Clinical Applications of Micro- and Nanoscale Biosensors

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17.1 INTRODUCTION

The ability to detect pathogenic and physiologically relevant molecules in the body with high sensitivity and specificity offers a powerful opportunity in early diagnosis and treatment of diseases. Early detection and diagnosis can be used to greatly reduce the cost of patient care associated with advanced stages of many diseases. These costs have been estimated to be ~\$75 billion [1] and ~\$90 billion [2] for cancer and diabetes, respectively. Currently, cancer can be detected by monitoring the concentration of certain antigens present in the bloodstream or other bodily fluids, or through tissue examinations. Correspondingly, diabetes is monitored by determining the glucose concentrations in the blood over time. However, despite their widespread clinical use, these techniques have a number of potential limitations. For example, a number of diagnostic devices have slow response times and are burdensome to patients. Furthermore, these assays are expensive and cost the health care industry billions of dollars every year. Therefore, there is a need to develop more efficient and reliable sensing and detection technologies.

A biosensor is commonly defined as an analytical device that uses a biological recognition system to target molecules or macromolecules. Biosensors can be coupled to a physiochemical transducer that converts this recognition into a detectable output signal [3]. Typically biosensors are comprised of three components: (1) the detector, which identifies the stimulus; (2) the transducer, which converts this stimulus to a useful output; and (3) the output system, which involves amplification and display of the output in an appropriate format [3].

One of the earliest references to the concept of a biosensor appeared in 1962 when Clark and Lyons [4] coupled glucose oxidase to an amperometric electrode to measure oxygen pressures. The enzyme-catalyzed oxidation of glucose lowered the oxygen pressure in the test solution, which was then sensed by the electrode. This oxygen pressure reduction was then shown to be proportional to the declining glucose concentration in the sample. An early example of the use of cells as biosensors occurred in 1977 when Rechnitz et al. [5] coupled living microorganisms (*Streptococcus faecium*) on the surface of an ammonia gas-sensing membrane electrode. Rechnitz's electrode biosensor was capable of detecting the amino acid arginine.

The emergence of micro- and nanoscale technologies for biology has a great potential to lead to the development of next generation biosensors with improved sensitivity and reduced costs. Nanotechnology is the study, manipulation, creation, and use of materials, devices, and systems of dimensions less than 100 nm [6]. Nanoscale technologies could be developed either by using bottom-up molecular processes or by scaling down traditional microfabrication processes that have been commonly used in microelectronics [7].

Modern biosensors based on micro- and nanoscale techniques have the potential to greatly enhance methods of detecting foreign and potentially dangerous toxins and may result in cheaper, faster, and easier-to-use analytical tools. Furthermore, microscale biosensors may be more portable and scalable for point-of-care sample analysis and real-time diagnosis. The goal of this chapter is to give a brief description of the different types of biosensors and their roles regarding *in vitro* and *in vivo* diagnostics. Specifically, we will discuss the applications of micro- and nanotechnologies in the development of future biosensors and discuss the current and future clinical applications of these technologies and analyze their viability.

17.2   CLASSES OF BIOSENSORS

17.2.1   Method of Biological Signaling

Biosensors can be classified either by the type of biological signaling mechanism they utilize or by the type of signal transduction they employ. The biological signaling used by biosensors can be divided into five major mechanisms (Fig. 17.1). Here, we will discuss each of these mechanisms:

(a) *Antibody/antigen:* The high specificity between an antibody and an antigen can be utilized in this type of sensor technology. Biosensors utilizing this specificity must ensure that binding occurs under conditions where nonspecific interactions are minimized [8]. Binding can be detected either through fluorescent labeling or by observing a refractive index or reflectivity change [9].

(b) *Enzymes:* Enzyme-based biosensors are composed of enzyme bioreceptors that use their catalytic activity and binding capabilities for specific
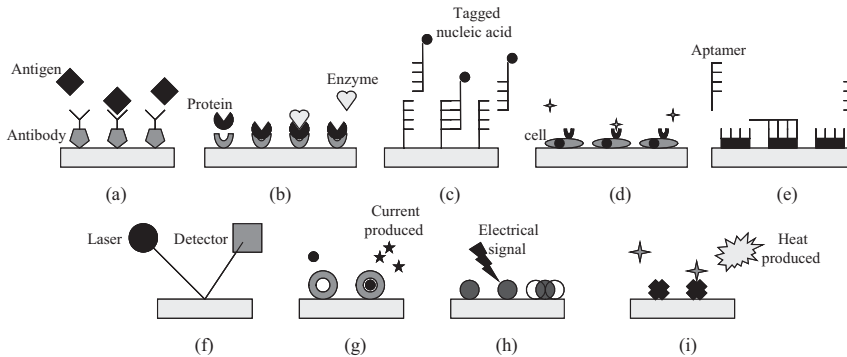
FIGURE 17.1   Biosensing and transduction classes for *in vitro* biosensors. Methods of biosensing: (a) antibody/antigen; (b) enzyme catalysed; (c) nucleic acid; (d) cell-based; (e) biomimetic. Methods of transduction; (f) optical; (g) electrochemical; (h) mass-sensitive; (i) thermal.

detection. The products of reactions catalyzed by enzymes can be detected either directly or in conjunction with an indicator [9]. The catalytic activity of the enzymes provides these types of biosensors with the ability to detect much lower limits than with normal binding techniques. This catalytic activity is related to the integrity of the native protein structure [10].

(c) *Nucleic acids:* The complementary relationships between adenosine and thymine and cytosine and guanosine in DNA form the basis of specificity in nucleic acid-based biosensors. These sensors are capable of detecting trace amounts of microorganism DNA by comparing it to a complementary strand of known DNA [8]. By unwinding the target DNA strand, adding the DNA probe, and annealing the two strands, the probe will hydrolyze to the complementary sequence on the adjacent strand [10]. If the probe is tagged with a fluorescent compound, then this annealing can be visualized under a microscope. For accurate analysis, polymerase chain reaction (PCR) is often used to create multiple copies of the sample DNA.

(d) *Cells and viruses:* Microorganisms such as bacteria and fungi can be used as biosensors to detect specific molecules or the overall "state" of the surrounding environment [10]. For example, cell behavior such as cell metabolism, cell viability, cell respiration, and bioluminescence can be used as indicators for the detection of heavy metals [10]. Furthermore, proteins that are present in cells can also be used as bioreceptors for the detection of specific analytes [11, 12].

(e) *Biomimetic materials based:* A biomimetic biosensor is an artificial or synthetic sensor that mimics the function of a natural biosensor. These can include aptasensors, where aptasensors use aptamers as the biocomponent [8]. Aptamers are synthetic strands of nucleic acid that can be designed to recognize amino acids, oligosaccharides, peptides, and proteins [13].

17.2.2   Method of Transduction

Biosensors can also be classified according to their method of signal transduction. Typically, biosensors belong to one of following classes of signal transduction:

(a) *Optical-detection:* Optical detection biosensors are the most diverse class of biosensors because they can be used for many different types of spectroscopy, such as absorption, fluorescence, phosphorescence, Raman, SERS, refraction, and dispersion spectrometry [8]. In addition, these spectroscopic methods can all measure different properties, such as energy, polarization, amplitude, decay time, and/or phase. Amplitude is the most commonly measured as it can easily be correlated to the concentration of the analyte of interest [8].

(b) *Electrochemical:* Electrochemical biosensors measure the current produced from oxidation and reduction reactions. This current produced can be correlated to either the concentration of the electroactive species present or its rate of production/consumption [8].

(c) *Mass-sensitive:* Biosensors that are based on mass-sensitive measurements detect small mass changes caused by chemical binding to small piezoelectric crystals. Initially, a specific electrical signal can be applied to the crystals to cause them to vibrate at a specific frequency. This frequency of oscillation depends on the electrical signal frequency and the mass of the crystal. As such, the binding of an analyte of interest will increase the mass of the crystal and subsequently change its frequency of oscillation, which can then be measured electrically and used to determine the mass of the analyte of interest bound to the crystal [10].

(d) *Thermal detection:* Thermal biosensors measure the changes in temperature in the reaction between an enzyme molecule and a suitable analyte [14]. This change in temperature can be correlated to the amount of reactants consumed or products formed.

17.3   TYPES OF *IN VITRO* DIAGNOSTICS

Micro- and nanoscale technologies can be used to improve diagnostic efficiency and to develop more portable devices for point-of-care applications. These devices can be used for a variety of common medical conditions such as diabetes, which currently comprises ~85% of the world biosensor market [15]. Here we will discuss three examples of these techniques.

17.3.1   Cantilever-Based Biosensors

The detection of molecular interactions between biomolecules by measuring their nanoscale mechanical forces offers exciting opportunities for the development of highly sensitive, miniature, and label-free biological sensors

[16]. Microscale cantilever beams can be used to detect biomolecules by deflecting upon interaction with a specific biomolecule. By measuring the amount of bending each cantilever beam experiences in response to interactions with the molecules, the amount of analyte in the solution can be quantified. Generally, there are three mechanisms to transduce the recognition of the analyte of interest into micromechanical bending of the cantilever. These include bending in response to a surface stress, bending in response to a mass loading, and bending as a result of a temperature change [17] (Fig. 17.2).
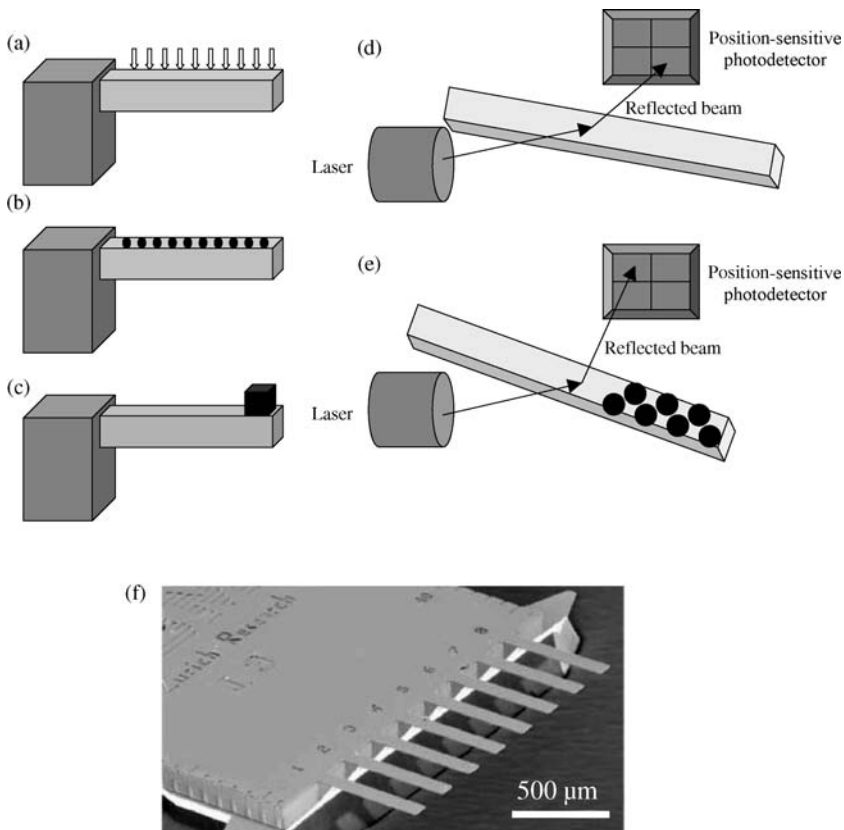

FIGURE 17.2 Microfabricated cantilevers for biosensing. Panels (a−c) illustrate various means of signal transduction: (a) a temperature and heat sensor, (b) a surface stress sensor, and (c) microbalance due to mass loading. Panels (d, e) illustrate the optical readout technique commonly used to measure deflections of cantilever biosensors; (d) optical deflection during normal cantilever conformation; (e) Optical deflection after analyte binding to the end of the cantilever panels beam. (f) is an SEM micrograph of an array of eight silicon microcantilevers. (Reprinted with permission from elsevier [115].)

Cantilever bending induced by surface stresses is caused by binding of the molecules of interest to one side of the cantilever. For example, specific binding of molecules such as streptavidin and biotin, L-cysteine [18], and prostate-specific antigen [19] can be detected by this mechanism. In addition, mass loading can be used to detect nanoscale deflections since the attachment of a larger mass causes the cantilever to oscillate at a different frequency. Also, a rapid, extreme temperature change around the cantilever can also cause it to bend.

Implementing different materials into the structure of the cantilever enhances its sensitivity to the aforementioned conditions. The most commonly used materials used for the construction of commercial micro- and nanoscale cantilevers are silicon, silicon nitride, and silicon oxide [17]. Although these cantilevers are extremely sensitive to different masses and stresses, they offer no chemical or biochemical selectivity. By coating the surface of the cantilever with biological recognition molecules such as peptides, self-assembled monolayers, DNA probes, or antibodies, cantilevers can be built that detect specific molecules [20].

The degree of bending of the cantilevers can be registered using a wide range of detection techniques including optical laser based, piezoresistive, piezoelectric, and capacitive [21]. Deflection measurements based on optical beams are an efficient readout method for cantilevers with reflecting surfaces [22]. Here, a laser diode is focused at the free end of the cantilever (usually coated with gold) and the reflected beam is detected by a position-sensitive photodetector (Fig. 17.2) [22]. For additional sensitivity at nanoscale regimes, electron transfer methods can be used with cantilevers that are only a few hundred nanometers in length [22]. For piezoresistive detection, a resistor is embedded into a silicon cantilever, which changes its resistance as the cantilever bends. Accordingly, when the silicon cantilever is deformed, the change in resistance of the device reflects the degree of deformation [21]. These cantilevers typically have two legs that enable the resistance of a boron-doped channel to be successfully measured by wiring two conductive paths to the cantilever base next to the legs. Correspondingly, the piezoelectric method of detection requires the placement of a piezoelectric material, such as ZnO, onto the surface of the cantilever. When a stress is applied to piezoelectric materials, they respond by generating a voltage, which can then be measured and correlated to the amount of stress applied. Finally, the capacitance method of detecting cantilever bending is based on measuring the capacitance between a metal plate on the cantilever surface and another plate fixed on the substrate [22, 23]. The capacitance is inversely proportional to the distance between the substrate and the conductor on the surface of the cantilever. As the cantilever bends, the distance between the tip of the cantilever and the substrate changes, which results in changes in capacitance and can be correlated to the mass loading. This detection method is highly sensitive, yet only applies to small displacements and does not work in liquid solutions.

17.3.2   Cell and Protein Arrays

The use of live cells for biosensing applications is an exciting alternative to traditional biosensing approaches. These techniques may potentially enhance biosensor specificity and sensitivity [24]. Cell-based biosensors are also particularly useful in detecting unknown compounds and toxins since the behavior of the candidate molecules can be directly observed in tissues. Specific examples include the use of liver [25], cardiac [26–28], or immune cells [29, 30]. By using micro- and nanoscale devices it may be possible to mimic the function of cells *in vitro* as a means to develop more efficient sensor and transduction technologies [31].

By engineering the response of B lymphocytes, cells responsible for humoral immunity, Rider et al. have demonstrated that immunosensors can be developed using live cell arrays [24]. In this work, B lymphocytes were altered to recognize surface proteins of several different pathogens. They found that upon stimulation with specific test samples, the engineered cells responded within few seconds. These responses were visualized through the bioluminescence of a calcium-sensitive protein that the cells were engineered to express.

In addition, multiphenotype cell arrays have been fabricated for biosensing [32–34]. In one example, a biosensor was fabricated that incorporated B cells for the detection of specific analytes and T cells to convert the B-cell output signal into a readable form. This was accomplished through a similar mechanism as discussed previously. Kim et al. used the dose-dependence response of calcium released into the cytosol upon stimulation with T-cell receptor to engineer real-time biosensors [29]. By inserting calcium-sensitive dye (fura-2) into the T cells they were able to visualize the T-cell response to the peptide presentation from the B cells.

In addition to cellular arrays, there is significant interest in the use of protein arrays in biosensing applications [35]. Most studies on protein array biosensors have focused on the use of antibodies to create biosensors with the capability of performing multiple analyses simultaneously [36]. Ligler et al. reported their work regarding a single biosensor array capable of detecting multiple analytes on the same chip [37]. This was accomplished by immobilizing capture molecules onto the surface of an optical waveguide in stripes resembling "bar codes". Each strip in the "bar codes" was directed against a different analyte of interest. The sample of interest was then loaded perpendicularly to these bar codes using flow chamber modules. This enabled each sample to encounter the "bar code" of columns and the specific binding of multiple analyte molecules to their corresponding capture molecule was achieved.

17.3.3   Nanoparticles

Nanoparticles have emerged as powerful and widely applicable materials in biosensing. Nanoparticles are generally defined as particles that range in size from 1 to 100 nm in diameter [38]. By conjugating specific molecules to the surface of nanoparticles, it is possible to engineer their biological functionality.

An exciting product of the progress in nanoparticles for *in vitro* diagnostic tools has been the emergence of probes encapsulated by biologically localized embedding (PEBBLEs). PEBBLEs are nanoscale polymer beads specifically designed to provide minimally invasive monitoring of specific analytes in single, viable cells with applications for real-time analysis of drugs, toxins, and environmental effects on cell function [39–45]. PEBBLEs typically encapsulate a dye sensitive to the analyte of interest and a dye to function as a reference. By encapsulating these within their biologically inert matrix, PEBBLEs avoid potential chemical interference from other cellular constituents [46]. Although PEBBLEs have been designed under multiple platforms [45, 47−49], most follow a parallel sensing format: analytes present in the cell diffuse through the PEBBLE matrix (which can be made from polyacrylamide, poly(decyl methacrylate) (PDMA), sol−gel or modified silicates [46]) and interact with the dyes contained within the nanoparticles. Interactions between the analytes and the sensing dye initiate conformal changes in the dye that are detectable through variations in excitation intensity. The reference dye is also affected by interactions and varying excitation intensities; however, the ratio between the two dyes helps to eliminate misrepresentation of the data [46]. In PEBBLE nanosensors, the sol−gel matrix can be modified to enable the encapsulation of both hydrophobic and hydrophilic dyes [39]. These gels can be heated to form high purity oxides that can then be combined with oxygen-sensitive dyes. Upon injection into rat C6-glioma cells, it was possible to detect intracellular oxygen concentrations with high accuracy and reproducibility [49]. This example shows the promise of PEBBLEs to detect and quantify the concentration of specific analytes.

Another group of nanoparticles for *in vitro* experimentation are quantum dots (QDs) [50–53]. QDs are semiconductor crystals (between 2 and 10 nm in diameter) that have unique electrical and optical properties. QDs are made from nanocrystals of CdS, CdSe, CdTe, or CdSe/ZnSe synthesized using different methods [54–57]. These materials exhibit unique optical and electronic properties through quantum mechanical scattering of valance shell electrons by the atomic cores [58] made possible due to their size. When excited with a beam of photons, they emit bright light at a distinct frequency on their own and hence are a promising technology for many biosensing applications [52]. In comparison to fluorescent labelling, QDs are less susceptible to photobleaching. Moreover, QDs have longer emission lifetimes and can be used in tracking cells for extended periods of time, and since they are small and emit light at a distinct color, multiple tagging experiments can be done simultaneously. Although examples of QDs in biosensing application are quickly emerging, the true promise of QDs is yet to be realized and they may lead to revolutionary advances in biosensing technology. We will discuss specific uses of QDs in Section 17.4.1.

17.4   *IN VIVO* DIAGNOSTICS

The advances in micro- and nanotechnologies can also be used for *in vivo* biosensing applications. With miniaturization, it is possible to fabricate novel

devices at low cost, with greater functionality and more reliability [34]. For instance, the ability to track the presence of fluorescent nanoparticles *in vivo* offers significant improvements in the detection, diagnosis, and treatment of diseases. Two types of nanoparticles that have been used *in vivo* for biosensing applications are QDs and MRI contrast agents.

17.4.1   Quantum Dots

QDs can be surface modified to enhance their biocompatibility, solubility, and functionality (Fig. 17.3a). For example, surface-modified QDs can be used as *in vivo* imaging tools capable of binding to specific targets [59]. The biological molecules can include peptides, antibodies, nucleic acids, or small-molecule ligands [60]. Recently, this technology has been used to image tumors *in vivo* (Fig. 17.3b) [60]. QDs have also been used for cell and tissue labeling [61], long-term cell trafficking, and multicolor cell imaging [62]. To increase the biocompatibility of QDs, the surface of QDs has been engineered with polyethylene glycol (PEG) molecules [60]. Using surface-modified approaches,
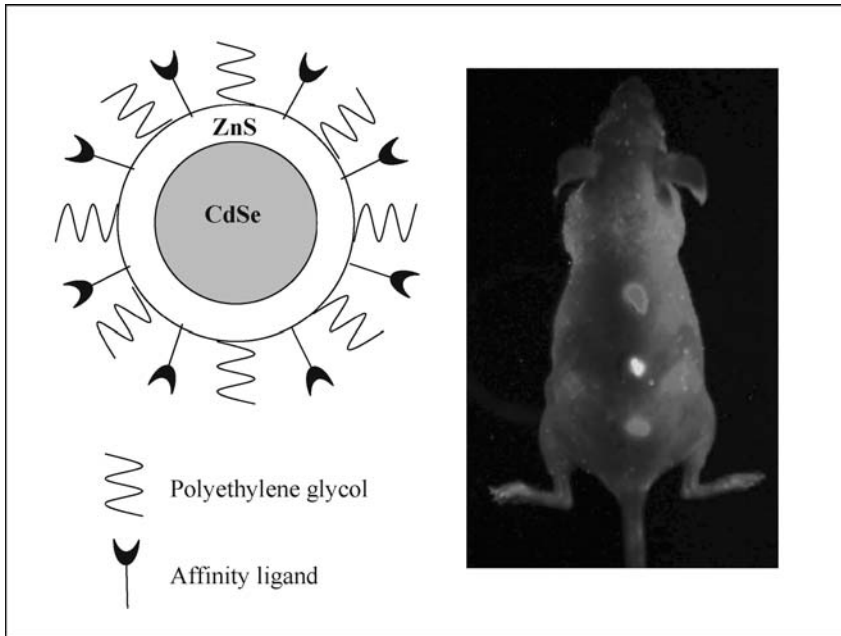

FIGURE 17.3   The basic structure of a quantum dot is provided. A cadmium selenide (CdSe) core is coated with zinc sulfide (ZnS) and polyethylene glycol (PEG) and affinity ligands are covalently coupled to the surface. The second image shows the capability of simultaneous *in vivo* imaging of QDs. The image was attained with either tungsten or mercury lamp excitation. (Used with permission from Gao et al. [60].)

Derfus et al. have demonstrated that QDs complexed with cationic liposomes can be used to target HeLa cells [63]. Thus, QDs complexed with transfection reagents are capable of entering the cytoplasm of cells.

Despite their success, a number of challenges exist for using QDs for *in vivo* applications. One of these challenges is that the core material for most QDs is a heavy metal that is toxic to cells, and accordingly, research is currently underway to produce nonheavy metal-based alternatives. Therefore, it may one day be possible to use QDs to detect, diagnose, and treat diseases in a minimally invasive manner.

17.4.2 MRI Contrast Agents

Cellular imaging with MRI contrast agents is used for many imaging and diagnostic applications [64–66]. MRI contrast agents are gaining popularity for *in vivo* diagnostics due to their high spatial resolution [67]. These particles are typically made from ferrous or ferric oxide coated with a polymeric material, such as dextran [67]. These particles have been shown to be nontoxic and inert for short durations, and after injection accumulate in the organ of interest [68]. In addition, these particles could be impregnated with a fluorescent agent to enable analysis by both fluorescent microscopy and MRI [69]. There are, however, a number of limitations to MRI tracking of cells. To effectively image a cell, a large number of nanoparticles need to be internalized by the cell. This requires a highly efficient labeling scheme [70]. Additionally, cell division dilutes the label once a cell has been effectively tagged [70]. These difficulties can hamper studies that aim to examine the long-term stability of cell labeling. A great deal of research is currently underway to overcome these limitations by engineering new contrast agents that are more stable, nontoxic, and functional *in vivo*.

17.5 CURRENT AND EMERGING CLINICAL APPLICATIONS OF MICRO- AND NANOSCALE BIOSENSORS

There are many applications of biosensor technologies in health care and for the treatment of infectious diseases. The current status and future potential of four of the most relevant applications are discussed below.

17.5.1 Glucose Detection *In Vivo*

One of the main clinical applications of biosensors is to develop point-of-care glucose concentration measuring devices for patients suffering from diabetes [71]. Originally introduced in the early 1980s [71], the latest generation of handheld glucose sensors has revolutionized the lifestyles of those suffering from diabetes. Patients are now able to self-monitor their glucose concentrations and self-administer insulin injections as required.

Most enzyme-based biosensors to detect glucose concentrations use enzymes known as oxidoreductases [72]. The most common enzymes used for glucose detection are glucose oxidase and glucose dehydrogenase [73]. Glucose biosensors generally make use of electrochemical transducers in their designs as they provide appropriate specificity and reproducibility and can easily be manufactured in large volumes at low costs [73].

These traditional amperometric-based biosensors have undergone recent miniaturization to enable subcutaneous implantation. In the minimed-medtronic continuous glucose monitoring system (CGMS), a needle-type amperometric enzyme electrode is coupled to a portable data logger [74]. The sensor is based on the aforementioned sensing technology and the data recorded from the logger can be downloaded to a portable computer after 3 days of sensing [74]. The monitor is implanted in the subcutaneous tissue to measure interstitial fluid glucose concentrations. Although interstitial fluid and blood concentrations are similar at steady state [75], there is a significant delay when the blood glucose concentration is rapidly changing as occurs after a meal.

Another microscale *in vivo* glucose monitor is the GlucoWatch (Cygnus, Inc.). This sensor operates by reverse iontophoresis, which utilizes a glucose-containing interstitial fluid that is lured to the skin surface by a small current passing between two electrodes [75]. Hydrogel pads containing a glucose oxidase biosensor are present on the surface and measure the glucose concentration present in the interstitial fluid. Again, the delay between the glucose concentrations variations in the interstitial fluid and corresponding changes in the blood creates a significant disadvantage.

There is a clinical need for future glucose sensors to become increasingly noninvasive and sensitive to rapid changes in glucose concentrations. It is anticipated that the development of microscale devices as well as emerging nano-based detection strategies will be useful for these techniques.

17.5.2 Bacterial Urinary Tract Infections

Bacterial infection in the urinary tract is the second most common organ system infection in the human body [76]. Microbial culture techniques are currently employed to identify urinary tract pathogens. These methods, however, are cumbersome and are accompanied by a 2-day lag period between the collection of the specimen and the identification of the pathogen [77]. As such, the development of tools to effectively decrease this lag period and increase diagnosis accuracy and efficiency is very appealing from an improved health care and reduced cost standpoint.

Electrochemical DNA biosensors have been documented in the literature to detect and identify pathogens [78, 79]. In these designs, a layer of oligonucleotide probes functions as the sensory receptor and the sensory input is detected through the use of an electrochemical transducer. There are two basic modes to detect DNA with this configuration. The first method requires target

immobilization followed by detection with a labeled probe [80]. In the second method, known as "sandwich" hybridization, the DNA target initially binds to a surface oligonucleotide through hybridization. This is followed by hybridization to a marker probe for signal transduction [80].

Liao et al. have used these concepts and methods to rapidly detect and identify molecular pathogens in clinical urine samples [77]. The authors successfully developed pairs of capture and detection oligonucleotides in an array for the detection of a 16S rRNA target. This "microchip" required 45 min after applying the sample to provide readout signals and did not require amplification or labeling of the target sequence. This biosensing technique confirms the capabilities of direct detection techniques for the identification of bacteria present in clinical samples and could be of great clinical potential.

17.5.3 Human Immunodeficiency Virus (HIV) Detection

More than 30 million HIV-infected people live in the developing world, where resources are scarce. In 2002, the U.S. National Intelligence Council (NIC) predicted that the number of HIV-infected individuals in the developing world would rise to 80 million by 2010. Effective antiretroviral therapy (ART) for HIV has been available in developed countries for more than a decade; however, only a small fraction of the infected people are currently receiving treatment due to lack of diagnostic tools and cost-effective therapies. To increase access to HIV care and improve treatment outcomes, there is an urgent need for low cost diagnostic tools that could be implemented in developing countries [81, 82].

Traditionally, HIV infections are diagnosed by either direct fluorescent antibody assays or viral load testing [83]. Direct fluorescent antibody assays, such as enzyme-linked immuno sorbent assay (ELISA), use two antibodies to identify the presence of a virus [84]. HIV presence *in vivo* can also be detected using viral load testing [85]. This technique detects cell-free plasma viral RNA with the use amplification techniques such as PCR. These types of diagnostic techniques provide rapid results, however, are generally not sensitive enough to provide reliable and consistent results [86].

The application of surface plasmon resonance-based (SPR) optical techniques could greatly enhance the understanding of HIV and lead to superior detection and quantification mechanisms [87]. In SPR, the surface of the biosensor is initially covered with immobilized ligands. Microfluidic channels then carry an analyte across the ligand and specific binding between the ligand and the analyte occurs. The SPR detector then measures changes in the refractive index of the biosensor as ligands and analytes bind and detach from one another [88] (Fig. 17.4).

This process has already had a tremendous impact on the understanding of HIV infections. Fagerstam et al. [89] initially used SPR to complete epitope mapping of monoclonal antibodies opposed to the HIV capsid protein. Subsequently, Alterman et al. studied the interaction of 17 inhibitors with differing structures on HIV protease immobilized onto electrodes during SPR
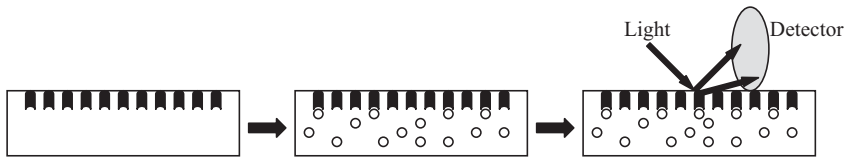
FIGURE 17.4 A schematic illustrating an SPR experiment. The ligand is initially immobilized on the surface of the biosensor chip. A microfluidic channel carries the analyte across the surface of the ligand and specific binding occurs. As the analyte binds and dissociates from the ligand, the refractive index is measured and recorded.

analysis [90]. This has potentially powerful applications in the development of HIV protease inhibitors, which may have profound impacts in the progress of therapies aimed at inhibiting the HIV replication cycle.

Another way to evaluate HIV-infected patients is to measure the absolute number of $CD4^+$ T lymphocytes in blood. The CD4 count is used to initiate treatment and to monitor the response to treatment. For instance, when the CD4 count falls below 200 cells/μl, HIV-infected patients are at risk for severe opportunistic infections, and HIV treatment is drastically needed. In high income settings, CD4 counts rely on flow cytometry, which is expensive and not suitable for resource-limited countries. Handheld, reliable, and low cost CD4 counting devices for use in resource-scarce regions of the world are needed. There have been recent efforts to develop affordable CD4 counting methods by flow cytometry. Although these tools are more affordable than standard flow cytometers, they remain complex for district hospitals or point-of-care use in developing countries, require expensive reagents, involve several sample preparation steps, and are labor intensive and low throughput. The need for simple CD4 counting solutions that meet design specifications appropriate to point-of-care and developing world testing—such as high throughput, low fabrication cost, and device disposability—are suitable for microelectromechanical systems (MEMS). Microfluidics-based devices are being developed as tools to overcome these limitations since they can be fabricated cheaply, are portable, and have been engineered to perform variety of functions required to make biological measurements [91]. For instance, Demirci et al. demonstrated a microfluidic device that can separate and image CD4 T lymphocytes on a polycarbonate filter, to measure their concentration in the blood as shown in Fig. 17.5. In microfluidic devices, blood samples could be screened and partitioned. For example, by using an array of posts of defined sizes, red blood cells can be filtered from leukocytes [91]. Subsequently, leukocytes can be immobilized in microwells and stained to measure the number and frequency of the desired cell types.

17.5.4 Cancer Cell Targeting

Currently, 60% of patients diagnosed with breast, colon, lung, or ovarian cancer already have cell metastases forming in other locations of their body
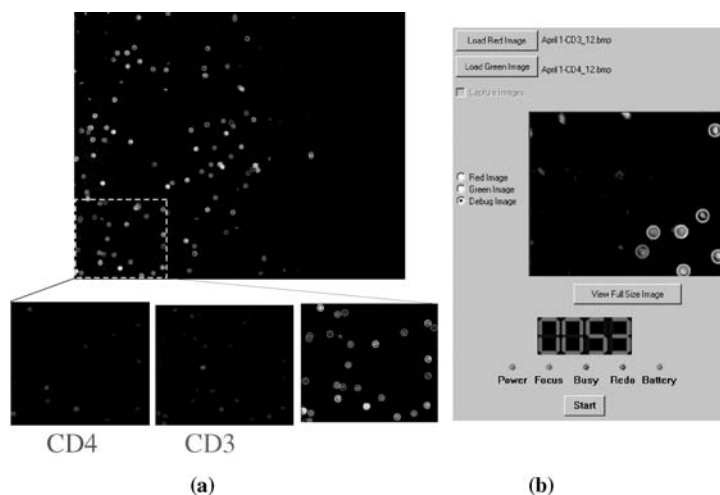
FIGURE 17.5 Detection of CD4$^+$ T lymphocytes in microfluidic-based devices for monitoring HIV. (a) Each location on the image is identified, where red marked cells correspond to CD3$^+$ cells, green marked cells correspond to CD4$^+$, cells, and the yellow marked locations on the image correspond to CD3$^+$ and CD4$^+$ T lymphocytes. (b) The software output is shown; the yellow circles are automatically drawn by the image recognition software and the CD4$^+$ T-cell count is displayed as 53 cells/μl of whole blood for this sample.

[92–103]. The development of effective diagnostic tools to detect these cells has been difficult due to the low number of circulating cancer cells and the lack of suitable markers to identify these cells. However, *in vivo* and *in vitro* applications of nanotechnology may be used to increase the selectivity and resolution and to make such diagnoses possible.

Currently, there are several techniques to isolate tumor cells. These require laborious manual sample preparation steps that result in variable results and low sensitivity. Circulating tumor cells (CTCs) are rare even in patients with advanced cancer, representing as low as 1–10 cells/ml [104] such that a reliable cell sorter for CTCs needs to detect approximately one CTC in one billion blood cells. The conventional cell separation methods rely on properties such as size, density and differential expression of surface antigens to isolate desired cell subpopulations, density gradient centrifugation, preferential lysis of red blood cells, ficoll-hypaque density, porous filters, immunomagnetic bead sorting, and cell filtration [105]. Molecular methods have also been developed that rely on PCR-based detection of tumor-associated RNA in blood as evidence of CTCs, including in melanoma, breast cancer and prostate cancer [14–17]. Moreover, methods that allow recovery of living or intact cells for further morphological, immunocytochemical, genome-wide expression profiling, or functional evaluation are significant. These studies could add to the potential benefits of CTCs and circulating metastatic precursor cells.

There have been attempts to standardize the preparation steps, which target reduction in variations. For instance, Immunicon, Inc. has developed a semiautomated sample preparation and analysis system and has used it in multiple clinical or preclinical trials, which has been applied to several cancers [94, 96, 97, 101, 106]. The sample preparation steps include isolation of leukocytes followed by the incubation with antiepithelial cell adhesion molecule (EpCAM)-coated ferrofluid particles. Finally, the magnetic incubation and washing steps follow. EpCAM is a homotypic cell adhesion molecule with expression limited to and highly expressed by cells of epithelial origin [107–109]. It has also been shown to be expressed on CTCs [110]. To capture cells with low density of EpCAM receptors, the ferrofluid was modified to contain two distinct receptors, one monoclonal antibody for EpCAM and another receptor (biotin analog) that binds to a multivalent aggregator. This method increases the number of magnetic particles per target cell through controlled and reversible aggregation of the ferrofluid, which increases the capture efficiency.

The above studies demonstrate the potential use of EpCAM to isolate CTCs from blood. There is room for improving the sensitivity of CTC detection and removing the variability for clinical applications. Among the new technologies with increasingly broader impact in biology, microfluidic lab-on-a-chip-type devices have potential for blood analysis. The use of physical properties for separation of cells takes advantage of the heterogeneity of blood cells [91]. The differences in the mechanical properties of cells, such as size, were tested for separation of larger tumor cells from blood samples [111]. Increased rigidity of blood cells was also used to distinguish and sort cells [112]. The advances in understanding of blood sample preparation and technological developments in microfabrication and microfluidics enable new capabilities for blood analysis.

In addition to antibodies for cancer cell detection, other mechanisms such as peptides and aptamers can be used. For example, nanoparticle−aptamer bioconjugates have been used to specifically target prostate cancer cells [113]. It was determined that nanoparticles with RNA aptamers experienced a significantly enhanced uptake in cells that expressed the prostate-specific membrane antigen, a prostate cancer tumor marker that is overexpressed on prostate cancer cells. This work is an exciting first step in targeting prostate cancer cells and could potentially be used to numerous other important human diseases.

The early stage detection of certain cancer cells *in vivo* is difficult since these cells generally do not metastasize [92]. As such, there have been significant advances in the development of *in vivo* techniques of cancer cell imaging. For example, iron oxide particles have been used to identify lymph node metastases in male prostate cancer [114]. This group found that the distribution of the iron oxide particles was disrupted by malignant tumors present in the prostate, and that this disruption was detectable with MRI. Furthermore, prostate cancer cells have also been imaged using quantum dots in nude mice [60]. In this case, quantum dot accumulation was achieved through two

different mechanisms: by enhanced permeability and retention of tumor sites as well as by antibody binding to specific cancer cell surface biomarkers. These *in vivo* techniques are examples of noninvasive cancer imaging tools that may be enhanced to include additional cancer cell types with future research.

17.6 CONCLUSIONS

Biosensors are widely used in medicine to monitor or detect biological molecules for applications ranging from diabetes to cancer. The recent progress in micro- and nanoscale technologies shows significant promise in enabling a number of novel biosensing applications. For example, microcantilevers have been used to detect desired molecules without the need for a labeling agent, while nanoscale particles and cell/protein arrays have shown promise for improved sensing applications in biomedicine. Furthermore, through miniaturization, it is possible to fabricate biosensors that are portable, cheap, and highly sensitive that can be used for resource-poor settings for diseases such as HIV/AIDS. Therefore, the continued progress in the development and use of micro- and nanotechnologies for biosensors shows great potential in improving methods to diagnose diseases or to monitor their progression in medicine.

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