electrodes is essential for achieving high sensitivity and specificity of electrochemical biosensors. This section provides an overview of the current trend in electrochemical sensors for medical diagnostics.

4.1 Recognition of pathogens

Point of care diagnostic devices present a viable option for rapid and sensitive detection and analysis of pathogens. Biosensors can play an important role in the early diagnosis of acute viral disease and confine the spread of virulent disease outbreaks. Biosensors can also play an important role in early detection and diagnosis of cancer and autoimmune disorders based on specific biochemical markers.
As discussed in section 2, separation and isolation of large quantities of a specific analyte would be preferable for many medical applications.
Patient samples comprise of a heterogeneous population of particles and cells, hence challenging the isolation of a single species in a high background concentration. For this reason, biosensors must be very specific and sensitive, allowing precise detection of very small quantities.

4.2 Antibodies

Many techniques for preparing functional biological surfaces for studies of cells, viruses or disease markers have been described in the literature. Refer to 3.3 for an overview of different coupling methods.
Immunoglobulins (IgG) are large Y-shaped proteins produced by the immune system, and are most abundant in blood plasma. Two identical antigen binding sites are formed from several loops of the polypeptide chain. These loops allow many chemical groups to close in on a ligand and link to it with many weak (non-covalent) reversible bonds. An antibody-antigen bond is highly specific because of the molecular structure of the protein.
Antibodies are the most common recognition molecule in biosensors. It is a naturally occuring protein and can only be produced in a host against immunogenic substances, giving rise to batch variation and a limited target range. For research purposes, monoclonal or polyclonal antibodies can be applied as recognition molecule. Typically, monoclonal antibodies will ensure a higher specificity than polyclonal antibodies.
In medical sensor applications, functional orientation of the antibodies on the surface is crucial to ensure high sensitivity and specificity. It can be achieved by immobilizing the proteins on a supporting layer of protein A.

4.3 Aptamers

For many years, antibodies have been applied for surface functionalization in biosensors, ensuring specificity and sensitivity of sensors. Artificial nucleic acid ligands - known as aptamers - can cover the same field of application as antibodies. In the recent years, the use

of aptamers has increased (Han et al. (2010); Syed & Pervaiz (2010)), and they are in many ways superior to antibodies, as it will be disscused in this section.

4.3.1 The properties of aptamers

Aptamers are oligonucleotides with a typical length of forty to eighty basepairs, and were discovered in the 1980's as naturally occurring regulation elements in prokaryotic cells, and they showed high affinity for viral and cellular proteins.
In 1990, Tuerk & Gold developed a convenient process for in vitro aptamer production, the so-called systematic evolution of ligands by exponential enrichment (SELEX, see in section 4.3.2).
Aptamers are in many regards better than antibodies as summarized in table 2. The affinity for the target molecules of aptamers is similar to antibodies, and in some cases even higher compared to antibodies. The specificity is also higher for aptamers, as they can distinguish between targets of the same family, like it was shown for the molecules caffeine and theophylline (Zimmermann et al., 2000). Selection and production of the nucleic acid ligands can be done in vitro, and once the correct sequence has been determined, the oligonucleotide can be synthesized in an automated chemical procedure. The range for possible target molecules is very wide and - in comparison with the mentioned biomolecules - comprises all kinds of smaller ions, organic compounds and even whole cells. Contrary to antibodies, aptamers can be selected against toxic compounds.
Due to the chemical synthesis, there is no significant batch variation and it allows for easy chemical modification, like attachment of certain end groups for surface immobilization.
Reversible thermal denaturation makes aptamers potentially recyclable and their very high stability promises long self life (Lee et al., 2008).

4.3.2 The SELEX process

Aptamer production is accomplished in the SELEX (Systematic Evolution of Ligands by Exponential Enrichment) process (see figure 9). A pool of single stranded oligonucleotides with a random section of about 25 to 70 basepairs (the library) is incubated with the target molecule. Some nucleic acid strands will interact with the target molecule and form strong non-covalent bonds. Target-DNA-complexes are partitioned from unbound DNA. After dissolution of the complex, the selected oligonucleotides are amplified in a standard PCR process. DNA strands are separated and the whole procedure is repeated for up to 20 times in order to select the best fitting sequences.
If RNA is used, a transcription step must be inserted before and after PCR. In order to increase specificity for the target molecule and exclude unspecific binding, counter selection steps can be employed. In those selection rounds no target is used and DNA strands with affinity to the support and container material are removed from the pool

4.3.3 Biosensor applications

In order to eliminate systematic problems with sandwich assays, the development of label free biosensors is an interesting topic. Xiao et al. (2005) modified a thrombin specific aptamer with a thiol group for immobilization on a gold surface. The strand was partially hybridised with a not fully complementary strand bearing a methylene blue (MB) tag.
In presence of thrombin, the strands separated and the MB redox tag was approximated to the Au surface (figure 10(a)).

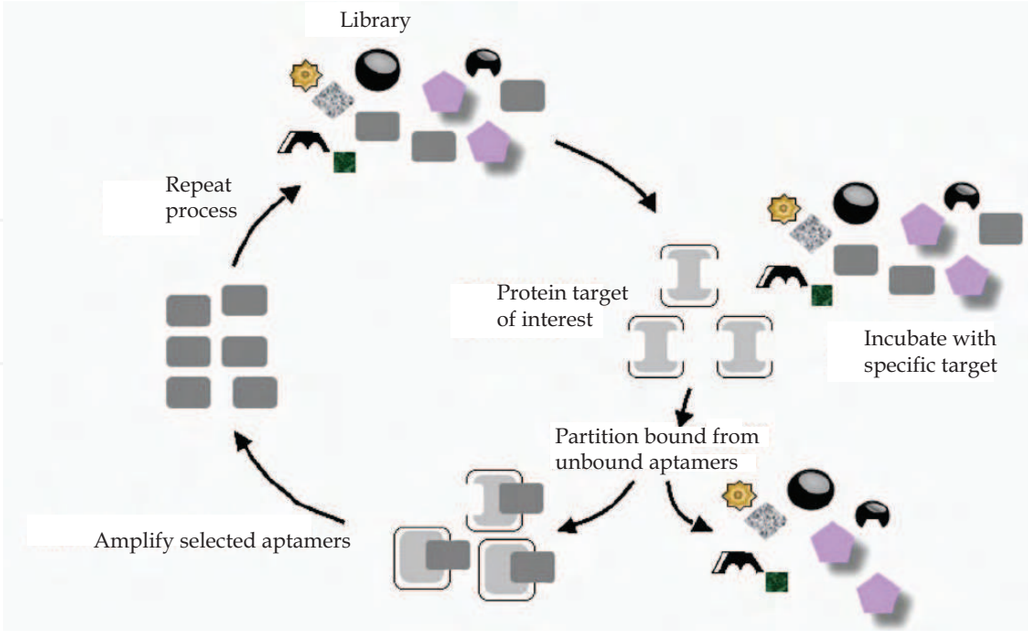


Fig. 9. The SELEX process for use with a DNA library (Nimjee et al. (2006)).

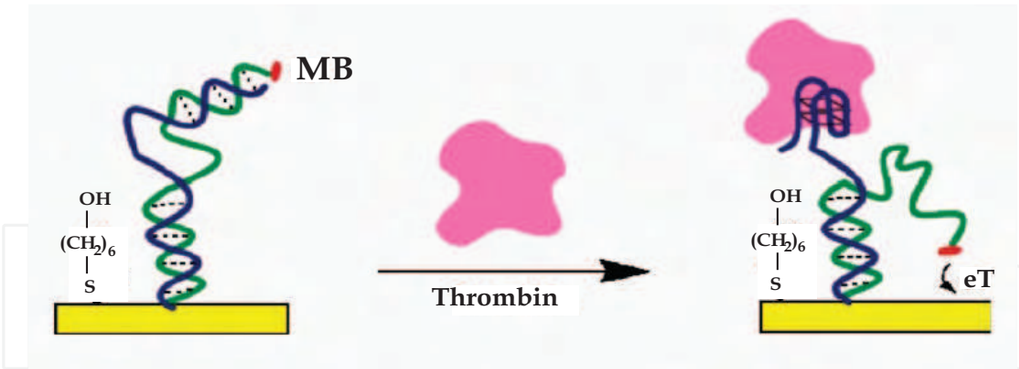
Similar signal-on detectors were developed by Baker et al. (2006) and Lai et al. (2006). The single stranded aptamer had the ability to hybridize with itself and form three loops upon target binding (see figure 10(b)). The conformational change brings the MB tag in proximity to the gold surface and allows for an electrical measurement. Both systems could be regenerated to a high degree, and thus are potentially reusable. Baker 's system could detect cocaine concentrations as low as 500 µM in biological fluids even in the presence of contaminants.

So et al. (2005) attached thrombin binding aptamer to a single walled carbon nanotube (SWNT) which connected two electrodes.

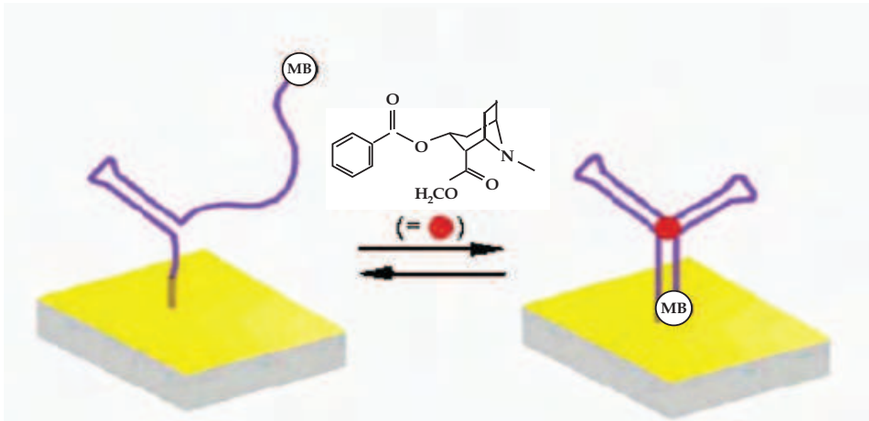
Binding the charged protein induced an electrostatic gate potential and changed the source-drain current. The field effect transistor (FET) biosensor was able to detect thrombin in a concentration range of 10 – 100 nM.

	Aptamers	Antibodies
Affinity	Low nM – pM	Low nM – pM
Specificity	High	High
Production	In vitro chemical process	In vivo biological process
Target range	Wide: ions – whole cells	Narrow: immunogenic compounds
Batch to batch variation	Little or no	Significant
Chemical modification	Easy and straightforward	Limited
Thermal denaturation	Reversible	Irreversible
Shelf life	Unlimited	Limited

Table 2. Differences between aptamers and antibodies. Advantages are emphasised (Lee et al., 2008



(a)



(b)

Fig. 10. Aptamers with a methylene blue redox tag for thrombin (a) and cocaine detection (b). The binding event induces a conformational change in the aptamer and brings the redox active tag closer to the gold surface Baker et al. (2006); Lai et al. (2006). An analogue sensor was described earlier in section 3.2. Xie et al. (2009) used carboxylic acid modified PEDOT nanowires instead of SWNTs as FET.

5. Electrical detection methods

The electrical detection has traditionally received the major share of the attention in biosensor development. Such devices produce a simple, inexpensive and yet accurate and sensitive platform for patient diagnosis. The name electrochemical biosensor is applied to a molecular sensing device which intimately couples a biological recognition element to an electrode transducer. The purpose of the transducer is to convert the biological recognition event into a useful electrical signal.

Electrochemical systems are extremely sensitive to the processes that take place on the surfaces of the electrodes, and in this sense the electrodes are direct transducers in biomedical applications. Several types of electrochemical methods are used in biosensors; the two most common ones are the amperometry and impedance spectroscopy (EIS) (Lazcka et al. (2007)). Recently, all-polymer field effect transistors for biosensing have been introduced (Lee et al. (2010)).

5.1 Amperometry

Amperometry is a method of electrochemical analysis in which the signal of interest is a current that is linearly dependent upon the concentration of the analyte. As certain chemical species are oxidized or reduced (redox reactions) at the electrodes, electrons are transferred from the analyte to the working electrode or to the analyte from the electrode. The direction of flow of electrons depends upon the properties of the analyte and can be controlled by the electric potential applied to the working electrode.

Amperometric biosensors operate by applying a constant potential and monitoring the current associated with the reduction or oxidation of an electroactive species involved in the recognition process. The amperometric biosensor is attractive because of its high sensitivity and wide linear range.

5.2 Conductivity and impedance spectroscopy

Electrochemical impedance spectroscopy (EIS) combines analyses of both the resistive and capacitive properties of materials, based on the perturbation of the system by a small-amplitude sinusoidal AC signal. The impedance of the system can be scanned over a wide range of AC signal frequencies. The amplitude of the current, potential signals, and the resulting phase difference between voltage and current dictates the system impedance Therefore, the impedance signal is dependent on the nature of the system under study.

Equivalent circuit models fitted to the impedance curves are useful tools for characterizing the system. Although this methodology is widely accepted because of ease of use, extreme care must be taken to ensure that the equivalent circuit obtained makes physical sense. An advantage of EIS compared to amperometry is that redox labels are no longer necessary, which simplifies the sensor preparation.

5.3 Organic field effect transistors

Organic field effect transistors (Organic FETs) have a potential being the active matrix for many electronic devices, including biosensors for biological material. An organic field-effect transistor consists of a source and drain electrode, an organic semiconductor (which is in this case a conductive polymer), a gate dielectric, and a gate electrode. A number of different studies have demonstrated conductance-based sensors employing a molecular receptor layer immobilized on the surface of a semiconductor device. The receptor molecules provide the means to achieve highly selective sensing because they can be engineered to have much higher binding affinities with the desired target molecules than the other species in the analyte solution (see section 4). Although the organic FET is a promising candidate for biosensor applications, optimization of the device structure and operating conditions is still required.

6. Outlook

In recent years, fascinating developments of a wide range of commercial applications have occurred. Elegant research on new sensing concepts has opened the door to a wide variety of microsystem based biosensors for clinical applications. Such devices are extremely useful for delivering diagnostic information in a fast, simple, and low cost fashion, and are thus uniquely qualified for meeting the demands of point of care systems, e.g. for cancer screening. The high sensitivity of the modern biosensors should facilitate early detection and treatment of diseases, and lead to increased patient survival rates.

In the future, one of the main challenges is to bring the new biosensor techniques to bed side for use by non-laboratory personnel without compromising accuracy and reliability. The internal calibration and reference is also a major requirement, and provoke researchers to reshape the existing methods. From a clinical point of view, the in vivo biosensors that are biocompatible and can remain in the body for weeks or months will also be a demand. Special attention should be given to non-specific adsorption issues that commonly control the detection limits of electrochemical bioaffinity assays. The stability of biosensors remains an important issue in the fabrication and use of these devices for many application areas.
By measuring abnormalities within few minutes, disposable cartridges containing electrode strips and simple sample processing could offer early and fast screening of diseases in a point of care setting.
It has become apparent that the field of polymer biosensors has reached a new level of maturity. In the near future it is highly likely that pathogen detection will undoubtedly benefit from the integration of biosensors into all-polymer microdevices, and thus in some regards revolutionize the medical diagnostics.

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Environmental Biosensors

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This book is a collection of contributions from leading specialists on the topic of biosensors for health, environment and biosecurity. It is divided into three sections with headings of current trends and developments; materials design and developments; and detection and monitoring. In the section on current trends and developments, topics such as biosensor applications for environmental and water monitoring, agro-industry applications, and trends in the detection of nerve agents and pesticides are discussed. The section on materials design and developments deals with topics on new materials for biosensor construction, polymer-based microsystems, silicon and silicon-related surfaces for biosensor applications, including hybrid film biosensor systems. Finally, in the detection and monitoring section, the specific topics covered deal with enzyme-based biosensors for phenol detection, ultra-sensitive fluorescence sensors, the determination of biochemical oxygen demand, and sensors for pharmaceutical and environmental analysis.

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