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Medical Biosensors for Point of Care (POC) Applications

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Introduction to medical biosensors for point of care applications

1

K. Baryeh, S. Takalkar, M. Lund, G. Liu
North Dakota State University, Fargo, ND, United States

1.1 Biosensors and medical biosensors

A biosensor is a self-contained integrated device capable of providing specific quantitative or semiquantitative analytical information using a biological recognition element which is in direct spatial contact with a transducer element[83]. Fig. 1.1 presents the basic scheme of a biosensor. It consists of three parts: the sensitive biological element, the transducer, and the associated electronics or signal processors that are primarily responsible for the display of the results in a user-friendly way. The biological recognition element plays a vitally important role because it determines the specificity of the biosensor. The biorecognition events can be recognized by various transducers to produce a signal (optical, electrochemical, and piezoelectric) proportional to a single analyte which is then conveyed to a detector. Biosensor research and development has been an area of increased research interest for over the last half-century. The first medical biosensor, an enzyme electrode for oxygen sensing, was reported by Professor Clark at the New York Academy of Science in 1962,[1] and was utilized for the continuous monitoring of oxygen levels in blood during cardiovascular surgery. Since then, there have been numerous advances in the field of biosensors drawing expertise from physics, chemistry, biochemistry, as well as engineering.[2] Biosensors have wide applications in the medical field for the detection of cancer, genetic disorders, and pathogens alike. The most widespread example of a commercial biosensor is the blood-glucose biosensor. These are known as medical biosensors and seek to increase the sensitivity, selectivity, and reliability of the biosensors, while reducing the time for sampling and the acquisition of test results. With the improvement in these areas, medical biosensors could become an essential tool in the field of medicine. Therefore, research is gearing toward miniaturizing and simplifying these for use in home-based preliminary screening.

1.2 Biosensors for point of care testing

One of the most important applications of biosensors is the point of care testing (POCT). POCT is the practice of performing a diagnostic or prognostic test near the patient to provide rapid results, meaning that the test itself has to be quick and easily performed without the use of expensive or complicated instrumentation. It also means

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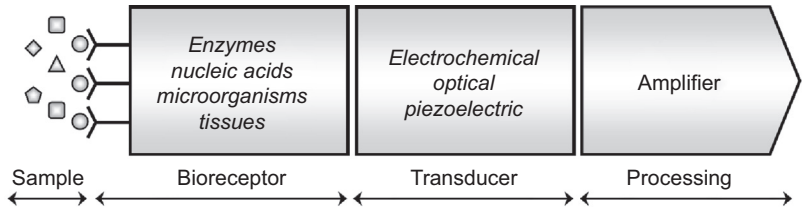

Figure 1.1 Basic scheme of a biosensor.[3]

that samples do not require the attention of any skilled technician as there is no need of the laboratory analysis, as well as no wait time for the collection and analysis of the results.[4,5] The person who is conducting the test (doctor, nurse, or the patients themselves) initiates the test and receives results on the spot, thus saving time. The POCTs are feasible in various environments; in general, these are the practitioner's surgery, hospital clinic, hospital ward, emergency room, intensive care unit, or even a patient's home. The need for sensitive, robust, portable, and inexpensive biosensor platforms is of significant interest in clinical applications for disease diagnosis and treatment monitoring at the point of care (POC) settings. In developing countries with constraints such as limited laboratory infrastructure, nonavailability of trained personnel, and—the major reason—lack of financial support, POC diagnostic assays play a crucial role. Millions die each year in the populated countries like India and China due to infectious diseases like malaria, AIDS, and tuberculosis. These developing countries are still struggling for access to treatment options available in developed countries.[6,7]

Accurate and early diagnoses play a crucial role in identifying the actual cause and nature of any disease. Currently, the emphasis and major focus are being shifted toward the early detection of a disease to effectively manage the treatments, thus reducing the mortality rates among patients. POCT and its ability to generate immediate results in nonlaboratory settings supports more patient-centered approaches for health-care delivery and is a prospect in the field of early detection.

1.3 Biorecognition elements of medical biosensors

The utility of any biosensor relies centrally on its ability to distinguish the target analyte from interfering molecules. As such, various biorecognition elements including antibodies, nucleic acids, cells, bacteriophages, and proteins have been employed by researchers over the years. This section seeks to review these biorecognition elements and show the strengths of each.

1.3.1 Antibodies

Traditionally, antibodies have been the main biorecognition elements used. Antibodies have been applied in various biosensor platforms including electrochemical,[8] fluorescent,[9,10] and colorimetric assays.[11] Antibodies provide varying stringency of interactions depending on whether they are monoclonal or polyclonal. Monoclonal antibodies

have the advantage of recognizing a single epitope of a target molecule, whereas polyclonal antibodies will recognize different epitopes of the same target. Because monoclonal antibodies are specific to a single epitope, they are less prone to cross-reactivity than polyclonals; however, the cost of production of polyclonals is more economical so polyclonals are still used. The most important application of the antibody-based biosensors is the development of immunochromatographic test strips for POCTs. The detailed POC applications of immunochromatographic test strips will be discussed in Section 1.4. However, antibodies as a whole suffer some drawbacks that limit their POC applications, namely they are relatively expensive to produce and must be stored under controlled low-temperature conditions. When not stored properly they denature and lose their binding ability which limits their utility in making assays for infield applications. This can be a big problem in poor communities with erratic power supply where continued refrigeration is not feasible. As a result, other biorecognition elements have been used in biosensors as shown in Fig. 1.2.

1.3.2 Nucleic acid probes

Nucleic acid research and detection continues to receive enormous attention from researchers because of their association with various genetic diseases and disorders as well as cancer. Various pathogens also show unique nucleic acid sequences that have been utilized in biosensors for their detection. Nucleic acids including single-strand DNA, peptide nucleic acids (PNA), locked nucleic acids, G-quadraplexes, and DNAzymes have been utilized as biorecognition elements to construct DNA biosensors.

1.3.2.1 Single-strand DNA

Typically, nucleic acid detection with a biosensor is based on DNA hybridization events, in which single-strand nucleic acid probes are immobilized on a solid support, acting as biorecognition elements. A lateral-flow biosensor for POC nucleic acid test was reported by Mao and colleagues[13] (Fig. 1.3). A capture DNA probe and a control DNA probe were immobilized on a nitrocellulose membrane to form a test line and control line. A detection DNA probe was immobilized on the gold nanoparticle surface by self-assembling process. The DNA–gold nanoparticle (DNA-AuNP) conjugate was placed on a conjugate pad. When a sample solution containing target DNA was applied, the solution moved toward the strip by capillary action and interacted with (DNA-AuNP) conjugate. The DNA probe on the AuNP hybridized with the target DNA to form DNA-DNA-AuNP complex. This complex then continued to migrate on the strip and was captured on the test line by the second DNA hybridization event between the capture DNA probe and the target DNA. The accumulation of AuNPs in the test line was visualized as a characteristic red band. Then, the excess DNA-AuNP conjugates continued to move and were captured on the control zone by the hybridization events between the control DNA probe and detection DNA probe on the AuNP surface, thus forming a second red band. In the absence of target DNA, no DNA-AuNP conjugate was captured on the test line, and no red band was observed.

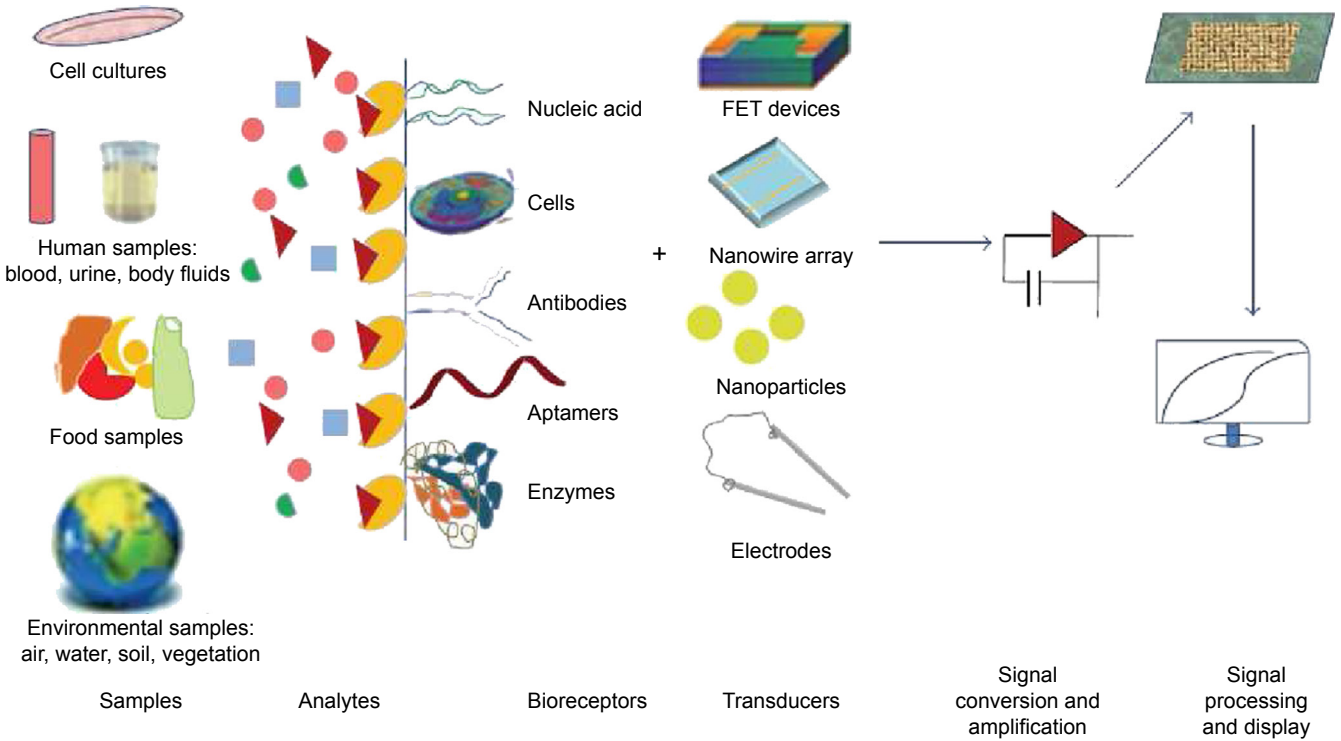

Figure 1.2 Scheme showing the components of a biosensor.[12]

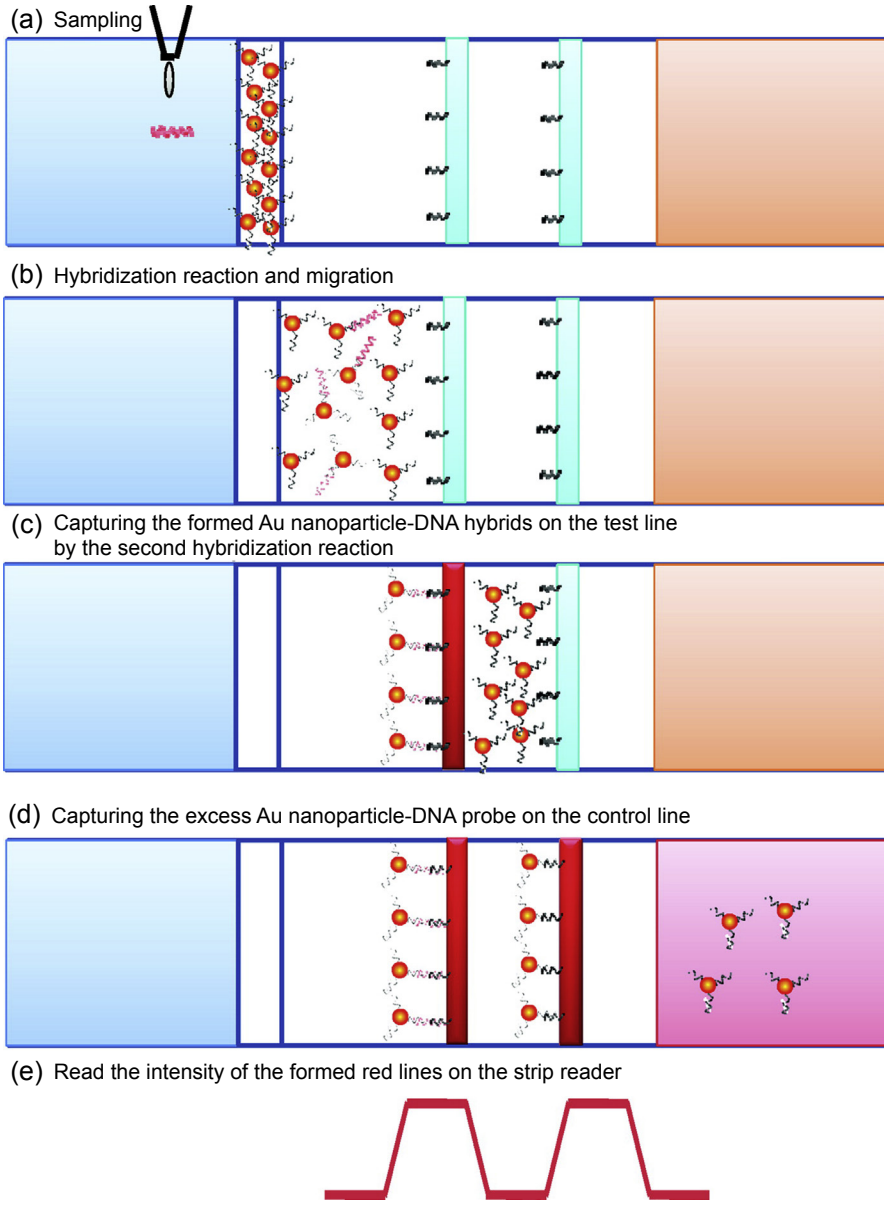

Figure 1.3 Schematic illustration for the visual detection of DNA on a lateral-flow test strip.[13]

1.3.2.2 Hairpin DNA

Single-strand nucleic acid with a hairpin structure has also been used as a biological element to develop DNA biosensors. The hairpin structure, often referred to as molecular beacon, is labeled with a fluorophore at one end and a quencher at the other end. The hairpin structure brings the labels into close proximity and causes fluorescence

resonance energy transfer (FRET) that quenches the fluorescence, whereas the hairpin structure will open up in the presence of target DNA/RNA leading to increased fluorescence as quencher is separated from the fluorophore. This is an example of a very common nucleic acid manipulation, whereas another way to manipulate nucleic acid is by the replacement of phosphodiester backbone.

1.3.2.3 Peptide nucleic acids

The nucleic acid backbone can be replaced with an N-(2-aminoethyl) glycine peptide backbone to form what has been termed peptide nucleic acids (PNAs). With this replacement, PNAs lack the negative charge from the phosphodiester backbone of regular nucleic acid analog and hence bind more effectively with complementary sequences because of the lack of electrostatic repulsions.[14] As a result, PNAs have been used in biosensors to increase sensitivity and DNA mismatch discrimination.

1.3.2.4 Locked nucleic acids

Another form of modified nucleic acids is the locked nucleic acid which is an analog of RNA with O_2 and C_4 and its ribose sugar furanose ring coupled by a methylene linkage. This modification maintains the three endo conformations of the ribose sugar which increases its affinity for target DNA or RNA.[15] The modification is important because it makes nucleic acid resistant to nucleases, making them more applicable in real samples as biorecognition element in biosensors.

1.3.2.5 G-quadraplexes

Guanine-rich, single-stranded DNA is able to fold and form structures called G-quadraplexes which are composed of four guanine bases linked to adjacent guanine by Hoogstein interaction through double hydrogen bond.[16] A single plane of a four-membered ring is referred to as g-quartet which can be formed in stacks as shown in Fig. 1.4. The unique property of G-quadraplexes that makes them valuable biorecognition elements in medical biosensors is their ability to bind anionic porphyrins which have aided the detection of targets including metal ions (eg, K^+, Ca^{2+}, Hg^{2+}, Cu^{2+}, Pb^{2+}), nucleic acids, and proteins.[16]

Binding of G-quadraplexes to probes results in change in either peroxidase activity, optical properties, or electrochemical signals, which are used to quantify target molecules.[16]

1.3.2.6 DNAzymes

DNAzymes are another class of nucleic acid probes that have been widely used in medical biosensors because of their ability to catalytically cut specific substrate when a designated cofactor is introduced.[17] DNAzymes were discovered by Breaker and Joyce in 1994 who found that in the presence of Pb^{2+} a particular single-strand (ss)DNA strand could catalyze transesterification reactions[18]; since then, various reports have been made discovering that DNAzymes catalyze cleavage, ligation, and phosphorylation of nucleic acids. DNAzymes are beneficial because of their ease of synthesis and stability compared to their RNA and protein counterparts.[18]

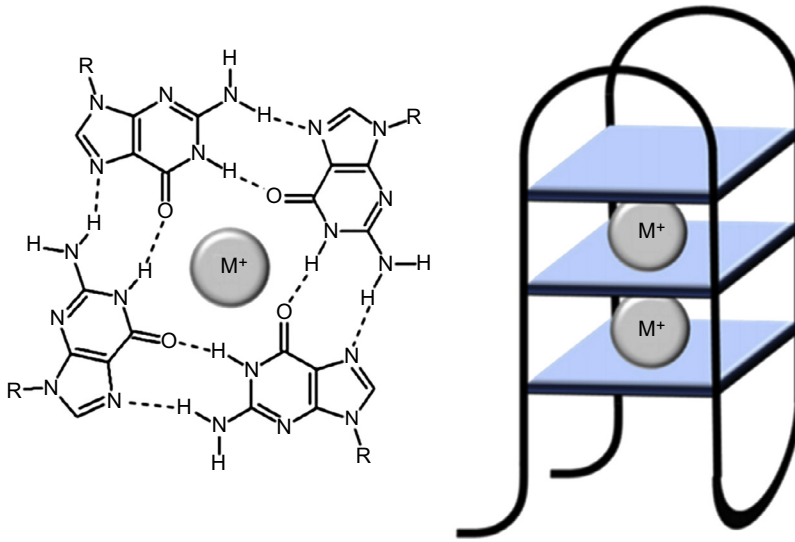

Figure 1.4 (Left) G-quadruplex with a centralized monovalent metal ion. (Right) A unimolecular stack of three quartets.[16]

1.3.2.7 Aptamers

Another class of nucleic acid probes used as biorecognition elements in biosensors are aptamers, short single-stranded nucleic acids, with distinctive binding characteristics to their target analyte unlike traditional hybridization-based nucleic acids assays.[19] Aptamers, under three-dimensional (3D) folding that assists in their specificity, bind to targets through Pi bond (π—π) stacking, electrostatic interactions, London forces, and hydrogen bonding, often in various combinations.[20] Aptamers are identified by a procedure termed Systematic Evolution of Ligands by Exponential Enrichment (SELEX) which was first reported by Gold and Tuerk[21] in 1990. In the SELEX procedure, the target molecules are incubated with a random pool of nucleic acid oligonucleotides (usually $>10^{12}$ sequences) over multiple steps of selection to produce a small pool of sequences that bind the target with high affinity and specificity. Depending on the complexity of the target, the selection can involve about 4–7 rounds in pure proteins[22] to between 20 and 23 rounds for complex targets such as whole cells.[23] This process has been used as a wide array for the identification of targets spanning heavy metals,[24] small molecules,[25,26] proteins,[27,28] and whole cells.[23,29] In addition to the wide variety of targets, aptamers offer the advantages of low cost of synthesis, ease of chemical modification, high thermal stability, and high binding affinities (K_d in low nano- to picomolar ranges). In a simple colorimetric test as shown in Fig. 1.5, aptamers for Ramos cells were captured between immobilized aptamers on a lateral-flow strip and aptamer-coated gold nanoparticles (AuNP-TD05 Aptamer).[34] The presence of AuNPs resulted in an intense red-colored test line, the intensity of which was correlated with increasing Ramos cells concentration. Excess AuNP-TD05 Aptamer then moved on to bind to a control line by base pair complementarity to give a second red line for quality

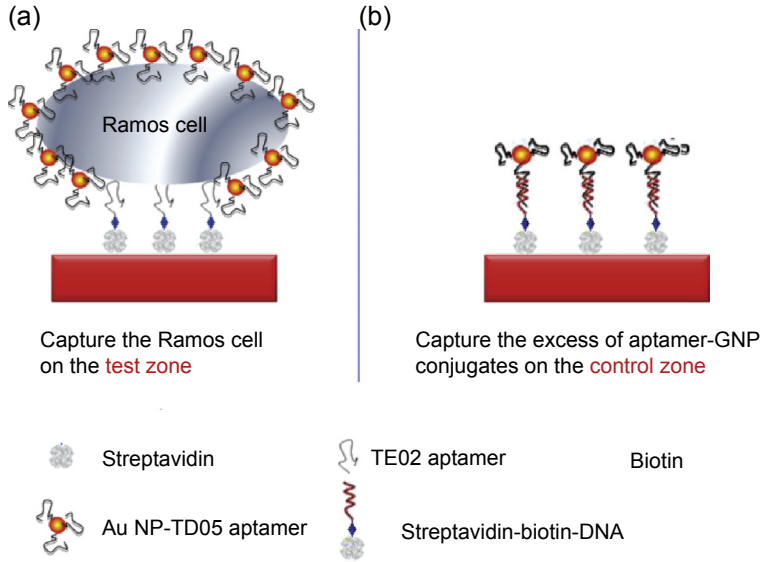

Figure 1.5 Aptamer-based lateral-flow detection of Ramos cells. (a) Ramos cell capture on the test line between immobilized aptamer and AuNP-TD05 Aptamer conjugate, and (b) hybridization-based capture of excess AuNP-TD05 Aptamer on a control line.[34]

control of the assay. Aptamers have been coupled with various transduction systems such as electrochemical,[30–33] optical,[34–37] and piezoelectric[38–40] for the detection of a variety of targets.

1.3.2.8 Other biorecognition elements

To a lesser extent, bacteria, bacteriophages (phages), and proteins (enzymes) have been applied as recognition elements in biosensors. Bacterial whole cells have been mainly used as biorecognition elements for toxic screening. Measurable gene products of reporter genes are monitored upon exposure of bacteria to toxins that induce cellular stressors. This has shown much promise for environmental monitoring.[41] Phages are viruses that attack bacteria and use the bacteria replication system for phage duplication and bind specifically to bacteria cell-surface receptors, thus they have the capacity to be used as biorecognition elements in sensors for the detection of clinically important bacteria. Phages have the advantage of easy immobilization and high specificity to their target bacterial cells.[42] For signal read-out of phage biosensors, both labeled and label-free assays have been reported. Labeling with fluorophores, however, tends to be a tedious, long process,[43] hence label-free methods are predominant.

Enzymes have been widely used as biorecognition elements in biosensors as they are able to catalyze reactions that produce electrochemical, optical, and thermal signals.[44] Most popularly, glucose oxidase is widely used in electrochemical glucose biosensors that have found wide usage in clinical and medical settings. Enzymes offer ease of immobilization on transducers by physical adsorption, covalent linkage, and

entrapment in hydrogels. By taking advantage of these biorecognition elements, multiple POCTs have been designed and are now commercially available.

1.4 Medical biosensors for point of care applications

Lateral-flow devices are among the most-established POCT platforms as they fulfill most of the aforementioned characteristics of an ideal POC platform. The idealized concept of a biosensor (Fig. 1.6)[45] in POC applications includes a transducer coupled with a recognition element. The recognition event is then converted into a useful analytical signal (mainly electrochemical or colorimetric). The recognition elements include proteins, nucleic acids, and many more as elaborated in Section 1.3.

The first lateral-flow immunoassay was developed in the form of latex-agglutination assay by Singer and Plotz in 1956.[46] Since then, significant advancements have been made in the manufacturing of the lateral-flow strips for the detection of various diseases. This idea of detection is being implemented into various fields for early detection of cancer, treatment of infectious diseases like Human Immunodeficiency Virus (HIV), malaria, influenza, and many more.

An excellent example of protein detection test strip available in the market and being used is the home-based pregnancy strip. These strips measure the presence of human chorionic gonadotropin (hCG), a hormone secreted in urine during pregnancy.[47] The kits rely on the reaction of a monoclonal antibody with the hCG present in the urine causing a color change. This test is administered by the application of urine to the latex-coated test strip that is pretreated with antibodies. The hCG antibody in the urine of a pregnant women reacts with the anti-hCG antibody dispensed on the membrane which is coupled with an indicator turning it into a bright red-colored band,

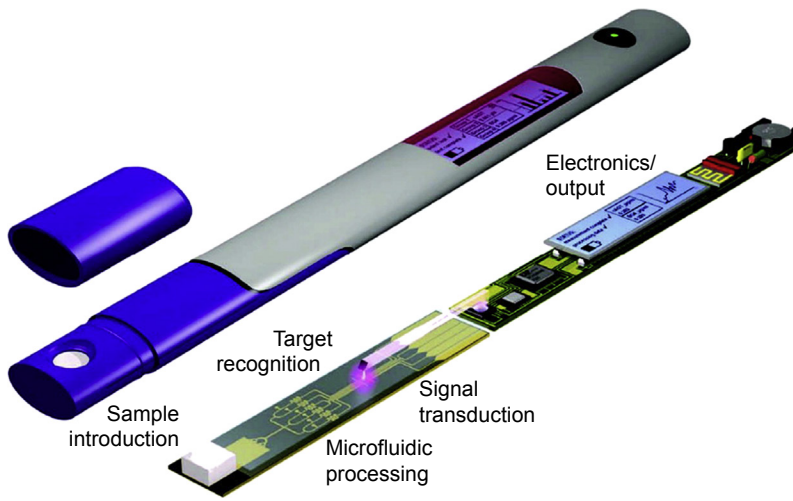

Figure 1.6 Idealized concept of a biosensor.[45]

which is often observed in the test window of the strip. The antibody will only bind to hCG, and the other hormones will not give a positive result. Therefore, in the absence of hCG hormone in the urine sample, only a control line is observed indicating that the test is negative, and the strip provides a valid test.[48] These test strips have gained a lot of attention, especially in the developing countries, because of its advantages such as stability (for more than a year), easy interpretation of the results (not requiring trained personnel), and usage at room temperature (no refrigeration required for shipping and storage).

Initially, lots of rapid tests were introduced for different disease targets like HIV, tuberculosis, and hepatitis-B in the dip-stick format, but gradually the immunochromatographic strips gained a lot of importance and attention in the medical field. Many protein-based biosensors have been developed, like hCG for pregnancy as described earlier, such as prostate specific antigen for prostate cancer, cardiac troponin I (cTnI) for myocardial infarction, to name a few. The general design and properties of these biosensors is as shown in Fig. 1.7.

Another well-established, leading POC test available commercially are glucose assays. According to a report from International Diabetes Federation, the numbers of diabetics worldwide are expected to reach 380 million by 2025 outnumbering the present 246 million. With this increasing demand, the production and usage of glucometers is very high making the business for companies like Abbott, LifeScan, and

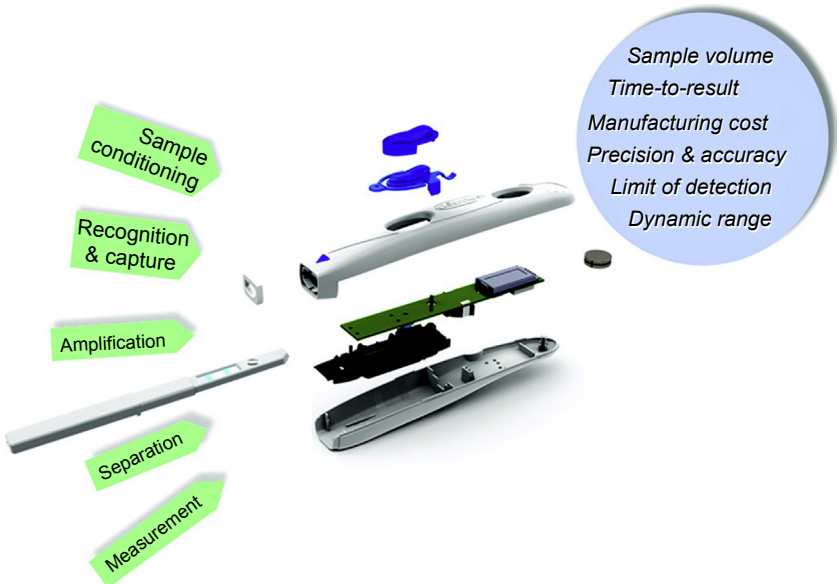

Figure 1.7 The pregnancy test-strip which gives the optical and digital read-out in a single-stick format (left in the picture) works with the interaction of the overall unit operations and specifications.
Image by Unipath, Ltd. Gubala V, Harris LF, Ricco AJ, Tan MX, Williams DE. *Anal. Chem.* 2012;**84**:487–515.

Bayer very profitable. The two major types of glucometers are colorimetric and electrochemical read-outs which depend on the type of enzymatic reaction. The enzyme portion of the glucometer is generally packaged in a dehydrated state on a disposable strip or reaction cuvette, so in the presence of glucose in the patient's blood sample, the enzyme gets rehydrated resulting in a detectable reaction product. Some meters generate hydrogen peroxide or an intermediate that can react with a dye, resulting in a color change proportional to the concentration of glucose in solution. This is an example of a colorimetric biosensor, whereas other meters incorporate the enzymes into a biosensor that generates an electron producing an electrochemical signal that is detected by the meter. There are three principal enzymatic reactions utilized by current glucometers: glucose oxidase, glucose dehydrogenase, and hexokinase.[49] Efforts have been continuously focused on miniaturizing the design of the glucometers with the needle-type to the current finger-prick ones. Not only has the finger prick-type biosensors been utilized for glucose monitoring but has also been extended for the detection of other metabolites like creatinine, urea, lactate, uric acid, cholesterol; most recent development has been the simultaneous detection of more than one metabolite.

Numerous rapid onsite tests with these biosensors are used in developing countries. Some of these examples include tests for malaria (*Plasmodium falciparum*) in countries like India and Africa, tetanus (*Clostridium tetani*) immunity test in Iran, Ora-Quick Rapid HIV antibody test, C-reactive protein test for meningitis in South Africa, a test for trypanosomiasis in Angola, and a test for *Leishmaniasis* in Brazil, India, Kenya, and Sudan,[50] naming just a few. Presently, research is being focused in the areas for the development of medicinal biosensors for infectious diseases including measles, mumps, influenza, respiratory viral infections, HIV, meningitis, rubella, gonorrhea, *c*hlamydia, filariasis, tuberculosis, *Legionella*, and *Streptococcus*.[51]

These POCTs have presented many advantages; however, doctors and medical practitioners have reported some disadvantages when it comes to using these biosensors in real-sample diagnostics, and their growing popularity raises safety concerns among the patients. Some medical practitioners who give importance to the inclusiveness rather than the speed are still of a notion that the POC testing is no replacement for laboratory testing, because compared to the full lab workups, the range of the values a device can test is definitely more limited. Although some are of the opinion that, though the rapid results represent an advantage, there is a compromise on the quality of the testing and that the POC devices will not always deliver the best-quality results. Although rare, sometimes there exists an issue of false negatives. For example, it is possible to get a negative result from a home pregnancy test when someone is actually pregnant and vice versa.[52]

However, there is improvement and the current developments in the field of biosensing certainly outweigh the disadvantages mentioned earlier. Over the past few years, numerous biosensors for POC applications have been developed in the field of medical diagnostics, food-quality monitoring, industrial quality control, environmental monitoring, and biological warfare detections. Though most of these biosensors are still limited to research and development, efforts are being devoted to their commercialization.

Industrial firms like Alere, Trinity Biotech, Quidel Corporation, to name a few, are involved in the production of these lateral-flow biosensors with Alere being a leading producer. Examples of the commercially available lateral-flow biosensors by Alere include tests for lymphatic filariasis, malaria, Acquired Immune Deficiency Syndrome (AIDS), dengue fever, influenza, tuberculosis in HIV-positive patients, pneumonia, bladder cancer, and colon cancer. Other industries have made substantial contributions tests for Legionnaire's disease and myocardial infarction by Trinity Biotech, infantile bronchiolitis by Quidel Corp., prostate cancer by CTK Biotech., myocardial infarction by Boditech Med Inc., congestive heart failure by LifeSign, hepatocellular cancer by Innovation Biotech, acute myocardial infarction by Response Biomedical and American Screening Corporation.[53] Based on the areas that need improvement, multiple POCTs have been developed and heavily researched as discussed in the next section.

1.5 Overview of types of point of care techniques

Taking advantage of biorecognition and applying this to the POC objective, an abundance of methods is available in which fast, sensitive testing can be accomplished. To make this easier to understand, the POC techniques have been divided into six broad categories including: lab on chip, label based, label-free based, nanomaterial based, wearable, and wireless. Table 1.1 gives an overview of the types of POC techniques, the analytical methods applied to these techniques, as well as demonstrating the versatility in what these POC techniques can detect and the amount of time required.

For the rest of this section, these categories of POCT will be briefly introduced and an example will be expanded upon; however, more information will be discussed in further chapters.

1.5.1 Lab on chip

Lab on a chip is classified as a miniaturized device that has the capability to analyze one to multiple parameters. This is done by taking advantage of microfluidics and molecular biotechnologies, a chip can fabricate numerous microchannels. In these microchannels, antibodies, antigens, or oligonucleotides are embedded allowing for thousands of biochemical reactions to be analyzed.[12] It is this reason that multiple reactions can be analyzed from a single drop of blood. Numerous materials can fabricate lab-on-chip biosensors including: glass, silicon, thermoplastic polymers, paper based, and polydimethyl siloxane (PDMS). However, paper based and PDMS are more widely used for the simple reasoning that these have low cost and are easy to fabricate.[12] There are many applications that can be taken advantage of when using lab on a chip. For instance, a main application from a molecular biology perspective is that lab on chip allows the fastest way of PCR by performing a high-speed microscale thermal shift. Lab on a chip is very versatile and has an ability to be quite useful in the POC technique; for example, a POC can be used to diagnose nonsmall-cell lung cancer. This was done by using a working principle of nanoporous glass integrated in

Table 1.1 Summary of point of care testing

POC technique	Method of detection	Target	Time	Limit of detection	References
Lab on chip	Microfluidic colorimetric	Nonsmall-cell lung cancer biomarker: CEA antibody	30 min	~50 pg/mL	54
	Magnetic integrated microfluidic electrochemical detector (MIMED)	Influenza virus: H1N1, RNA	3.5 h	10 TCID50	55
	Microfluidic electrochemical quantitative–loop mediated isothermal amplification (MEQ-LAMP)	*Salmonella* genomic DNA	<50 min	16 copies/μL	55
	Colorimetric	Urinary protein to assess kidney function	25 min	8.5 μg/mL	56
Labeled	Lateral-flow assay: fluorescent probe	Nucleic acid for HPV types: 6, 11, 16, and 18	30 min	10 copies/μL for types 6, 11, and 16, and 10^2 copies/μL	57
	Electrochemical detection labeled with silver nanoparticles	Study between nanoparticles to use (silver nanoparticles)	~7 min	2.1 pM	58
	Fluorescent lateral-flow immunoassay	Hepatitis B virus genotypes: A, B, C, and D	20 min	Genotype A: 2.5 IU/mL Genotype B & C: 5 IU/mL Genotype D: 10 IU/mL	59
	Electrochemical	Enzyme-based E-DNA		10 fM	60

Continued

Table 1.1 Continued

POC technique	Method of detection	Target	Time	Limit of detection	References
Label-free	Colorimetric	*Mycobacterium tuberculosis* (Mtb) DNA	75 min	1 cfu	61
	Colorimetric	Potassium DNA (metal ion)		1 mM	62
	LSPR	Tobramycin	Real-time	3.4 μM	63
	Fluorescence	*Vibrio parahaemolyticus* ssDNA and *Salmonella typhimurium* ssDNA	2 ½ h	*V. parahaemolyticus*: 35 cfu/mL and *S. typhimurium*: 25 ecfu/mL	64
	Sur surface-plasmon resonance	Cortisol antibodies	8 min	38 ppt	65
Nano-material based	Labeled with gold nanoparticles, colorimetric lateral-flow assay	Influenza A: H1N1 RNA	45 min	10^6 copies/mL	66
	Single-walled carbon nanotubes (SWCNTs) electrochemical	Thrombin protein through the use of aptamers	Real-time (within 75 s)	88 ag/mL	67
	graphene-DNA biosensor electrochemical	Cu^{2+}	60 min	0.5 fM	68
Wearable	Electrochemical	Alcohol in perspiration	5 min after consumption	0.0005 g/L	69
	Electrochemical tattoo	Lactate in perspiration	Continuous monitoring up to 8 h	1 mM	70
	Soft-contact lens electrochemical biosensor	Glucose concentrations as hydrogen peroxide, product of glucose oxidase reaction	Real-time	0.03 mM	71
Wireless	Electrochemical graphene on tooth enamel	*Staphylococcus aureus* cells	Continuous	1 bacterium/μL	72
	Magnetic resonance relaxometry	Cardiac biomarkers antigen doxorubicin	Continuous, up to 72 h	–pg/mL range	73
	Optical spectroscopy	Paraoxon: Neurotoxin	–	2.9 nM	74

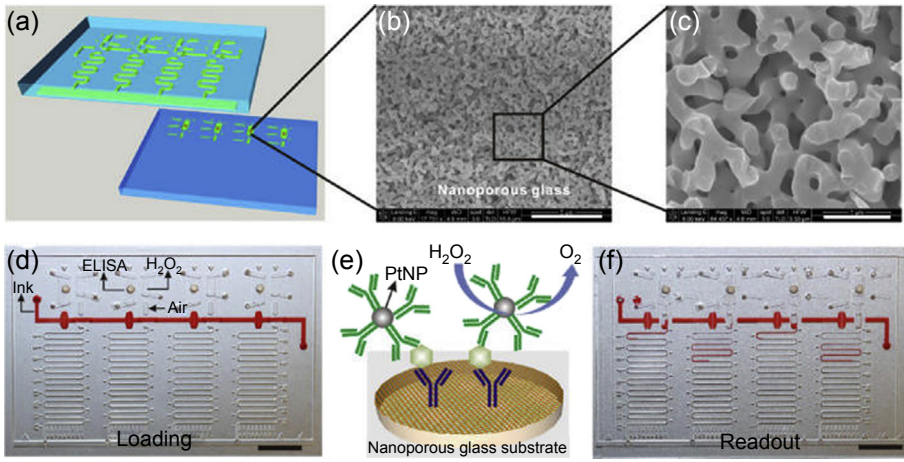

Figure 1.8 (a) Schematic of NPG-V-Chip b and c, SEM images of plate, 5um (b) and 1 um (c), (d) loading phase of the device, (e) principle of biomarker detection, (f) readout phase of the device.

volumetric bar-chart chip (NPG V-Chip) in which carcinoembryonic antigen (CEA), a biomarker for lung cancer, was conjugated onto the NPG surface; when CEA antigens are present in the solution they would bind to the capture antigens (see Fig. 1.8). Then, using platinum nanoparticles conjugated to a secondary antibody specific for CEA the antigen would bind, thus creating a sandwich. The reaction is catalyzed by adding hydrogen peroxide to produce oxygen gas. This would then propel red ink through the serpentine channels to form a bar chart, indicating that the farther the red ink travels, the higher the concentration of CEA in the sample.[54] Essentially, this method could also be classified as a labeled method, because the detection utilized by Enzyme-Linked Immunosorbent Assay (ELISA) has taken place on an NPG V-Chip, it allows a multitude of reactions to be observed and analyzed and is therefore classified as lab on chip. However, not all lab-on-chip techniques utilize a labeled technique.

1.5.2 Labeled

A labeled POC arises when the target itself is not the transducer; instead, when the target is present, it starts a series of events which then leads to a signal. The most appropriate example of labeled POC systems would be by using ELISA or enzyme-linked immunosorbent assay.[75] This works in a simple manner as described in the previous section for the detection of CEA antigen; briefly, an antibody is bound to a surface, which will bind only to a specific antigen. When the target antigen is present and binds to the capture antibody, then a secondary antibody binds to the capture antigen, creating essentially a sandwich between the bound antibody, antigen, and the secondary antigen. The secondary antigen is labeled with nanoparticles, enzymes, or micron-sized beads. Not all labeling methods are accomplished through the interactions between antibodies and antigens; another common labeled technique can also be accomplished between DNA strands. This was demonstrated in the simultaneous

detection of four different types of the human papillomavirus (HPV) using fluorophore labels. The four different types that were detected were HPV types 6, 11, 16, and 18 genomic plasmids with lateral-flow assay (LFA) (see Fig. 1.9). Samples in this technique had to perform a PCR in which primers would be labeled with fluorophores specific toward each type of HPV selected in this study. The nitrocellulose membrane contained four test lines, one specific toward each type of HPV, and then a control line to ensure that the LFA was working properly. The capture probe in this case was ssDNA that was specific toward individual strains of HPV, after the PCR in which the target was labeled, the samples were then tested on the LFA. Samples traveling through capillary action, the target ssDNA would then be captured, thus forming a sandwich between the capture ssDNA probe, target ssDNA, and the fluorophore signal. Then the fluorophore signal would be evaluated by fluorescence reader.[59] This is just one of the many ways in which a labeled POC technique can work; however, a huge drawback of this method was the time it takes to label the sample. After this process is complete, the given study was accomplished within 30 min. Another huge drawback is that the instrumentation needed for labeling would be limited to specific laboratories. It is this reason that label-free techniques are also studied.

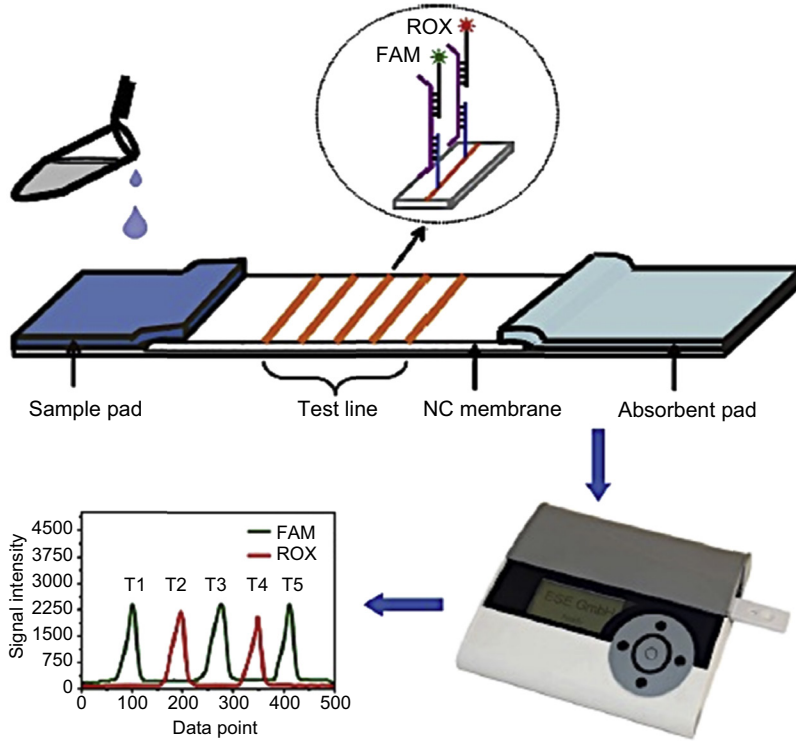

Figure 1.9 Configuration and measurement principle of the fluorescent probe-based nucleic acid lateral-flow assay. Where FAM(fluorescein) and ROX(6-Carboxyl-X-Rhodamine) are the fluorescent labels and how measurements are quantified.

1.5.3 Label-free

In a label-free POC technique, the target itself contains the transducer; therefore, no label is needed for detection. This technique has an advantage because it eliminates the need for labels and thus the need for sample processing steps.[75] In the previous section in which the sample had to be labeled through a PCR, reducing the time in which the targets could be analyzed would be cut down immensely as well as cost for the test itself. However, a downside to this method is that samples often need to be amplified before use, and, without this, a higher limit of detection can be observed. An example of a label free technique is accomplished by taking advantage of a plasmon shift measured by surface plasmon resonance. A typical SPR works when a specific wavelength is shown at the interface between a dielectric and a thin layer of conductive material. Typically this layer of conductive material is gold however other metals can be used. This yields an evanescent plasmon wave, which is sensitive to the refractive index neighboring the interface. However there is another SPR approach called a localized surface plasmon resonance or LSPR. The way in which this is made is shown in Fig. 1.10. This works very similar to a typical SPR however an LSPR creates a disconnect pattern of the conductive metal to generate a non-propagating plasmon. Therefor the characterization of the sample can be made from the transmitted light giving a normalized signal. The greater differences in the normalized signal can be correlated to the amount of concentration in the plasmon. In this method, tobramycin was detected through the use of aptamers with a LOD of 3.4 μM from filtered undiluted blood sample. This method has been proven to measure several biomarkers in 50% blood serum and has been proven to work in either direct or sandwich type assays.[63] This is just one of the many ways in which a label-free detection method can work. Many of these methods have been taking advantage of nanomaterials for detection, which leads into the next section in which POC techniques are based on nanomaterials.

1.5.4 Nanomaterial based

Nanomaterials are classified as small particles that are 10^{-9} m in size. These nanomaterials can be metals such as gold, silver, copper, or platinum, to semiconductor

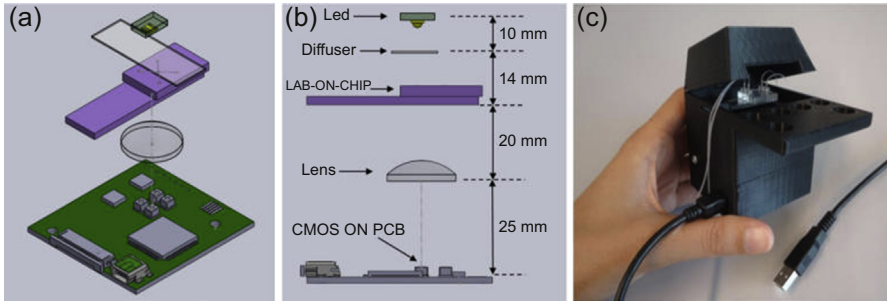

Figure 1.10 Schematic representation of the LSPR: (a) Digital rendering containing components. (b) Side view of components with relative distance. (c) Side view of complete set up

quantum dots like cadmium, tellurium, or selenium such as carbon or magnetic.[76] In most cases, metals and the semiconductor quantum dots are typically used as labels in the POC techniques; however, there are techniques that take place on nanomaterials such as single-walled carbon nanotubes (SWCNTs) and graphene. These types of materials are very useful in POC-type devices because they are low-dimension carbon nanomaterials that are composed entirely of sp^2-hybridized carbon atoms that are arranged in a honeycomb lattice. This type of lattice offers a compatibility with organic and biochemical molecules. Graphene and SWCNTs are atomically stiff, and naturally have an end functionalized with carboxylic acid groups allowing an ease of covalent linkage to molecules.[77] Nanomaterial-based methods are advantageous in that nanomaterials themselves have a high surface-area-to-volume ratio, high electrical conductivity, magnetic properties, and unique physiochemical properties. These allow nanomaterials to have multiple uses.[78] However, nanomaterials have a few disadvantages such as possibly toxic, their small size and large surface area can lead to particle aggregation, and they can have limited loading capacities.[79] With the knowledge of the advantages and disadvantages of nanomaterials, a nanomaterial-based POC technique can be designed to be quite effective. This was shown when individual DNA aptamers were coupled with SWCNTs at point contacts to form single molecule devices allowing selectively and reversibly detecting thrombin in real time (see Fig. 1.11). In this study, the SWCNTs contained a nanogap, which would fit exactly one aptamer specific toward

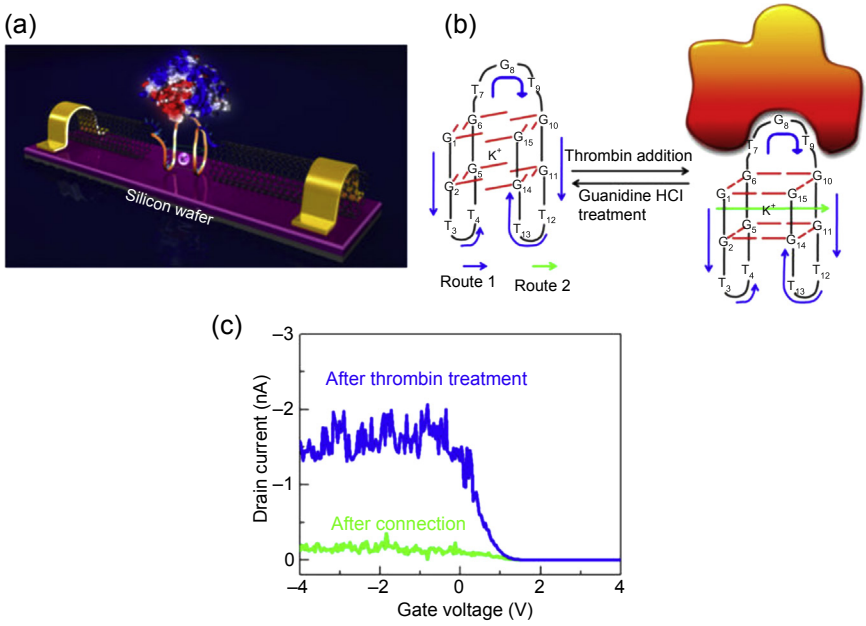

Figure 1.11 (a) Depiction of the single-walled carbon nanotube (SWCNT) and the nanogap, (b) interaction between the aptamer and the thrombin, and (c) electrochemical resistance readout for the measurement of thrombin.

thrombin. When thrombin was present, it would bind specifically to the aptamer and thus keep the aptamer in a rigid conformation thus enhancing the charge transport, giving an increase in current.[67]

This study leads to a very low detection limit and can offer results within 75 s as well as being reusable. The disadvantage of this device is that it still needs a technician to understand the results; however, with more research accomplished and applying this technology to other POC techniques, it can prove to be quite useful.

1.5.5 Wearable

A wearable POC technique offers attractive alternatives to other POC techniques because these types of devices are not as bulky, expensive, and can offer simpler analytical instruments that are typically used in the health care industry. Most sensors used today rely on blood samples, however this can be difficult to obtain especially if the objective is to have continuous monitoring for an extended amount of time. A wearable POC allows for continuous noninvasive chemical sensing by taking advantage of physical sensor devices, this is done by examining saliva, tears, and perspiration.[80] This can be very useful for people with diabetes or cancer patients recovering from surgery, but it can also be helpful in monitoring blood-alcohol content (BAC) levels (see Fig. 1.12). This can be vitally important because alcohol-impaired driving crashes account for nearly one-third of all traffic-related deaths in the United States.[81] Therefore, designing a wearable device in which blood-alcohol levels could be monitored by ethanol in perspiration could offer a reliable way to track alcohol intake and thus enhance road safety. When alcohol is consumed, the body treats it as a toxin and is broken down by the enzyme alcohol oxidase. The product of this reaction is secreted through perspiration and upon being exposed to oxygen produces hydrogen peroxide. With an increasing amount of hydrogen peroxide produced, it will start a cascading reaction and produce an electrochemical current. With more hydrogen peroxide present in perspiration, the higher the electrochemical signal indicating a higher blood-alcohol content concentration. This has the ability to have continuous monitoring of BAC after 30 min of ingestion for up to 8 h.[69] This is a perfect example of a wearable device.

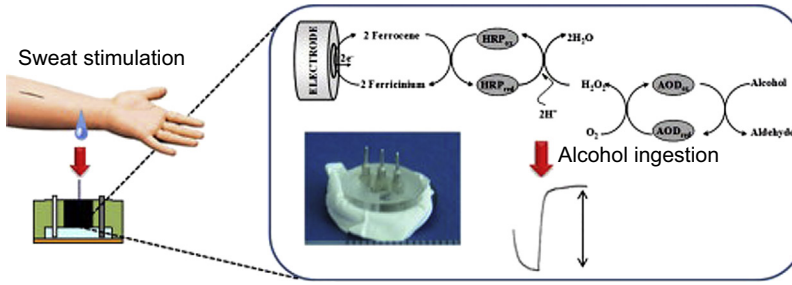

Figure 1.12 Depiction of perspiration detection of blood-alcohol content (BAC) through the electrochemical detection of the alcohol oxidase.

1.5.6 Wireless

A wireless POC is a device that can provide a continuous monitoring of the wearer's health, tracking exercise activity, assessing a soldier's performance, as well as analyzing the wearer's environment. There are two main categories of wireless devices, invasive and noninvasive.[82] An invasive device is one which typically monitors blood samples; however, this can pose a major hurdle to the patient and impedes the temporal information acquisition that is desired for diverse biomedical applications. This can be true in the case of neonatal, the elderly, and patients for whom blood sampling is challenging; therefore, there has been a lot of interest in noninvasive wearable biosensors. Noninvasive biosensors rely on samples such as saliva, perspiration, and tears. A perfect example of a wireless device is integrating POC techniques onto a smartphone. This is proven possible by the detection of neurotoxins that inhibit cholinesterase which can lead to abdominal cramps, neuromuscular disorders, hypotension, coma, and even seizures; therefore, the detection of this inhibition can be vitally important. This was performed by the Smartphone Optosensing Platform (SOP) which essentially allows for the attachment of a cuvette onto a smartphone and the spectrum of samples to be observed from 450 to 600 nm (essentially the spectrum for visible light). The decrease in visible light indicates that more neurotoxins are present; this was quantified through the measurement of absorbance.[82]

Wireless devices could be potentially used for biomedical devices which offer many advantages over classical testing, because wireless devices can offer an improvement to quality of life in patients with chronic disease who are subjected to daily analytical biosensing.[80]

In this section, six broad categories of POC techniques have been summarized with examples in Table 1.1. The categorization of the POC techniques can be quite difficult because technologies/methods used often overlap, and, for instance, the lab on chip method can often use ELISA, which is also a labeled technique. However, depending on what the target is for the detection method and overall objective for the POC, one can easily design a test for that purpose by taking advantage of any one or a number of the techniques described.

1.6 Conclusion

Many countries, especially those developing, have been reported to have high mortality rates which can be attributed to cancer, HIV, and many more diseases that are infectious. Cancer, if diagnosed at an early stage, is more likely to be treated successfully because adequate time has not been given for the cancer to spread. If the cancer starts to spread, treatment becomes more difficult, thus lowering the chances of patient survival rates. Delay in cancer diagnosis can be prevented if there is a reliable, inexpensive, and easy-to-use diagnostic tool like the medical biosensors, thus improving the mortality rates.

To combat this ever-growing need of the early detection, there exists a need to develop POC diagnostic tests for convenient use among patients. There are some

medical biosensors reported for the detection of various diseases like HIV, malaria, and typhoid. Use of novel recognition elements and various transducing elements as mentioned in the earlier sections have contributed in solving the associated weaknesses like sensitivity and accuracy. Nevertheless, there still exist areas for improvement such as reproducibility, elimination of false positives, and to provide tests that may be more reliable for the early detection of deadly diseases like cancer. Further interest in the research and development of these medical biosensors for POC applications would enable routine health checks at home, thereby detecting the abnormalities at an early stage, thus saving millions of lives.

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Validation and regulation of point of care devices for medical applications

2

S. Misra, J. Huddy, G. Hanna, N. Oliver
Imperial College, London, United Kingdom

2.1 Introduction

Point of care test (POCT) devices are increasingly developed for numerous biological analytes and medical applications [1,2]. POCT devices are attractive in clinical practice as they enable rapid diagnosis or clinical decision-making on the basis of results obtained in real time, which may then impact on clinical pathways [3]. However, before implementing POCT devices for clinical applications, a number of steps are required to comprehensively validate the method [4,5]. These steps are applied to any new laboratory analytical methodology as part of formal acceptance testing, but are equally applicable to POCT and aim to verify the manufacturer performance specifications of the method. Providing performance specifications for in vitro diagnostics is a legal requirement that manufacturers must fulfil and for laboratories to be fully accredited, validation or verification of these specifications must be undertaken [6]. It is debatable whether proof of simply meeting pre-specified criteria is sufficient to validate a new test; therefore, before new tests (either POCT or laboratory) are implemented, there is often a second phase of evaluation that assesses clinical performance of the test in a specific context such as in a relevant patient group or location. This second phase ideally measures clinical effectiveness of the test in comparison to a gold standard for diagnosis or another intended use.

The spectrum of what constitutes a validation of a POCT device is broad and may range from a superficial examination of the device to an extremely detailed and comprehensive assessment of both analytical and clinical performances. The extent of validation may also depend on the device itself; for example, the next generation of an established POCT device may only need to demonstrate non-inferiority from the previous model. However, a POCT device for a new analyte may require a more in-depth assessment that assesses analytical performance and compares the device to existing testing strategies and clinical pathways. Validation plans for POCT devices should be completed before implementation with involvement of local laboratories as stipulated by local accreditation bodies.

It is also important to consider whether the provision of POCT methods to measure analytes is required in the clinical context. One of the first steps, therefore, in implementing these devices is to establish that a clinical need exists and integration of POCT devices into a clinical pathway is warranted.

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2.2 Analytical method validation

Method validation is a process that provides objective evidence that requirements for a specific intended use of the method can be fulfilled consistently; in other words, that the method meets stated performance [7]. Method verification (a term often used interchangeably with validation) is the provision of objective evidence that a given item also fulfils specified requirements [7]. Together, these two processes underpin the evaluation of a methodology, which defines the analytical and clinical performance characteristics and ensures they meet pre-specified criteria. In the context of POCT devices, validation is likely to be undertaken once the device has been regulated and is available on the market. In most cases, commercial POCT methods will already have been validated by the manufacturer. These are the assay parameters that are often stated on the kit insert that accompanies the POCT device. The user (a laboratory in most cases), however, must independently verify that these characteristics can be reproduced locally.

2.2.1 Why is validation required?

Any analytical method requires an evaluation before being implemented. This is to ensure, as a minimum, that the manufacturer-stated performance characteristics are met, but the job of validation is far greater as it also, for the first time, demonstrates real-world performance undertaken by end users in the chosen clinical scenario and location (see Fig. 2.1). It is worth noting that the published studies validating the POCT methods are not always performed in the settings in which they will ultimately be used and may be undertaken by trained laboratory professionals rather than end users [8]. This disparity is likely to underpin the variability in the way POCT methods perform in user hands [9]. The performance of assays may not meet manufacturer-stated references for numerous reasons that span from reagent instability and storage issues to user expertise and lack of quality control and quality assurance. Establishing how, and by how much, an assay in user hands deviates from the stated performance criteria is a critical part of method validation, and is important for users to establish as it may influence interpretation of assay results, reference ranges or, if the degree of deviation is unacceptable by clinical criteria, may preclude implementation of that particular test (Fig. 2.2).

The validation of POCT technologies is arguably even more important as POCT devices have features that make them vulnerable to poor performance, these may include:

- Compromises to the quality of the analytical methodology (in some cases)
- Pre-analytical variability: change in sample matrix and patient condition
- Specimen, reagent and control degradation
- Less-controlled user environment (outside the laboratory)
- Variety of users with wide spectrum of credentials, backgrounds and experience (see Box 2.1)
- Inadequate and inconsistent quality maintenance (internal quality control and external quality assessment)

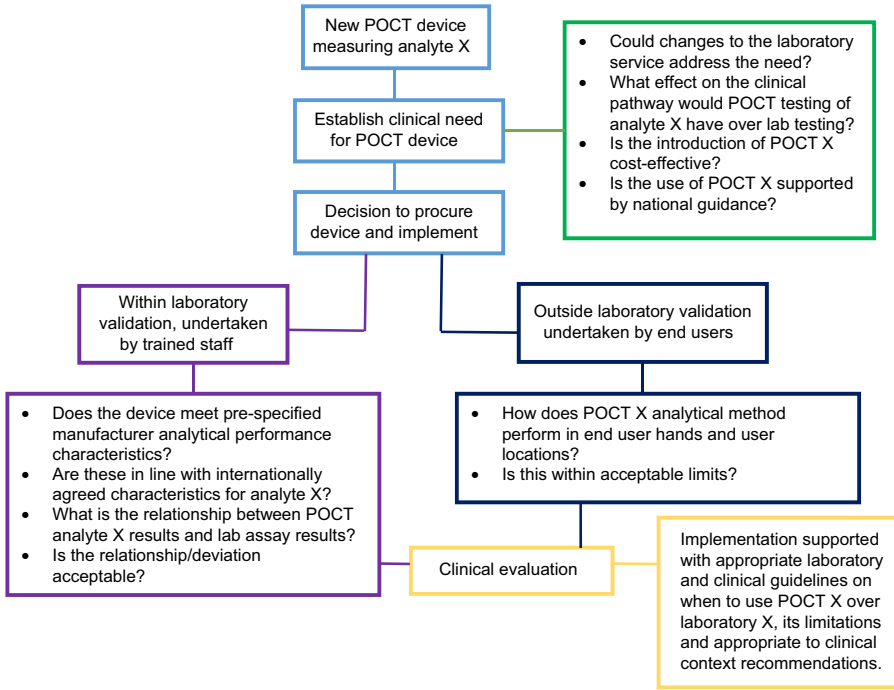

Figure 2.1 Key components of an ideal point of care test device evaluation.

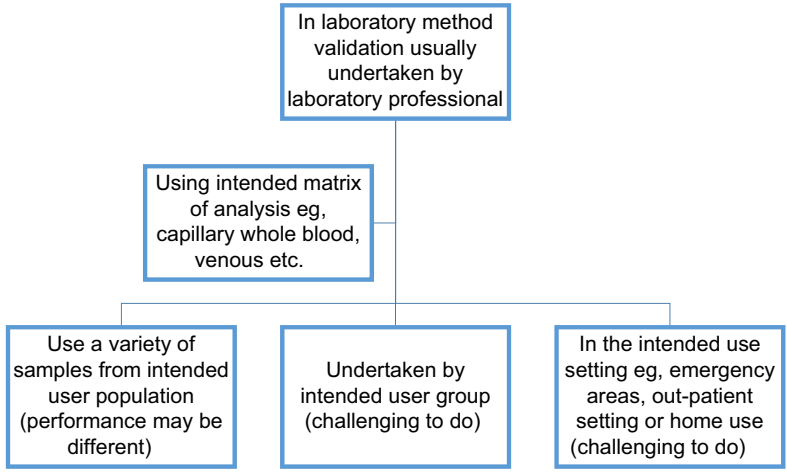

Figure 2.2 Schematic of the variable features of point of care testing that may impact on the validation plan.

Box 2.1 Potential location and operators of POCT devices

- Within hospital: performed by doctors, nurses, laboratory staff and allied healthcare professionals
- In community settings, eg, general practice, pharmacies, community clinics: performed by doctors, nurses, allied healthcare professionals and administrative staff
- In the home: performed by patients, family members or other caregivers

- Variety of regulatory pathways by which a device can come on the market
- Underestimation of risk in busy clinical environment
- Underestimation of error by non-laboratory-trained users

The other key reason to evaluate POCT methods is to define the relationship between the POCT assay result and the local laboratory assay, remembering that POCT methodologies seldom replace existing laboratory assays, but are more likely to be used in conjunction with the existing laboratory assay, for example POCT cardiac markers available in emergency units and the laboratory [10]. As all assays have an inherent bias compared to a gold standard, it is critical that this relationship is understood and defined so that variation in results from POCT method to local laboratory does not lead to erroneous clinical decision-making. This might initially be defined during method validation, but subsequently underpins the need for external quality assessment [11].

2.2.2 The extent and scope of method validation

A variety of national and international guidelines have been formulated to support method validation. Requirements for the components of a method validation may vary between countries depending on the level of approval that has been obtained. For example, in the United States, Food and Drug Administration (FDA)-approved devices must meet Clinical Laboratory Improvement Amendments (CLIAs) requirements for method validation, if they are deemed of medium or high complexity; laboratories operating these tests are subject to inspection and must meet the CLIA quality system standards [12]. However, some POCT devices deemed of low complexity by the FDA may be CLIA-waived such that areas operating only these low complexity tests must simply follow the manufacturer's instructions for those tests to meet CLIA requirements. However, it is not always clear if CLIA-waived devices meet the quality specification for the analytes being measured.

The goals of method validation are not simply to demonstrate comparability to the manufacturer's stated performance, but to also show that any variability is smaller than internationally agreed performance criteria and appropriate for patient care in the clinical context it is to be used. The extent of method validation that is undertaken may also relate to where the device sits in the repertoire of other available devices and the extent to which these other devices have undergone evaluation and been established in clinical care pathways.

2.2.3 Before starting method validation experiments

There are a number of steps to undertake before a method validation. These are outlined in the following section.

2.2.3.1 Establishing a clinical need

Identifying the clinical need of a point of care device should be at the forefront of evaluating any POCT. Clinical need assessments consider how a diagnostic or testing strategy or service is currently provided, the population it applies to and where pathway limitations can be overcome [13,14]. Technological advances allow for an increasing number of tests to be feasible at the point of care. The evolution of these tests stems either in response to an unmet clinical need (clinical pull) or as a result of technological developments (technology push). Particularly when there is technology push, if an unmet clinical need cannot be demonstrated, consideration will need to be given to whether the device has market potential before investing in further evidence generation. Within clinical services, the main drivers for POCT devices that measure analytes for which testing is already available within a laboratory is the faster turnaround time. Establishing a clinical need in this scenario may require demonstration that changes to laboratory work flow or demand management cannot address this to ensure the option of POCT remains cost-effective.

2.2.3.2 Choosing the correct device for the clinical application

After a decision is made to implement POCT for a specific clinical purpose, a decision must be made as to which device to commission. There are usually a variety of devices available for any one clinical application. This process is initiated by setting out the basic requirements or ideal specifications and assessing which devices meet these and may even form part of a tendering process.

The requirements for devices used in medical applications are broad; considerations may include performance of the device in published studies, potential interferences, usability, and provision of connectivity to electronic patient records [15]. It is likely that these decisions will be made jointly between end users and the local laboratory, or ideally in conjunction with a local specialist multidisciplinary POCT expert group. In some cases, in which there is insufficient evidence from published studies as to the performance of POCT devices, a laboratory may choose to evaluate two or more of the available devices in tandem to determine which device to implement.

2.2.3.3 Decide which samples to test

Choosing the correct samples to validate the method is a key step (see Table 2.1) as validation parameters may vary according to the matrix used. The experiments described in the table which underpin method validation can be undertaken on a variety of samples [16]. It is often preferable to use the same matrix to validate the test as will be used for actual POCT measurements; however, this may not always be possible. For example, it is not feasible to fully evaluate a POCT device that uses capillary whole blood, using only capillary whole-blood samples. Using anti-coagulated whole

Table 2.1 Sample types that can be used in method validation

	Example
Same matrix as the device will use	Urine, capillary whole blood, whole blood, serum or plasma from healthy volunteers or patients of interest
Different matrix if same matrix unfeasible	Ethylene diamine tetraacetic acid (EDTA) whole blood instead of capillary whole blood from healthy volunteers or patients of interest
Manufacturer-provided control material	High, medium and low controls or standards
Alternative manufacturer controls	High, medium and local control or standards from a different kit
Certified reference materials	If available, certified standards of the measured analyte

blood as a substitute for capillary whole blood may be an option, but has its own issues as these samples may not be stable when refrigerated and, if frozen, there is evidence that freeze–thaw cycles can affect stability of analytes [17]. Some parameters, for example imprecision, may be initially assessed using control samples provided by manufacturers and in some cases reference standard samples, but these should still be replicated using the intended sample collection to highlight any matrix effects.

The other important consideration is which patient population to select. Again, it is ideal to validate a POCT method using samples that are taken from the target patient group in whom the test is to be used, as there may be patient-specific interference or matrix considerations. For example, a POCT that measures blood ketones may perform differently in extremely dehydrated patients admitted with ketoacidosis to a healthy diabetes out-patient population. However, it may also be valuable to undertake the test in a different population. At the very least, users undertaking a method validation, should consider which sample matrix to test and which sample population to study (see Box 2.2 for a case example).

2.2.3.4 Consider who should undertake the validation and where

The final consideration before commencing validation experiments is who should perform the evaluation. The reported performance of POCT devices according to manufacturer-stated references is seldom poor, but, despite this, significant real-world variability is reported. This variation is often apportioned, in part, to the spectrum of users performing the test, particularly those tests that are undertaken infrequently or by staff with sub-optimal training. The first part of a method validation, which assesses analytical performance, is usually undertaken within the lab by laboratory-trained professionals; however, subsequent parts of the method validation process, for example, assessing bias, may be better undertaken in user hands.

2.2.4 Parameters of method validation

A variety of factors can be assessed when undertaking POCT method validations. These are defined in this section (see Box 2.3 for summary) and also apply for

Box 2.2 A case study

Case description

A paediatric consultant approaches the pathology laboratory in their hospital to discuss the provision of POCT-glycated haemoglobin (HbA1c), a test that assesses diabetes control, in the paediatric clinics using capillary whole blood. HbA1c is already offered by the laboratory on site with a 24-h turnaround time using an anion-exchange high-performance liquid chromatography (HPLC) method on EDTA whole blood. After a workflow analysis, it is agreed to implement POCT HbA1c in paediatric clinics.

How should the lab/paediatric team decide on which analyser to procure?

- A review of literature that has evaluated POCT HbA1c devices.
- Assessment of ease of use and other performance criteria that may be relevant, for example sample volume required may be particularly relevant for a paediatric population. As may be the sample-turnaround time.

What form of method validation should be undertaken?

- The usual parameters of imprecision, linearity, interference accuracy and bias should be assessed. The method comparison with the lab assay is critical as there is likely to be a bias between the two methods and the laboratory must establish the size of this.

Which populations and samples should be studied?

- The initial method validation could be undertaken on whole-blood EDTA samples received in the laboratory for analysis on the lab method. However, when initial parameters have been assessed, the POCT device should be relocated to the paediatric clinic and within-clinic capillary-blood samples should be obtained (if ethically permissable).

Where should the evaluation be undertaken and who should conduct it?

- Initially in the laboratory by trained staff who can assess the analytical performance.
- Then, within the clinic by the health-care professionals who will be trained to run samples.

Box 2.3 Parameters assessed experimentally in a POCT method validation

Major parameters	Other
• Linearity	• Cross-reactivity
• Imprecision	• Recovery
• Accuracy and bias	• Lower limit of quantification
• Lower limit of detection	
• Interference studies	
• Reference range verification	
• Usability	

laboratory-based assays. There is no international agreement on how to assess these parameters, and some of the more popular approaches are described.

2.2.4.1 Imprecision [18–20]

The random error in an assay is defined by its imprecision and is usually assessed by measuring a range of (at least two) samples in replicates (between $n=5$ and $n=20$) and calculating the coefficient of variation of the replicate results. This provides a measure of analytical variability. Imprecision can be assessed within a day and between days to give a total analytical imprecision. Imprecision may be assessed on control samples that are stored appropriately and provided by the manufacturer (eg, a high and a low control) or patient samples to assess the impact of matrix effects, and is preferably assessed in both. It may also be meaningful to assess imprecision not just in high, medium and low samples, but specifically around levels that may be of clinical interest – for example, around a diagnostic cut point. The results of imprecision studies should be compared to what the manufacturer reports, what is deemed a clinically meaningful variation (often difficult to define) and if there are internationally agreed targets for imprecision of a particular analyte, then these should be met. Setting goals for imprecision is challenging and depends on the analyte in question [21,22].

2.2.4.2 Linearity

Linearity (analytical range) is assessment of the range over which results can be obtained without the need for dilution, reflecting the range over which there is a proportional relationship between analyte concentration and signal [23]. This parameter may be difficult to assess in POCT methodologies as the traditional way to assess linearity is through dilution of high-concentration samples and it may be problematic to dilute common POCT samples – whole blood for example, without introducing matrix effects. However, a high standard could be used as a surrogate sample type. Quoted linearity should be verified by running a minimum of two replicates at five to seven concentrations over the claimed measuring interval [20].

It is important to validate the reported linearity of quantitative devices against quoted ranges. This was shown very effectively in a study that compared a capillary-blood ketone metre result to a serum laboratory assay and found that linearity was lost at >3 mmol/L rather than the manufacturer-stated level of 6 mmol/L with clinically significant imprecision in values obtained above 3 mmol/L [24,25]. Identifying a different range of linearity to the manufacturer may not preclude the use of the POCT device, but may change the analytical measurement range or the cut-off at which ‘greater than’ values are reported, or at which a laboratory assay corroborative measurement is advised.

2.2.4.3 Accuracy and bias

An assay that is precise may not be accurate and an assessment of the agreement between the POCT result and the ‘true’ result must be undertaken to establish the accuracy and relationship (bias) between the POCT results and a reference [20]. The reference may be an alternate method, and this is usually the case in POCT-device

validation in which the result from the POCT test is compared to the laboratory method in operation, which is fully validated, quality controlled and quality assured. There are inherent challenges in this as the laboratory method may often be a different assay type to that utilised by the POCT device [26]. An alternative approach (that can be assessed in conjunction with a method comparison) is to assess results on the POCT device using certified reference materials. This may be the favoured approach when there is no alternative method available and may be useful to undertake even when there is, as the method comparison makes the assumption that the laboratory assay is superior and this may not be the case. It is critical to undertake a method comparison study to establish the bias between the POCT and laboratory method, remembering that the two assays may be used interchangeably within any single clinical service. Though assays for most routinely measured analytes have been standardised, there is still a significant assay-to-assay variation, which if undefined, could lead to erroneous clinical decision-making on the basis of a step-change in results perceived to be an improvement or deterioration [27].

At its simplest, a method comparison involves testing between 20 and 200 test samples across the entire testing range on the POCT device and the reference comparator. Results obtained may be analysed by linear regression (eg, Passing and Bablok regression) and bias plots (eg, Bland–Altman method) [28]. If there is a significant bias between POCT methods results and the comparator, it may be inadvisable to implement the test due to step changes in the results obtained. These decisions are best made by the multidisciplinary POCT expert group reviewing the data with local laboratory staff or in the POCT committee (see Implementation).

2.2.4.4 Interference studies

The presence of an interfering substance in the patient sample, interacting non-specifically with the assay, may result in an artefactually higher or lower result. Interference studies compare the results obtained by an assay in two aliquots of the same sample with and without the interferent. The experimental design should be carefully considered; the concentration of the interferent should be at a clinically meaningful level and the sample concentration of the analyte of interest should be varied, as the effect of the interferent may differ depending on the concentration of the underlying analyte [29]. For immunoassay-based POCT devices or those using colourimetric detection, the most commonly studied interferences include: bilirubin, haemolysis and lipaemia. It may also be necessary to test interfering drugs or metabolites. Which potential interferent to study will depend on manufacturer stated claims and published reports and also the likely interferents encountered in any specific patient groups.

2.2.4.5 Establishing a limit of detection

This may not be possible if there is a qualitative test. However, for quantitative devices and when a lower limit cut-off exists, it may be valuable to assess the limit of detection, which is defined as the lowest concentration of analyte in a sample that can be detected with a stated probability [30]. This is usually assessed by measuring a blank

sample at least 10 times, calculating the standard deviation of the results and multiplying this by 3. Assessing this may be problematic in POCT devices as manufacturers may have pre-set low concentrations to be reported as 'less than'. It is worth reflecting on whether this is a necessary analysis for the particular POCT device.

2.2.4.6 Verification of the reference ranges and cut-offs

Reference intervals (the range of values measured within 95% of the healthy population) are difficult to validate, not standardised and are often population specific [31]. Manufacturer reference ranges are ideally verified, but in practice this may be challenging, and if another laboratory has undertaken this already and the reference range is traceable then it may be practical to accept their verification. For many analytes, the normal reference range may not even have been established in the literature or, if it has, it may lack a firm evidence base to support it [32]. In the context of POCT devices, the reference interval stated should be similar to the laboratory assay comparator and, if it is not, some work should be undertaken to establish how to harmonise these. For laboratories that have an array of 'healthy patient' samples for the purpose of reference interval verification, CLIAs provide guidance on how to verify ranges [31]. Any cut-points that are stated by the manufacture should also be fully assessed in terms of imprecision and bias, but also the evidence base when possible should be reviewed, before clinically implementing this cut-off.

2.2.5 Validation of qualitative test

Similar to quantitative methods, qualitative tests should also undergo a method validation. The measuring system of a qualitative test usually transforms a quantitative result into a negative or positive report or in some cases a semi-quantitative outcome; however, the absolute numerical concentration of the analyte itself is not reported. Qualitative tests can be validated by using a series of samples with known concentrations of analyte that fall either side of the positive–negative cut-off. These known values may be assigned by an alternative method, or may be reference material. It is particularly important to assess reproducibility of results around a concentration of the analyte of interest that is clinically important such as around a diagnostic cut-off. A method comparison study can also be undertaken with a comparator. The new method can usually be implemented when predefined criteria are fulfilled.

2.3 Clinical validation methodology

Evaluating POCTs and taking an evidence-based approach to their adoption is vital to clinical governance. However, diagnostic evaluation is challenging. Evidence on diagnostics is commonly produced in lower quantity and quality than for pharmaceuticals and technology progresses rapidly, meaning that, if large-scale clinical trials are undertaken, devices can be obsolete before results are reported. High-quality clinical studies in diagnostic test evaluation are rare, often due to lack of resources, large sample size requirements and unclear evidence requirements from involved stakeholders [33].

2.3.1 Clinical pathway mapping

Diagnostic tests cannot be evaluated in isolation. Instead, they should be considered as one step of a patient journey or *clinical pathway*. Patients rarely benefit from undergoing a diagnostic test in isolation but from the decision-making and interventions that follow because of the result [34]. Therefore, before the value of a POCT can be determined, the clinical pathway must be mapped. When new diagnostic tests are introduced to a clinical pathway they fall into one of three main roles: they can replace existing tests, triage patients for existing tests or be an add-on test performed in a subgroup of patients [34]. POCTs can be particularly disruptive as they can bring diagnosis earlier in the pathway, altering the process of care. Evidence-based clinical management and decision-making is usually based on gold standard (reference) or established tests. However, when POCTs are introduced, these pathways are altered, as diagnostic information is available earlier in a patient's journey. A comparison of the current clinical pathway and the new POC pathway is the basis of evaluating the impact of introducing these devices.

Potential clinical pathway benefits of a new point of care test
Reduced turnaround time
Blood sparing
Testing by a single practitioner (sample collection, testing and notification)
Immediate or faster decision-making
Earlier treatment
Improved adherence with treatment
Reduced complications
Quicker optimisation of treatment
Reduced use of healthcare resources (reduced admission, early discharge and patient management of disease)
Improved patient experience

2.3.2 Diagnostic accuracy studies

Diagnostic Accuracy Studies provide a cross-sectional comparison of the test under evaluation (index test) to a gold standard that acts as a surrogate for the 'true' disease status of the patient (reference test). The study should be undertaken in the population intended for the test. Results can then be presented in a 2×2 table (see Fig. 2.3) and used to determine the sensitivity (the proportion of people with a disease who have a positive test result) and specificity (the proportion of people without a disease who have a negative test result). The Standards for Reporting Diagnostic Accuracy (STARD) guidelines provide a set of reporting guidelines for diagnostic accuracy studies [35] and this has been accepted by many medical journals.

2.3.2.1 Randomised controlled trials

Randomised Controlled Trials are regarded as the gold standard of study methodology in pharmaceutical or interventional studies but are rare in the evaluation of diagnostic

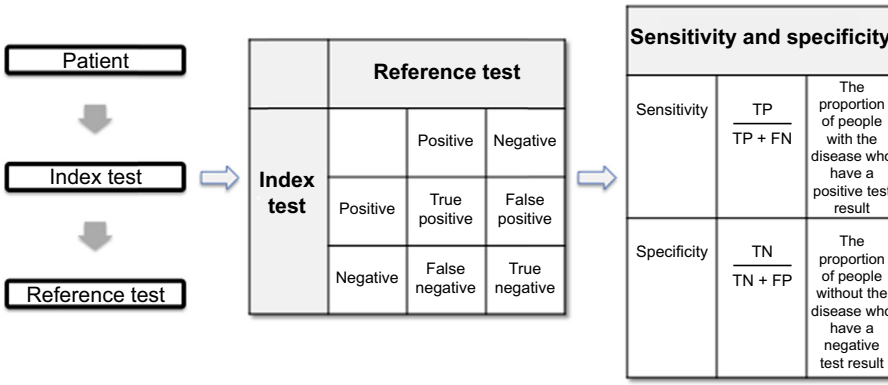

Figure 2.3 Diagnostic accuracy study.

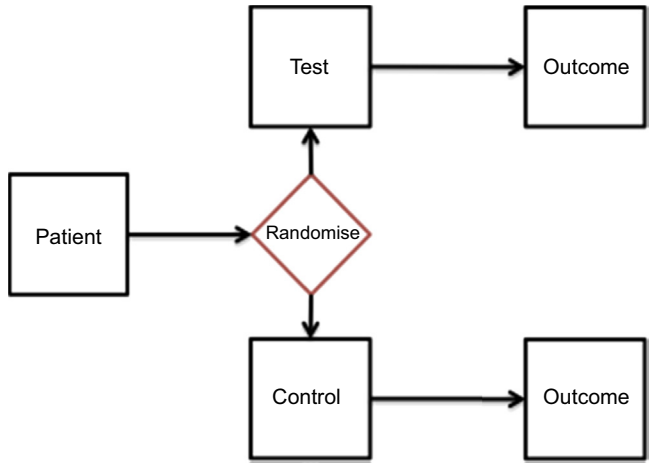

Figure 2.4 Randomised clinical trial approach to diagnostic evaluation.

tests. Advantages include the ability to evaluate the impact of a test device to patient outcomes, costs or organisation of care whilst eliminating bias by ensuring any differences between patient groups are because of chance (see Fig. 2.4). These studies provide an evaluation of clinical utility and therefore determine the clinical effectiveness of a test through the direct impact on patient or societal outcomes. However, randomised controlled trials are rare in the evaluation of diagnostic tests and policy, and decision makers frequently have access only to lower-grade evidence [36,37] when making decisions regarding policy and adoption.

Linked evidence approaches are a compromise between cross-sectional and randomised controlled trials by providing a methodology to combine systematic literature review and the narrative linking of evidence such as diagnostic accuracy studies, evidence on the selection of treatment options for patients and the effects of treatment to the health of the population under evaluation [38]. Whilst linking this evidence

provides an efficient means of evidence generation, it is important to consider the limitations of transferability of the combined evidence when evaluating such arguments.

Human Factors is a multidisciplinary science in which human behaviour, capacities and engineering principles are used to explore why errors occur, and how to reduce the likelihood of preventable harm to individuals, with the specific aim to support human performance and safety [39]. It analyses the components of a 'work system' (the tasks and processes, the environment and the equipment) and can be applied at individual, team and organisational levels. POCT devices move tests that have been traditionally performed in the clinical laboratory by expert staff into clinical areas or patient's homes where users may have less training and experience. Fundamental to their design success is usability, defined by International Organisation for Standardisation (ISO) guideline 9241-11 as '*The extent to which a product can be used by specified users to achieve specified goals with effectiveness, efficiency and satisfaction in a specified context of use'*. A specific guideline (ISO 62366) describes the design processes required for the usability engineering process of medical devices.

Errors in the use of POCTs may lead to inaccurate results that compromise patient safety and lead to harm. Using human-factors approaches such as simulation of the testing process (sample collection, test procedure and result output) and system (training, data transfer, patient safety and intervention) can identify these risks and mitigate them.

Economic Analysis is vital to justify the implementation of new diagnostic tests into resource limited health-care systems. POCTs are often more expensive on a test-by-test basis due to the scaling and portability of devices and not benefiting from the efficiency of large-scale multiplex laboratory testing of multiple samples. However, POCT can have benefits further down the clinical pathway such as those described in Table 2.1. These indirect cost savings can only be established by performing a comparative evaluation of the clinical pathway with and without the test.

2.3.3 Barriers to adoption

The introduction of a novel POCT involves a variety of stakeholders including patients, clinicians, laboratory services, regulators, commissioners and industry. These stakeholders will have different attitudes, beliefs and incentives relating to the adoption of a novel device. It is reported that there exists a 17-year time lag for research evidence in health innovation to translate to clinical practice [40,41]. Whilst progress has been made since the adoption of faecal occult blood sampling for colorectal cancer screening – that test took over 100 years from first being described to widespread implementation in colorectal screening programmes [42] – there remains an absolute need to reduce the timescale for the adoption of new technologies. Barriers to the introduction of new technologies are abundant and vary between stakeholders. A recent systematic review [43] explored the perceptions of primary care physicians on POC. Emergent themes included concerns regarding accuracy, misleading or wrong results, cost consequences, maintenance, over-reliance and increased anxiety to patients. Qualitative research techniques including interviews and focus groups can be undertaken with stakeholders to predict the incentives and disincentives to device adoption and mitigate for these [44].

2.3.4 Implementation

The successful implementation of POC testing strategies into clinical practice requires a structured multidisciplinary framework to ensure clinical governance. Most large health-care organisations have institution-wide POC leads and committees to oversee the use of POC devices; staffing varies between organisations but usually comprise clinical scientists, pathologists, laboratory representatives, clinicians, nursing staff, financial representatives and patients. Lines of responsibility and accountability need to be in place and policies reviewed at frequent intervals to ensure delivery of a high-quality service. Therefore, the responsibilities of the committee include determining if POC testing is required at a particular site, audit, procurement, preventing the uncontrolled purchase of POC devices, training and ensuring quality control and quality assessment schemes are adhered to [15,45]. Once the decision to implement POC in a given area has been made, there are further practical considerations that should include:

- How to choose the right equipment
- Maintenance of an asset register
- Location of equipment
- Required space
- Power source and battery life
- Limitations of test
- Storage of reagents and consumables
- Users and competence
- Maintenance

Training is vital to the successful implementation of POC testing strategies into clinical practice, and test use should be restricted to staff whose training and competence has been established. Training needs to cover all phases of the testing process including sample acquisition, device use and appropriate response to unusual test results. Training manuals and standard operating procedures for devices should outline operator dependent steps, hazards of sampling handling and disposal of biofluids and consumables.

Quality Assurance refers to the measures taken to ensure test results remain reliable and is broadly divided into internal quality control (IQC) and external quality assessment (EQA). Internal quality control is aimed at checking that patient results are reliable before issued to clinical teams. The process by which this is undertaken will vary dependent on the characteristics of the test and traditional laboratory IQC standards do not translate easily into POCTs [46] but may include the analysis of a reference set of specimens or internal electrical or optical checks undertaken by the device itself. All POCTs need to have a defined IQC protocol determined by risk management [47] and results should be documented appropriately with guidance available for when results are outside of acceptable limits. As well as routine IQC, further control should be triggered by events such as the use of a new batch of consumables, questionable patient results, following maintenance or a physical insult or damage to the device. Users of POCTs have a duty to participate in an EQA scheme to practice good clinical governance. Information technology can play an important role in ensuring quality by

auditing results, providing quality related statistics and monitoring users and patient details. However, devices have varying amounts of data storage and transfer capability.

Adverse Incident Reporting is an essential part of POC management and important to post-market regulation surveillance. Adverse incidents include any event that causes, or has the potential to cause, unexpected or unwanted effects involving the safety of device users including patients or other persons [Medicines and Healthcare Products Regulatory Agency (MHRA)] and reports should be made to the relevant local bodies [48].

2.4 Regulation of POCT devices

Increasing access to POCTs present challenges for regulation; tests are undertaken in different environments, there are several steps in which user errors can occur (sample collection, testing process and result interpretation) and there may be limited training of users. POCTs are defined as in vitro diagnostic medical devices and therefore in the majority of developed countries medical device regulation applies. Such directives apply to the test device itself in addition to consumables (including reagents and calibrators) and instructions for use. These requirements deal with the safety, quality and performance of the test. In the United States, the US Food and Drug Administration (FDA) is responsible for ensuring device regulation, and similar bodies exist in Europe. The European Conformité Européene (CE) mark represents that the device meets the relevant provisions of the Medical Devices Regulations. Currently, many POC devices can be self-certified by the manufacturer and a CE mark applied by manufacturers, once appropriate technical documentation of conformity is provided; only high-risk devices require the involvement of notified bodies. However, the landscape for the regulation of POC devices in Europe is likely to undergo significant revision in medical device legislation.

Regulatory requirements are usually mandatory prior to market launch and additional post-market monitoring is vital to ensure continuing patient safety with device use. Requirements vary by region but the route to approval is often determined by the perceived level of risk dependent on the relative danger to public health and patients if the device fails to perform as intended. As an example, enhanced requirements are often in place for home-testing devices. Regulatory compliance is typically demonstrated through a portfolio of evidence specified by the bodies which commonly addresses:

- Management structure for the organisation and management of programme
- Description of the test and specifications
- Packaging and user manuals
- Reagents
- Training and competence
- Design verification
- Quality assurance and quality control (including calibration and standards)
- Specimen handling
- Maintenance
- Risk analysis (to review whether the risks of test device use are acceptable when balanced with the potential benefits)

In addition to medical device regulation, laboratory services and pathology may be subject to accreditation that in some circumstances extends POCTs. The priority of accreditation is ensuring technical competence. In the United States, this is through Clinical Laboratory Improvement Amendments (CLIAs) legislation and several organisations exist to inspect laboratory and POC testing programmes [5]. In European countries, guidelines are based on the International Organisation for Standardisation (ISO) standard TC212.

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Materials for improved point of care biosensor–tissue interfaces

3

P. Vadgama
Queen Mary University of London, London, United Kingdom

3.1 Introduction

Point of care testing provides rapid availability of results, enabling early institution of therapy. The need for timely clinical decision-making is particularly important as regards the critically ill patient, but more generally point of care ensures tailoring and individualisation of therapy. This latter therapeutic flexibility is a part of the move towards stratified medicine, increasingly regarded as the model for future medicine and manifest in initiatives for storing and analysing biological samples on a wide population basis in biobanks [1]. Additionally, for the ambulatory patient, there has been a move to emphasise self-monitoring, enabling both closer surveillance of disease in the home and a route to patient empowerment. The classic use of this modification to the patient care pathway is for diabetes and anticoagulation management in which a plethora of handheld metres have been placed on the market. The aim of point of care testing is thus not to compete with the high throughput and analysis of the central hospital laboratory, but to deliver analytical immediacy and flexibility that is under the direct control of the patient and clinician. Ancillary advantages are the avoidance of sample transport, specimen-processing schedules and cumbersome data return loops [2]. The point of care testing market is now growing at the rate of 10% per annum, the highest growth rate within the in vitro diagnostic sector.

A unique assay sub-set, in which point of care comes into its own, is in vivo monitoring [3] in which continuous, real-time data can allow for the fine tuning of therapies and enable trends to be observed and compensated for at an early stage. This distinct area is a new encounter for medicine, and contrasts with well-established physical and electrophysiological systems for managing the acutely sick patient in critical care. However exciting as a field, there is also a more serious risk of operational failure given the need for chemical sensors to work reagentlessly and without sample preparation. If in contact with tissue, the sample matrix is also of a much higher level of complexity than is the case with plasma or even whole blood, particularly as regards on-going reactivity and biological remodelling, neither of which are easy to control once the device has been placed in vivo. Safe operation is another issue, quite apart from the challenge of sterilising a biological device, the material components, at least those that interface with tissues, need to give a performance that at the very least matches that of traditional biomaterials. This is technically a distinct area to transduction chemistry, bringing in imperatives of materials science, cell biology and pathophysiology to address the needs of implant biocompatibility. As such, only lately have material considerations entered

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the dialogue in biosensor design, led, moreover, by industry rather than by academic researchers. Inevitably, industry being product orientated, is more alert to the mosaic of disciplines needed to generate practical working systems. Industry also has the resources and long-term strategy for the concept of the bedside and effort needed to satisfy the requirements of the consumer, as well as for meeting the stringent safety requirements of the regulator. One drawback to the stimulation of general material advances, however, is that some of the design innovation and practical know-how is sequestered within industry and so less available for wider development of the field. From the early development of online blood monitoring, systems, particularly of pH, pO_2 and pCO_2 to new materials for tissue-based biosensors, we have now moved on, and the concept of a biocompatible material has advanced from being simply a surface that resists blood coagulation.

However robust a transducer and its attendant biorecognition molecule might be, the encounter with biological samples either in vitro or in vivo demands some form of interfacial stabilisation that is most commonly achieved through a carefully optimised covering barrier layer. Its formal value is in improving biocompatibility and controlling solute access to the transduction chemistry, and through the latter achieving selectivity and a response range tailored to analyte target values. Solute transport selectivity is especially needed for electrochemical sensors, in which a preset operating/polarisation voltage cannot fully eliminate either background electrochemical interference or electrode surface passivation; this contrasts with optical sensors in which wavelength and chemistry control offer a more secure option for selectivity. However, this chapter will emphasise external biointerfacing needs, as this is the special need for the point of care issue, whereas selectivity though addressable by materials is a common need, inclusive of those used in the laboratory setting. In addition, systems will be described that are either being used or that have potential viability rather than innovations at the chemistry concept stage. Arguably, the innovations that have had the real impact are those that have re-engineered classical assay chemistries in a way that they become reliable and accessible. Devices designed for blood are included as this is the primary sample for bioanalysis, and blood in any case should be regarded as a tissue in its own right.

3.2 Materials for in vitro sensors

3.2.1 Ion selective electrodes

In vitro measurement for point of care typically uses whole-blood samples. Though blood has reactive cellular constituents and is capable of fast surface coagulation masking the sensor surfaces, there is generally not a problem of sensor response deterioration. Exposure of the sensor surface is for only a limited time period, enough for a single reading and invariably the sensor is then disposed of. Moreover, for an in vitro measurement, it is possible to include a sample pre-processing routine, based on, say, microfluidics or lateral flow. However, even here, useful material modifiers have been reported for reducing the bioincompatibility of ion selective electrodes (ISEs).

ISEs have the advantage that they constitute a true interfacially responsive system in which it is facile equilibrium binding that defines the response; there is no analyte destruction, and so no need for continuous solute flux. The implication of this is that surface protein, or even cell, deposition does not compromise the response, unless the

surface adventitious coating is so heavy that solute access to the surface is not possible; this may mean, of course, that response does become slower. Usually, a polyvinyl chloride (PVC) membrane is used as the sensing surface, with an ion-binding agent such as a chelator or ionophore co-incorporated with a plasticiser to provide mobility. High molecular weight PVC has acceptable biocompatibility [4], inducing limited complement activation with the advantage of being uncharged and structurally inert. However, water uptake into PVC can lead to electrode drift, and if used in contact with tissue, a general inflammatory reaction [5] would be a problem. The primary risk is that the need for a high content of plasticiser leads to high surface hydrophobicity, reducing biocompatibility for in vitro measurement, and an induction of inflammatory change during tissue measurement, especially through leaching. The chloride moiety of PVC, however, offers synthetic routes for derivatisation [6] and the attachment of plasticiser, as well as for chemical cross-linking and bioreagent immobilisation; surface attachment of more biocompatible poly (ethylene glycol) (PEG) [7] is also possible. Aminated PVC has enabled extension of modifier reactions. In one example, for urea, urease was attached to PVC, with acrylate polymer to host the enzyme solid phase [8]; the PVC formulation, with plasticiser can be readily deposited on planar carbon electrodes for scale-up fabrication [9]. Few studies have explored material modifications or ISEs operating in undiluted blood, but this reflects, in part, the good working stability of these devices, at least during limited *in vitro* use.

3.2.2 Amperometric sensors

Amperometry, whether for O_2 measurement or for metabolites, requires a concentration-dependent flux to the electrode independent of any surface fouling. This is difficult to achieve in practice for extended use, but is achievable for short-term sample exposure. A reported cassette [9] with planar enzyme-based sensors to measure glucose and lactate, additionally to potentiometric urea, creatinine, and potassium potentiometric devices, demonstrates the utility of a thick-film platform for polymer deposition. This allowed for differential layering of polymers for selectivity and biocompatibility control. The design versatility of PVC was demonstrated here in the use of a vinyl acetate-vinyl-maleinate-polyvinylchloride-co-polymer as the outer low-fouling membrane. Additional fabrication refinements were the printing of polymerisable silver ink and Ag/AgCl to create the initial counter and reference electrodes; unusually, a working electrode incorporating MnO_2 operated as a catalytic surface for oxygen regeneration from the oxidase enzyme reactions and also allowed operation at reduced electrode polarisation voltage.

For any enzyme-based electrode, there is the problem of finite substrate affinity determined by enzyme Michaelis constant (K_m) that, typically, falls well below the analytical concentration range needed for a linear response in undiluted samples. For oxidases, there is also the need for oxygen co-substrate. Venous blood samples have relatively low pO_2 levels which could compromise response. A material-based solution to the problem is to use a membrane barrier that (1) reduces the transport of analyte to the enzyme and (2) maintains a relative permeability to O_2. In one early approach, substrate-restricting microporous membranes were used modified with O_2 permeable silicone [10]; in another, silicone emulsion was used [11]; both these barriers promoted the differential transfer of O_2. High-volume production of sensors is less

readily achievable with preformed membranes, and deposition of polymer from solution is preferable. This is a challenge if the bioreagent is also to be deposited; because of this, water-soluble phases have been tested. In one example, a water-based carbon ink loaded with cobalt phthalocyanine combined with glucose oxidase was used [12]. Alternatively, for controlling transport, Chen et al. [13] used SiO_2 nanoparticles, and thereby suppressed response transients at a screen-printed planar glucose electrode.

A three-enzyme electrode system, such as needed for creatinine measurement, poses a more difficult enzyme-immobilisation problem, in that different enzymes have different immobilisation requirements and their microenvironmental interrelationships need to be optimised. For one creatine sensor, the requisite creatine amidinohydrolase and sarcosine oxidase were immobilised in polyurethane pre-polymer and PEG-linked creatinine amidohydrolase was attached via diisocyanate pre-polymer to create a polyurethane adduct [14]. The likelihood of enzyme inactivation with chemical immobilisation is high, but provided an enzyme preparation survives this, long-term stability is feasible. In the case of these three particular enzymes, a loss of activity resulted from silver ions diffusing from the reference electrode; the material solution was to protect the enzyme layer with a diffusion-resisting cellulose acetate membrane.

Hydrogels offer multiple routes to bioreagent retention ranging from physical entrapment through to covalent binding to hydrogel polymer-pendent groups. Their high void volume allows for high loading yet with ready diffusive access for low molecular weight analytes. Additional functionalities for molecular selectivity through diffusion and partitioning control could be possible but have not been explored to date. Organisation of the gel phase can be as microspheres and films through to surface-attached brush polymers retaining a stable aqueous layer at the boundary with the sample. Poly(vinyl alcohol), polyvinylpyrrolidone and poly(methacrylic acid) are common starting materials, variously cross-linked, derivatised and photo-patterned for tailored use at biosensors. Their optical transparency has made them especially appropriate for optical biosensors [15]. A variable degree of swelling in samples of different pH and ionic strength, and certainly from the dry state, is a drawback and a potential source of response instability. With the correct synthetic structural flexibility, analyte-responsive 'intelligent' gel phases can be created, however, as well as materials for the controlled release of therapeutic agents [16].

A specialised hydrogel which has possibly escaped the operational instability of dry state reconstitution is a polyvinyl pyrrolidone with attached bisimidazole-coordinated osmium centres. The resulting osmium ion redox relay serves to transfer electrons from the flavin adenine dinucleotide (FAD) prosthetic group of glucose oxidase for direct electron flow and response to glucose substrate without need for oxygen. The special relevance of the hydrogel here is in the lowering of local viscosity and through this enabling the osmium centres to be sufficiently mobile for uninterrupted redox shuttling [17,18]. The further advantage for blood measurement here is operation at low polarising voltage in which electrochemical interference from sample constituents is not a problem. A variant of this has used Pt nanoparticles in polyaniline which enabled efficient hydrogen peroxide (H_2O_2) decomposition along with charge collection and transmission by the background polyaniline chain [19]. Conducting polymers for enzyme immobilisation are being increasingly used, a topic reviewed

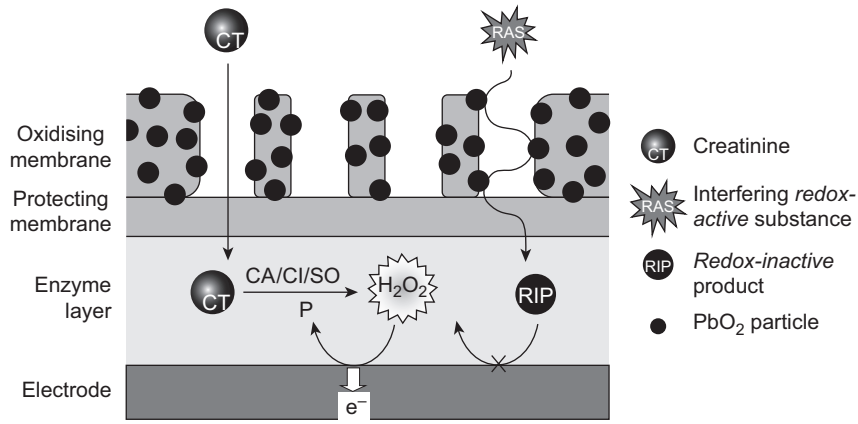

Figure 3.1 Interference removing oxidising membrane based on incorporated PbO_2 particles over a three-enzyme creatinine biosensor based on creatinine amidohydrolase (CA), creatine amidinohydrolase (CI) and sarcosine oxidase (SA).
With permission from Shin JH, Choi YS, Lee HJ, Choi SH, Ha J, Yoon IJ, et al. A planar amperometric creatinine biosensor employing an insoluble oxidizing agent for removing redox-active interferences. Analytical Chemistry 2001;73(24):5965–71.

by Ramanavicius et al. [20]. A further redox polymer composite has been used to eliminate interference; here, a hydrophilic polyurethane barrier loaded with PbO_2 chemically oxidised interferents, but being separated from the enzyme layer (Fig. 3.1) allowed amperometric measurement of creatinine via the three enzyme H_2O_2 product without undesired degradation of the latter [21].

3.2.3 Commercial systems

One of the most structurally complex, yet operationally simple, point of care systems is the i-STAT [22]. This enables measurement of Na^+, K^+, Cl^-, Ca^{2+}, urea, pH, pCO_2, pO_2, glucose and haematocrit using a series of self-contained cartridges. The key to reliable measurement here has been the incorporation of self-calibration fluidics, dilution and controlled sample presentation, all activated by the introduction of blood through a sample portal. However, what allows the devices to work in a self contained manner is the organisation of the membrane laminates (Fig. 3.2). Formally, there is no new chemistry; it is the way the membranes are created and some of their composition that is novel. For the ISEs, traditional plasticised PVC is used; for urea, urease is in a latex layer for potentiometric measurement. Other sensors, reliant on mass transport control, use silicone–polycarbonate for O_2, polydimethylsiloxane for CO_2 and silicone–carbonate for glucose. The compactness of the various sensing elements derives from the micro fabrication of the conducting surfaces and the superposition of thin electrolyte layers.

Glucose sensors are the most used, most established, highest volume and most diverse of all available point of care systems. They have a precedent in filter paper-based dry-reagent technology, originally used for optical readout [23], in some cases with an outer protective layer able to screen out red cells. It was, however, with the

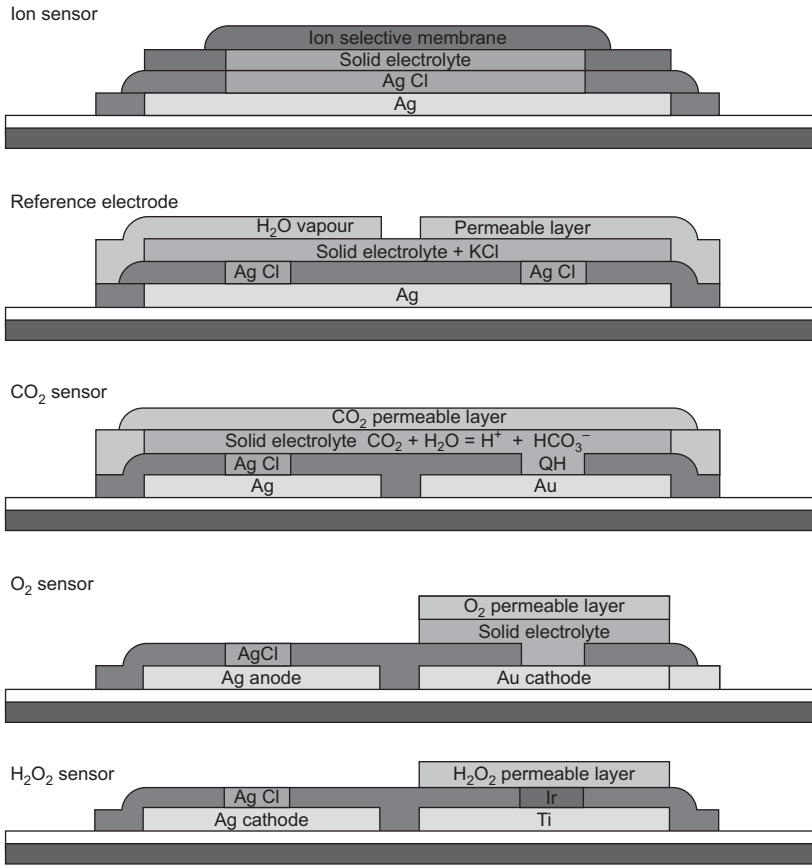

Figure 3.2 Cross sections of membrane laminates used variously for ion, CO_2, O_2 and H_2O_2 (for glucose) sensing, together with the reference electrode design, showing the repeat organisation of polymer, dry electrolyte and thin film-deposited metal conductor.
With permission from Lauks IR. Microfabricated biosensors and microanalytical systems for blood analysis. Accounts of Chemical Research 1998;31(5):317–24.

advent of the Yellow Springs electrochemical analyser (YSI 23A) [24] that more specialised membranes became featured in commercial systems. Here, an outer polycarbonate microporous membrane excluded the formed elements of blood and an inner molecular weight cut-off cellulose acetate membrane excluded organic interferents whilst allowing inward diffusive access to the H_2O_2 product of the enzyme layer. This special inner-plus-outer membrane configuration has been standard for all so-called first-generation sensors in which H_2O_2 is the measured entity, whereas for mediator-based systems an inner membrane is not usable and selectivity depends on low polarising voltages [25]. The outer blood-contacting surface for single use requires additional materials, for example, meshes and filters designed for sample spreading and to facilitate rapid fluid transfer to the inner layers. The volume taken up by red cells (haematocrit) can alter response, and layers containing silica and fatty acids with quaternary ammonium salts, for example, have been proposed for avoiding such loading effects [26].

With effort now directed to further point of care devices allowing single non-calibrated use, beyond any attention given to the core chemistry [27,28], the material components for variously retaining the biocomponent, accelerating sample uptake and improving selectivity are likely to feature increasingly. New materials for sample partitioning into the device could also help extend analytical range to the sub-millimolar range, or allow pre-loading with reagent.

3.3 Biocompatibility

3.3.1 The tissue matrix

Immediately on tissue implantation, a sensor surface becomes coated with a protein layer, and an acute inflammatory reaction is triggered which over time metamorphoses through various cellular phases into a chronic, avascular, wound site (Fig. 3.3). This entire evolution process can be summarised as a foreign-body reaction and manifests a distorted local biochemistry as well as morphology. Some level of distorted sensor

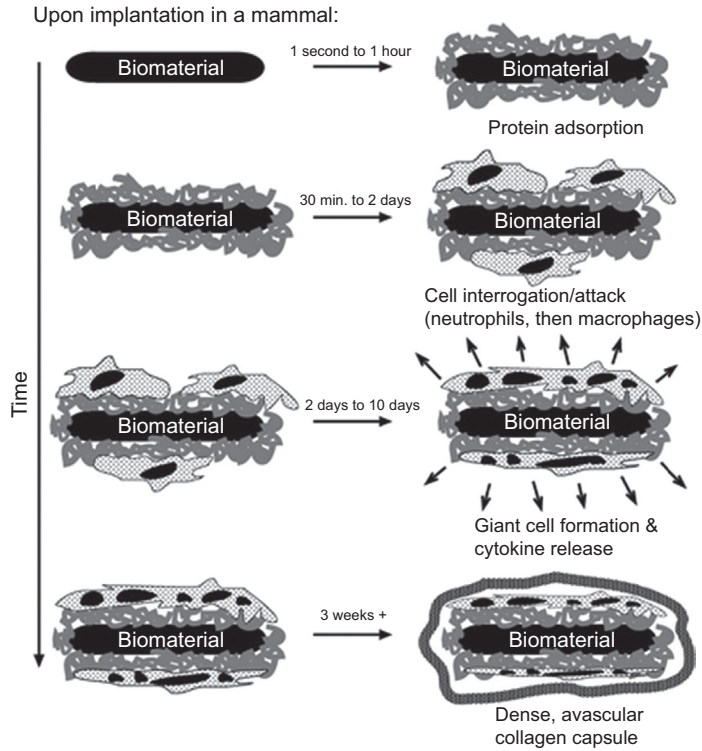

Figure 3.3 Schematic of foreign-body tissue response to any implant material showing the metamorphosis from the initial protein coating to a state of chronic cellular infiltration up to the biomaterial surface and the final fibrous, avascular collagen encapsulation.
With permission from Ratner BD, Bryant SJ. Biomaterials: where we have been and where we are going. Annual Review of Biomedical Engineering 2004;6:41–75.

response is, therefore, inevitable regardless of whether the device itself operates flawlessly despite a covering protein or cellular layer. At the chronic end stage, moreover, a masking fibrous capsule may fully preclude target molecule entry from the rest of the normal tissue environment and blood supply [29]. Biologically, the tissue has then achieved its desired goal, that of sequestrating a foreign body it has been initially unable to degrade [30]. The difficulty for both general implants and implanted biosensors is that this self-sustaining sequence takes place mostly independently of any surface chemistry or design change made to the basic implant, though interestingly, size and shape do have some influence. Tissue response to sensor outer membrane charge, polarity and topography at any tissue appears to be cooperative, complex and mostly self-directed. So there is limited insight that can be gained from in vitro analysis of surface correlates of, say, isolated cell adhesion, mobility and morphology; little of this translates in to predicting the biological whole tissue response. The next generation of reactive, smart and biomimetic materials could well address the problem more fully, but for now sensor intrinsic design has somehow to bypass the current problem. One option for amperometric systems is to reduce the flux dependence of the response so that any further biological distortions in tissue have less of an impact.

A range of signalling systems come into play when an implant disrupts the integrity of a tissue. The complement cascade is triggered, there is extreme local capillary vasodilatation, capillaries become highly permeable, there is chemotactic inward migration of phagocytic cells and, with a sustained foreign-body presence, their assembly into giant cells [31]; all this is followed by a local hyper metabolic state, higher fibroblast activity and deposition of collagen. At this acute inflammation stage, also, specialist white cells, mast cells, release histamine through degranulation of their cytoplasmic load, creating a local focus for histamine pharmacological activity, recruiting even more macrophages and further distorting the local tissue architecture. Given that all the associated cells are oxygen respiring and metabolically active, local pH, pO_2 and metabolite concentrations are inevitably distorted. Meanwhile, the implant surface itself is no longer the original one, but one coated by a complex mixture of tissue and plasma proteins that continues to denature, remodel and grow [31–33] with major contribution from tissue fibronectin and vitronectin [34,35]. This inflammatory response is actually, better considered a part of the immunological machinery of the body [31]. With any admixture of blood constituents through capillary damage, there may also be the generation of a local thrombus, the end result of interaction between the coagulation pathway, the complement system, kinin generation and platelet activity.

A week after the acute inflammatory phase, monocytes and lymphocytes accumulate at the implant surface [36–38]; if this is non-degrading and chemically benign, then this phase lasts two weeks and is then given over to highly vascular healing tissue populated by capillaries at high density, granulation tissue, which also has associated with it fibroblasts and macrophages [39]. Interleukins at this stage promote the aggregation of macrophages into foreign body giant cells, designed to degrade the implant [40,41]. In the absence of successful degradation being achieved, the giant cells release destructive oxygen free radicals and hydrolytic enzymes and establish an acidified local environment [31,42]. It is at this stage that the chemistry of the implant itself determines whether the next phase is a further degradative one or if the material remains intact behind a dense collagen wall.

3.3.2 Tissue effects on biosensors

With survival of an intact implant, the outcome is a cell-quiescent local tissue state with an unrecognisable locally remodelled tissue environment featuring dense, orientated bands of collagen fibres with few constitutive cells or blood vessels [43–45]. The contribution of cellular and capsular variables on subcutaneous tissue glucose sensor response has been modelled by Reichert's Group [46,47]. Histology of the implant site follows the pattern represented earlier, local vascularity was noted, and although there were inter-individual differences in histology, overall, a porous network of connective tissue with inflammatory cells was evident that later polarised towards the sensor surface. In this particular implant model, capsular thickness appeared to be the factor dictating a slowed response, whereas porosity and tissue vascular density appeared to determine the magnitude of the response.

With regard to glucose sensor performance within tissue, after an initial stabilisation period, commonly referred to as the run-in period, yet to be fully understood, the eventual response is delayed in relation to blood glucose change by 10–15 min. Also, the absolute responses, at least from the literature reports that actually provide such information, can be lower by up to 80% versus blood. These factors make it a necessity to repeat the calibration in vivo, and the former also continues to lend uncertainty and risk to any notion of close-loop, sensor-regulated insulin infusion. The micro-anatomical tissue distortion juxtaposed to the biosensor, though limited in volume, poses a continued and time-variable uncertainty to biosensor readings at chronically implanted devices [48]. Predictive modelling is too imprecise, as yet, to allow reliable correction of response for any single individual, let across individuals. Interestingly, it has been found, even with a sensor membrane designed specifically to limit dependence on glucose flux at a commercial enzyme electrode, steep glucose gradients were observed up to 100 μm away from the sensor tip and these were surprisingly unrelated to any surface deposition of metabolically active stromal cells [49]. With regard to the above observed under-measurement of tissue glucose by enzyme-based sensors, it is not yet possible to distinguish between the component that is truly due to physiological inter-compartmental difference, and that which is sensor artefact; independent measurement has not resolved the issue. What is clear is that the extent of the artefactual element differs between sensor types and sensor dimensions. As regards its clinical significance, provided a sensor readout, after in vivo blood calibration, can track blood glucose excursions, then the system is clinically viable; indeed, this is the basis for all currently marketed glucose sensors.

Manipulation of the tissue matrix is an option for response stabilisation. Enhanced hydration through local fluid microinfusion has provided for tissue responses with implanted sensors that subsequently appear to be a dynamic and quantitative match for blood glucose levels [50]. This Open Microflow system using a concentric cannula-delivered hydrating fluid may well have operated through localised tissue expansion and with this a reduction in interstitial tissue density, so it promoted higher glucose flux to the electrode surface, while at the same time diluting inflammation-promoting factors. Any technology able to eliminate in vivo calibration and inexorable recalibration steps would change the way such sensors can be are used in diabetes management and, thus, considerably change long-term outcomes [51] in an analogous way to the therapeutic augmentation achieved with reliable, programmable infusion insulin pumps.

3.4 Materials for in vivo sensors

3.4.1 Ion selective electrodes

Beyond the early studies with intravascular Ca^{2+}, K^+ and pH electrodes based on PVC catheter electrodes, eg, pioneered by Band in the 1980s for continuous monitoring [52–54], few studies have ventured to further evolve ISEs for practical, continuous monitoring. Physiological findings have been of the nature of ion-change dynamics, in normal physiology and following the administration of pharmacologically active agents. However, there have been some attempts, recently, to use ISEs for heart surface monitoring in cardiac ischaemia [55–57], and with appropriate redesign further clinical measurement opportunities may arise.

Attention has, however, been given to core material needs for in vivo ISEs [58], though in relation to vascular use rather than tissue implantation. For such use there would be clear advantages to reducing, say, plasticiser content. Certainly for tissue use inevitable inflammatory reactions due to plasticiser leaching would need be avoided; 2-nitrophenyl octyl ether, for example, has been shown to cause acute inflammatory changes [5]. As an alternative, polyester sebacate, is more easily retained in PVC with at least an extension of ISE lifetime [59], and similar advantages accrue with branched polyadipates and polymeric phthalates used as plasticisers [60]; biocompatibility would be uncertain at this stage.

Prospects for medical use has stimulated work on alternatives to PVC. Thus, hydroxylated PVC, polyurethane and silicone rubber have been tried with demonstrable improvement in haemocompatibility [61a,b]. Further alternatives with technological and operational advantages include polysiloxane, which allows chemical tethering of active ionophore [62]; aliphatic polyurethane, which offers mobile anionic sites for H^+ response; anisotropic cellulose acetate which has potential for organic interference rejection based on size [63]; photopolymerisable epoxy (diacrylates) that allow for precision electrode fabrication and scale up production [64]; silicone-modified polyurethane which is both more resistant to oxidative damage in vivo and less thrombogenic [65]. Surface treatments to improve haemocompatibility without disturbing the sensing membrane component have included polyethylene oxide (PEO)/poly(propylene oxide) co-polymer [66], heparin [67] and biomimetic phosphoryl choline [68] coatings.

In a more recent extracorporeal application, a PVC lithium ISE housed in an oxygenator circuit allowed measurement of cardiac output via continuous tracking of post-injection Li^+ dilution in the peripheral circulation [69]. The technique has been validated for clinical use and exeplifies the inherent analytical reliability of an ISE [70]. The sensor is disposable, sterilisable and used within a flow-through cell, the active sensor surface here electrolytically connected to the requisite reference electrode using a saline bridge [71].

Although ISEs are perhaps the earliest of practical medical sensors, and have good response capabilities for baseline, "housekeeping", ions of relevance to medicine, their use is mainly restricted to the automated central hospital laboratory. Such in vitro use has, nevertheless, stimulated re-evaluation of their responsive constituents and membrane materials, eg, with regard to blood compatibility, which may have relevance to future in vivo use. Therefore, interest in the avoidance of leachables, for example, and investigation of alternative polymers is ongoing.

3.4.2 Glucose sensors

3.4.2.1 Rationale for monitoring

As with handheld metres, glucose remains the dominant target for in vivo monitoring. This is not surprising given its near-unique medical status as a diagnostic variable that is, respectively, under active dynamic control, where the loss of such control can have rapid, serious consequences and in which therapeutic response cannot be entirely fully predicted. The extent of the added benefit for insulin-dependent diabetics is still a matter for some debate, but at least for selected subgroups such as those with brittle diabetes, or those under acute stress conditions and illness, there is definite added value. It is also clear now that the long-term complications of vascular disease, neuropathy and renal damage can be reduced in severity or at least delayed through better monitoring and control [72]. In practical terms, there is the benefit of fewer needle stabs, of special relevance for children with diabetes. Silent hypoglycaemic episodes leading to central nervous system (CNS) damage are also likely to be minimised through trend analysis and timely intervention [73].

A myriad of chemistries have emerged to monitor glucose, but in the main these employ a redox enzyme-degrading glucose with the direct or indirect generation of electron flow through an electrically polarised working electrode. The majority of implantable devices use H_2O_2-based sensing rather than an interposing mediator in the reagent layer of the device; it is difficult to have a mediator as reagent that is guaranteed not to be leachable. An important safety consideration is the avoidance of dangerous thromboembolism and disseminated infection; accordingly, sensors are nearly all designed for tissue use rather than for intravascular use.

3.4.2.2 Enzyme-based systems

In view of the potential antigenic nature of an enzyme protein, it is a given that the external surface of any enzyme-based sensor requires coating with a barrier layer that both masks the underlying enzyme and presents tissue with an inert, non-degrading contact surface. The majority of packaging materials used have been adapted from biomaterials, where they have been well tested under implantation conditions. However, other key considerations then come into play. Controlled permeability to glucose is required and, possibly, to O_2 as the co-substrate, so the enzyme reaction does not become O_2 limited and can operate well below enzyme K_m. Through appropriate diffusional transport control, a covering polymer can also facilitate operation that is independent of external physicochemical variables, notably sample convection, viscosity, pH and ionic composition. In reality, it is only possible to suppress, not eliminate, such adventitial effects, given that, ultimately, an enzyme biosensor is a mini-bioreactor, with all the mass transport constraints and needs of any immobilised reactor phase, a major contrast to the thermodynamically well defined response of ISEs. Tissue catalase is another confounding factor. Though rarely considered, it has the potential to alter response through rapid, external H_2O_2 degradation so promoting its outward diffusion, following production by the enzyme reducing the gradient for its inward, signal-generating transport.

Substrate diffusion and reaction modelling has, thus far, not permitted precision design of barrier membranes, and a semi-empirical approach has generally been adopted.

One guiding principle has emerged, the use of maximum-diffusion barrier materials, within the constraints of retained signal magnitude, to minimise the sink effect of target molecule degradation by the sensor in the constrained tissue environment.

Polyurethane coatings have proven to be the 'workhorse' barrier membrane material, with permeability control achieved variously from pores generated during the coating of pre-polymer, through electrospinning of nano-fibres or through inclusion of hydrophilic component groups opening a route for glucose transport [50,74,75]. Polyurethanes have been further adapted for targetted properties. Thus, enhanced oxygen transfer has been attempted using polydimethylsiloxane inclusion [76], and steroid release to suppress inflammatory change through loading with dexamethasone [77]. Degradation is a concern for their long-term use; it has been observed for a polycarbonate-based polyurethane through hydrolytic tissue induced degradation, with degradation products being a further trigger factor for cellular and free enzyme attack [78]. The material surface physical profile is of known importance for cell adhesion; myoblast adhesion, for example, is greatest at porous polyurethane as compared with smooth, solvent-cast material [79]. Key degrading agents are hydrolytic enzymes such as cholesterol esterase, found in macrophages, an ongoing presence regardless of polyurethane type, as was specifically demonstrated for the case of a poly(ether-urea-urethane) [80]. Hydrogen bonding has some protective effect against enzymic hydrolysis, and could be a basis for long-term stable membranes [81]. Also, in vivo studies on mesh cage-confined polyether polyurethanes, Anderson et al. [82] observed oxygen to accelerate the degradation of polyurethane soft segments, while incorporation of polycarbonate, polydimethylsiloxane, and polyether substitution using hydrocarbon chains slowed the process. Surface modification has been tried using established biocompatible polymers such as PEG [83], effecting reduced surface protein adsorption and local inflammation.

More specialist membrane systems have been tested, such as with the layer-by-layer assembly technique for depositing alternate cationic and anionic oligomers. The procedure allows fine tuning of permeability properties. Thus, in a comparison of a low-permeability, layer-by-layer assembly system with a high-permeability one (poly(diallyldimethyl ammonium chloride)/polystyrene sulphonate) [84], a paradoxical higher current response was seen with the former and helped to direct attention to the importance not just of glucose access but H_2O_2 exit from the reaction layer.

Although commercial glucose monitors have used polyurethane-based covering membranes, these are modifications utilising proprietary formulations, reviewed by McGarraugh [85]. The Minimed Guardian sensor uses an oxygen diffusion-sustaining barrier layer comprising a polyurethane polyurea block co-polymer with diisocyanate, siloxane and PEG as the core constituents. The DExCom STS device similarly uses a transport-differentiating membrane for glucose versus oxygen, but uses a hydrophobic polyurethane mixture in which one of the sub-units has attached PEG to facilitate the glucose transport. A match of the hydrophobic elements here enables homogenous polymer integration and a continuum of properties. The Abbott FreeStyle Navigator, by contrast, uses a gel membrane with crosslinked vinyl pyridine and styrene; hydrophilic side groups here enable rapid hydration. A range of other porous and homogeneous membrane formulations have been investigated [48], but it is unclear at this stage if an optimally advantageous material will emerge.

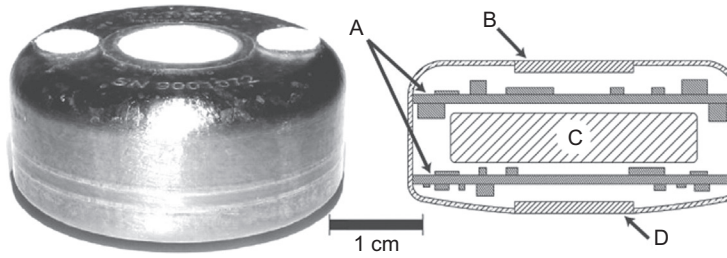

Figure 3.4 Implantable, self-contained titanium-housed battery, transmitter and sensor combination with two surface pads of polyester velour patches for fixation in tissue: (A) electronic sub-units, (B) telemetry transmission portal, (C) battery, (D) sensor location.
With permission from Gough DA, Kumosa LS, Routh TL, Lin JT, Lucisano JY. Function of an implanted tissue glucose sensor for more than 1 year in animals. Science Translational Medicine 2010;2(42).

Current semi-implantable commercial devices are designed to operate for limited periods, typically 3–10 days. For long-term monitoring, full surgical implantation would be necessary and the potential denaturation of enzyme by sustained exposure to the H_2O_2 product of an oxidase reaction, for example, would need to be considered. As a development from their earlier work on oxygen sensor-based intravascular electrodes, Gough's Group have developed a pillbox-type sensor (Fig. 3.4) using an oxygen cathode sensor [86]. The system has the advantage of monitoring the oxidase reaction using O_2 changes and so can incorporate catalase in the enzyme layer to eliminate H_2O_2. The critical oxygen-sensing membrane is gas-permeable polydimethylsiloxane (PDMS) over an electrolyte film. Over this is arrayed a series of wells containing immobilised glucose oxidase; the geometry of the PDMS wells is such as to present a higher surface area for oxygen transport, as opposed to reduced diffusive access for glucose, so avoiding oxygen limitation. The PDMS barrier, moreover, blocks the electrolytic path to tissue and so avoids internal polarised electrode electric field effects transferring to the tissue matrix whilst also eliminating access to solutes with the potential to poison the oxygen working electrode surface. It is feasible that electric field effects in tissue can promote tissue encapsulation. Tissue fixation of the device for long-term implantation was enabled through surface positioned polyester velour patches.

Independent of any membrane functional specifications, surface modification able to reduce bioincompatibility would be a strong asset. Phosphoryl choline (PC) is a zwitterion found on the external facing surface of the cell membrane lipid bilayer (BLM); PC head groups constitute up to 80% of the outer leaf of the red cell membrane, a proposed basis for its biocompatibility. One considered mechanism for this is that natural biofluid phospholipids adhere to these PC head groups through lipid–lipid interactions and the endogenous lipids are then able to mask the foreign surface. Relevant baseline observations have been made at glucose electrodes [87,88], eg, using a methacryloyloxyethyl phosposphoryl choline n-butyl methacrylate co-polymer. This particular coating tended to delaminate and disaggregate under haemodynamic flow, but PC bulk-phase integration with polyurethane proved more robust and also more effective, possibly through PC head group migration to the surface. Hydrogels also offer a tissue-tolerant surface, though not necessarily one that conveys

enhanced biocompatibility. One example is HEMA–DHPMA (hydroxyethyl methacrylate–2,3-dihydroxypropyl methacrylate) copolymer, but gel phases such as these would also need some form of reinforcing material, either as a 3-D scaffold or one that furnishes additional gel crosslinks [89].

3.4.2.3 Affinity sensors

Enzymic glucose degradation offers a popular, ready, means for generating an instrumented response. Affinity sensors are now being investigated in more detail as an alternative, and as with ISEs are able to reach an equilibrium response without degrading the target molecule. Early work on this affinity principle centred on the sugar-binding lectin Concanavalin A. Reversible lectin binding here required a semi-permeable hollow-fibre membrane to permit inward diffusion of glucose whilst retaining the lectin and labelled macromolecular indictor dextran molecules [90,91]. Becton Dickinson reports a prototype optical sensor with a galactose/glucose binding protein retained within a glucose-permeable matrix. Conformational molecular flexibility is required for a fluorescently mediated glucose binding response, and the matrix apparently permits this [92].

Boronic acid binds reversibly to vicinial hydroxyls, and so shows binding affinity for the hydroxyls on glucose, providing a non-protein, non-degrading binding vehicle. A variety of constructs have been proposed in which a fluorescently labelled boronic acid alters its fluorescent output, or its chromophore binding by glucose. Sequestration of lipophilic boronic acid in a hydrophobic matrix has allowed glucose binding with minimum adverse interaction with sample [93]. A gel-entrapped diboronic system has also been used for tissue glucose monitoring in experimental animals [94]. Here, a complex with a fluorescence-quenching anthracene was used; glucose-induced conformational change disrupted the quenching. Fluorophore injected as polyacrylamide microbeads became dispersed in tissue; by contrast injected fibres instead remained in position without dispersion, could be cut to required length and were retrievable. Through use of a PEG–polyacrylamide gel composite, protein adsorption and inflammatory response was suppressed, permitting improved optical signal transmission through the tissue [95]. The reporter gel has been tested as part of a micro fabricated complementary metal-oxide semiconductor (CMOS) image sensor with integrated light-emitting diodes (LEDs) that is of sufficiently small diameter to be injected [96]. A gel-loaded Concanavalin A reporter implanted subconjunctivally demonstrates the challenge of long-term use [97]. Alginate-entrapment gel was used, and though combined with biocompatible poly (vinyl alcohol) and a hydrophilic biocompatible coating, a tissue response still occurred that generated an avascular fibrous capsule.

An intravascular fibreoptic probe has been developed using gel-loaded boronate. This is intended for short-term use in critically ill patients (~40 h) [98]. The key materials goal is haemocompatibility, thus a dialysis membrane encloses the gel to serve as a barrier against proteins, close off this access route to glycated and non-glycated protein interference while a microporous membrane prevents ingress of blood cells (Fig. 3.5). The latter is also platinum loaded to destroy peroxides that are able to oxidise the boronate binder and combines an outer heparin layer to reduce initial blood cell adhesion.

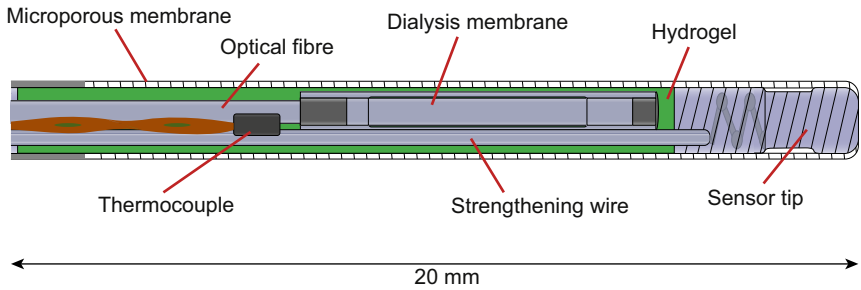

Figure 3.5 Cross section of boronate-affinity sensor for intravascular glucose monitoring showing the bornonate gel-covering dialysis membrane and outer haemocompatible, heparin-coated microporous membrane.
With permission from Crane BC, Barwell NP, Gopal P, Gopichand M, Higgs T, James TD, et al. The development of a continuous intravascular glucose monitoring sensor. Journal of Diabetes Science and Technology 2015;9(4):751–61.

3.5 Materials for ex vivo sensors

Sampling from the body is an alternative to sensor implantation, avoiding attendant tissue-interfacing concerns. The GlucoWatch system (Cygnus, California) attempted this [99]. Reverse iontophoresis was used to extract tissue fluid through skin, with sample taken up into an absorbent pad loaded with glucose oxidase, and formed from cross-linked PEG-polyacrylic acid. The assay cycle was a 3 min collection period and 7 min measurement, with the *total* amount of peroxide in the pad determined electrochemically. This promising development was discontinued, however, partly because of the unreliability of iontophoretic glucose extraction and also because of problems of skin irritation.

Microdialysis-based tissue sampling in the GlucoDay monitor (Menarini, Florence) has enabled use of an extracorporeal sensor in a flow cell for limited 48 h operation [100]. Predilution in the microdialysis step, together with its elimination of sample protein enabled a more traditional Yellow Springs Instrument type sensor to be used [24]. Although microdialysis is an important sampling methodology, and the hollow-fibre sample screen has the advantage of pre-selecting for microsolutes only, the level of solute recovery from tissue cannot be predicted, may be time dependent and can, moreover, create a solute depletion zone at the collection site, itself with attendant effects on solute recovery. With on-line monitoring of pH, pO_2 and pCO_2, again, more standard electrodes and electrode membranes can be used, equivalent again to their in vitro counterparts, but arterial , and not venous, sampling is is demanded and makes such monitoring more cumbersome as well as clinically higher risk [101].

3.6 Conclusion

This chapter has mainly focussed on tissue glucose biosensors; inevitably, given both the huge societal importance of such monitoring and the consequential multifaceted approaches for its measurement. The market pull and technological capabilities for

other parameters is much more limited. These have been given limited attention mostly because for most cases they are at an early conceptual stage, not a realistic proposition for medicine and materials strategies have not yet been described. This may change in the future, particularly with greater attention now being paid to sensors as an expression of materials science [102,103]. It is likely that what has been learnt for glucose sensor materials will translate to other micro- and macromolecular targets. In future, also, smart materials, plastic antibodies, and aptamers might change the outlook for implantable systems With this additional materials for effecting sample and reagent processing may come to the fore. What is clearly evident, though, is the emphasis that industry has given to materials science for biosensors and the practical successes they have been secured using this approach. The stage is now set for a more balanced strategy to biosensor design for tissue/blood monitoring with clear recognition of the need to first understand the behaviour of the biomatrix and from this to design better interfacing materials that can be useful right across the spectrum of point of care diagnostics sensors and instrumentation [27].

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Screen printing and other scalable point of care (POC) biosensor processing technologies

4

L. Gonzalez-Macia, A.J. Killard
University of the West of England, Bristol, United Kingdom

4.1 Introduction

The current increase in healthcare costs and an aging global population have generated a growing need for small, rapid, low-power and robust analytical tools for the remote monitoring of patients, either by the patients themselves or by medical doctors and healthcare providers (Campbell, 2011; Arlett et al., 2012; Chin et al., 2013; Vasan et al., 2013). Point of care (POC) biosensors are miniaturized portable devices that integrate all the functions of centralized laboratory testing without the need for trained staff. This unique characteristic becomes essential for collecting real-time responses in areas with low resources, such as developing countries or where clinical laboratory facilities are absent (eg, military expeditions and space missions) (Sia and Kricka, 2008). Typical device dimensions are in the order of millimetres to a few square centimetres, although some items may be larger – around credit-card size – and operate with sample volumes ranging from approximately 1 μL to 1 mL (Arlett et al., 2012; Gubala et al., 2012).

Ideally, POC devices should be inexpensive disposable devices based on 'chip' or cartridge formats that comprise: an *inlet* for sample introduction, *microfluidic features* to transport the sample to the *reaction cell*, *biosensor* for target recognition, *signal transducer*, *electronics* for control and communication (display), data management and storage, *power supply* and a *device identification system,* such as a bar code or similar (Price and John, 2008; Gubala et al., 2012). The combination of advances in areas such as microfluidics, biosensor analysis and microelectronics have been, and continue to be the driving force to enable the development of POC devices and broaden their application. There is continuing drive towards the miniaturization and microfabrication of the devices to minimize the consumption of costly reagents as well as sample volume and power consumption required for the analysis and to increase portability for mobile diagnostics (Chan et al., 2013; Chin et al., 2013). With respect to microfluidics, the application of microfabrication (microcontact printing, etching) and rapid prototyping [microinjection moulding, three-dimensional (3D) printing, and lamination] techniques to polymers such as elastomers and hard plastics has enhanced the integration of these systems in POC devices (Attia et al., 2009; Chin et al., 2012; Pataky and Brugger, 2014). In the microelectronics field, silicon-based systems have been at the centre of the miniaturization challenge. However, these have been shown to be unsuitable for

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disposable biosensor applications because of high cost, low biocompatibility with physiological fluids and absence of available rapid prototyping (Chin et al., 2013). Printed electronics is an emerging technology that enables printing of processable materials (organic, inorganic and hybrid) and electronic devices, such as power sources, biosensors and displays, for different types of applications (Lupo et al., 2013; Cen et al., 2014; Secor et al., 2014). Unlike conventional semiconductor electronics technologies, the devices are generally fabricated at room temperature and are compatible with mass production applications, often using roll-to-roll processes. Techniques such as screen and inkjet printing are some of the examples that allow the manufacture of lightweight, flexible and low-cost products (Sokolov et al., 2009; Aliaga et al., 2015). Screen printing had already been adapted in the early 1980s by Medisense for the fabrication of enzyme biosensors for home blood-glucose monitoring devices (Turner, 2013). The development of the biosensor industry has been intimately connected to the advances of blood glucose sensors for people with diabetes, a disease with which more than 150 million people suffer worldwide and that leads to about six billion glucose assays performed annually (Heller and Feldman, 2008; Hayat and Marty, 2014). In comparison with the worldwide in vitro diagnostics (IVD) market (currently worth $50 bn per year), the printed glucose biosensor industry for diabetes monitoring represents more than 10% of this market ($6.4 bn) (Taylor et al., 2014).

Half of the disposable glucose sensors are fabricated by screen printing, whereas the rest are produced using a combination of vapour thin-film deposition and laser micromachining. Despite all the improvements in this area, most of the glucose diagnostic devices still rely on disposable strips costing US$ 0.02– $0.06, combined with a pocket-size meter costing US$ 10–100. Recent developments have led to the design of promising all-printed sensor devices in which all the elements (battery, sensor and display) are printed using roll-to-roll techniques and integrated to generate a credit-card size device. Killard et al. have recently demonstrated the successful integration of multiple printed electronic components into a single device capable of the measurement of hydrogen peroxide and cholesterol (Fig. 4.1). The device was capable of measuring 8 µl samples of both hydrogen peroxide and cholesterol (using cholesterol oxidase) from 0 to 9 mM. It could operate for 10 min via a printed battery and display the result for many hours or days. A mobile phone ‘app’ was also capable of reading the test result and transmitting this to a remote health-care provider (Ritvonen et al., 2013). However, additional difficulties regarding organic semiconductors (eg, light sensitivity, oxygen and water degradation and compatibility with complex sample media) must be addressed, and minute silicon chips are still required to process the signals (Sokolov et al., 2009; Turner, 2013).

Despite being one of the most promising areas for biosensor application, POC systems face several challenges to compete with well-established traditional technologies for patient diagnostics (Choi et al., 2011; Arlett et al., 2012; Chan et al., 2013):

- High sensitivity and selectivity for the target analyte employing ultrasmall sample volumes;
- No special storage conditions should be required, particularly in developing countries;
- Easy-to-use by patients of any age without special training. Internal control should indicate when the measurement is not valid as well as eliminate erroneous results;

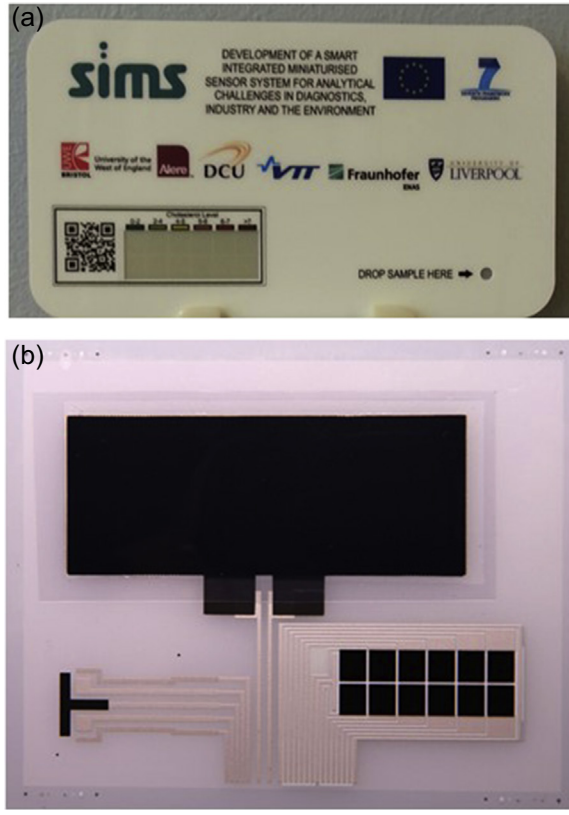

Figure 4.1 A highly integrated printed sensor system for application in biomedical diagnostics. (a) The assembled prototype showing printed cover with Quick Response (QR) code, concentration values and sample application zone; (b) integrated printed system components: sensor (bottom left), display (bottom right) and battery (top) were effectively integrated onto a single substrate using a combination of screen printing, inkjet printing, bonding and lamination processes.
Ritvonen, T., Gonzalez-Macia, L., Willert, A., Ylimaula, A., Donaghy, D., Tuurala, S., Suhonen, M., Myers, R., Smart, D., Morrin, A., Baumann, R., Raja, M., Kemppainen, A., Killard, A.J., 2013. SIMS: a smart integrated biosensor system. In: International Conference on Flexible and Printed Electronics_ICFPE2013, Korea.

- Miniaturization of the devices, which generally enhances physical and chemical effects, leading to more complex processes compared to lab equipment as well as low signal-to-noise ratios;
- Low-cost and rapid production, to achieve affordable and practical POC tests.

In this chapter, we review the state of the art of the current techniques employed for the fabrication of biosensor devices, from those commonly utilized in recent decades (ie, screen printing) to other more recent developments, such as 3D and inkjet printing.

Descriptions of the techniques and their applicability to the POC diagnostics field will be discussed. Examples of devices already commercially available in the POC biosensor industry, together with future perspectives and issues to overcome to reach the market for other techniques will be illustrated.

4.2 Printing techniques

The adaptation of printing techniques to the biosensor industry brought about a revolution in in vitro diagnostics with the introduction of disposable, low-cost strips for the individual monitoring of personal health.

Printing techniques can be classified as contact and noncontact methods depending on whether they involve direct interaction, often with high pressure, between the substrate and the surface from which the material is donated, or whether the transfer of materials occurs by ejection (Barbulovic-Nad et al., 2006; Dias et al., 2014). Advances in printing technologies have included the modification of traditional contact methods utilized in newspaper production and graphic art reproduction (eg, screen and gravure printing) and more recent noncontact processes oriented to highly detailed and more precise purposes. The deposition of biological samples on biosensor platforms by inkjet printing or the manufacture of paper-based analytical devices (PADs) assisted by wax-ink printing are some examples of the latest achievements of noncontact printing methods. Table 4.1 addresses the main characteristics regarding biosensor fabrication (resolution, substrate, speed and cost) associated with the printing techniques discussed in this section. Besides health monitoring, other potential applications of printed biosensors are: security, environmental sensors, smart clothing, sport doping and food industry (Dias et al., 2014; Taleat et al., 2014; Taylor et al., 2014).

4.2.1 Contact methods

4.2.1.1 Screen printing

Screen printing is a conventional thick-film technique that enables the deposition of both organic and inorganic materials on flexible substrates, making it compatible with rapid roll-to-roll processing (Pardo et al., 2000; Parashkov et al., 2005; Windmiller and Wang, 2012; Hoth et al., 2013; Cao et al., 2014). Briefly, a liquid paste is forced by a rubber squeegee through a mesh screen, with a mask which defines the pattern to be transferred to the substrate surface (Fig. 4.2A). The inks employed in screen printing typically consist of three basic components: a powdered metallic (gold, platinum, silver) or nonmetallic (graphite) functional material, a polymeric binder (eg, glass powder, resins or cellulose acetate) and a solvent (eg, terpineol, 2-ethoxyethanol, cyclohexanone or ethylene glycol). Adhesive and nonconductive materials can also be deposited by this technique. Screen-printing inks usually exhibit a thixotropic behaviour, ie, the viscosity of the ink decreases with an increasing shear rate and increases again when shear rate ceases, which is influenced by all the ink components. The thixotropic property of the inks fulfils the properties

Table 4.1 Printing methodologies and their characteristics

	Contact methods				Noncontact methods		
	Screen	**Gravure**	**Flexo**	**μCP**	**Inkjet**	**Wax-ink**	**3D**
Resolution (minimum feature size)/μm	50–100	20–50	50	0.2–1	≥1	850	16–100
Ink viscosity/Pa s	0.5–50	0.05–0.2	0.05–0.5		0.003–0.02		
Printing speed/m min^{-1}	10–15	20–1000	50–500		50–200		
Substrates	All (glass, plastic, metal)	Flexible (paper, plastic polymers, board)	Flexible (paper, plastic polymers, board)	Silicon, glass or ultraflat metal	All (glass, plastic, metal)	Paper	
Cost	+	++	+	++	++	+	+++

μ*CP*, microcontact printing.

Lawrence, D., Kohler, J., Brollier, B., Claypole, T., Burgin, T., 2004. Manufacturing platforms for printing organic circuits. In: Gamota, D.R., Brazis, P., Kalyanasundaram, K., Zhang, J. (Eds.), Printed Organic and Molecular Electronics, USA, Kluwer Academic Publishers; Gonzalez-Macia, L., Morrin, A., Smyth, M.R., Killard, A.J., 2010. Advanced printing and deposition methodologies for the fabrication of biosensors and biodevices. Analyst 135 (5), 845–867; Castrejon-Pita, J.R., Baxter, W.R.S., Morgan, J., Temple, S., Martin, G.D., Hutchings, I.M., 2013. Future, opportunities and challenges of inkjet technologies. Atomization and Sprays 23 (6), 541–565; Gross, B.C., Erkal, J.L., Lockwood, S.Y., Chen, C., Spence, D.M., 2014. Evaluation of 3D printing and its potential impact on biotechnology and the chemical sciences. Analytical Chemistry 86 (7), 3240–3253; Ihalainen, P., Maattanen, A., Sandler, N., 2015. Printing technologies for biomolecule and cell-based applications. International Journal of Pharmaceutics 494 (2):585–592, doi:10.1016/j.ijpharm.201502.033.

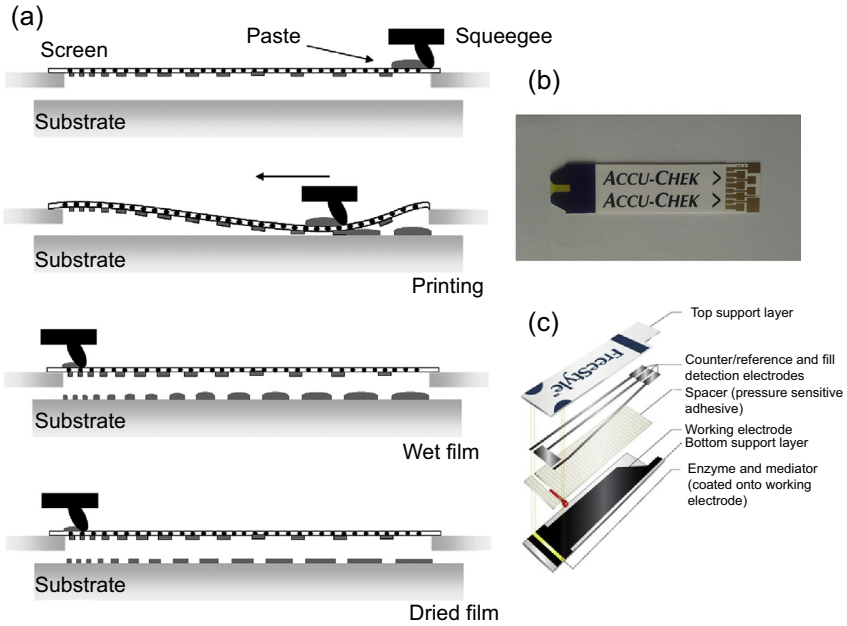

Figure 4.2 (a) Schematic diagram of the screen-printing process *(Reprinted from Lee, D.-H., Choi, J.S., Chae, H., Chung, C.-H., Cho, S.M., 2009. Screen-printed white OLED based on polystyrene as a host polymer. Current Applied Physics 9 (1), 161–164, with permission from Elsevier)*; (b) Accu-Chek® Aviva Plus Roche Diagnostics glucose biosensor strip; (c) diagram of the structural components of a commercially available blood glucose test strip (FreeStyle® Abbott Diabetes Care) *(Feldman, B., 2009. Electrochemical blood glucose test strips for people with diabetes. Electrochemistry Encyclopedia).*

required by the ink for the printing process. The ink should not flow during standstill to avoid dripping, whereas high shear rates are needed throughout the squeegee operation (Tafelmeier, 2015). The solvent is chosen based on its solubility for the binding agent and volatility for thermal curing, whereas the binding agent improves the mechanical strength and flexibility of the ink and its adhesion on the substrate. Variations of the size, type or loading of particles (functional materials and binders) in the ink have a significant effect on the overall performance of the printed materials (viscosity, thermal resistance, hydrophobicity and conductivity) and, therefore, can be used to customize the formulations to meet the final application requirements. After the printing step, the freshly deposited material is typically cured by oven drying or a photocuring process using UV irradiation (Zhang, 2003; Laschi et al., 2008; Gonzalez-Macia et al., 2010; Hayat and Marty, 2014).

Traditionally, screen printing has been used for the manufacture of biosensor platforms as the printed substrates can be easily modified (metal films, conducting polymers, nanoparticles and other nanostructured composites) for the recognition of different analytes. However, the biological molecules (enzymes, DNA, antibodies) are usually dispensed in some other way (Gonzalez-Macia et al., 2010; Mistry et al., 2014; Taleat et al., 2014). Slot-die coating and drop deposition are two of the conventional

techniques used for the commercial deposition of biosensor reagents. However, drawbacks such as manufacturing speed, material waste and quality issues have lately led the market towards a new improved technology (Jospeh, 2012). Techniques such as inkjet printing and microspotting are now commonly applied for the functionalization of biosensors at the research level and are promising methods for mass production applications (Pataky and Brugger, 2014). Besides the widely known glucose-biosensor market (Fig. 4.2B and C), there are many other literature examples of biosensors manufactured using screen printing. Commercial carbon screen-printed electrodes modified with carboxylic acid-functionalized single-walled carbon nanotubes (SWCNT-SPEs) have been employed for the monitoring of DNA hybridization using differential pulse voltammetry (DPV) and electrochemical impedance spectroscopy (EIS). An amino-linked complementary DNA sequence based on the breast cancer early onset gene mutant *BRCA1* was immobilized onto the carboxylic acid-modified electrode surface and was applied as a probe for the electrochemical quantification of the target *BRCA1* sequence. The use of screen-printed platforms has facilitated the ease, cost and speed of sensor fabrication, allowing an increased number of tests in a short time (Li et al., 2012). The same type of commercial screen-printed electrode has also been employed as a platform for the development of a sensitive biosensor for the rapid and inexpensive determination of lactose. Cellobiose dehydrogenase (CDH), which is involved in the lactose recognition process, was deposited onto the electrode surface and cross-linked with glutaraldehyde (GA) and poly(ethylene glycol) diglycidyl ether (PEGDGE) to increase the stability and sensitivity of the disposable device. The biosensor was successfully applied to the analysis of lactose in dairy products, including lactose-reduced and "lactose-free" milk with a high reproducibility [1.5–2.2% relative standard deviation (RSD)] (Safina et al., 2010).

Disposable immunosensors for the determination of bacteria based on screen-printed platforms have been also reported in the literature. Afonso et al. (2013) fabricated a screen-printed carbon-based biosensor in combination with magnetic separation for the rapid detection of *Salmonella* in milk samples. Magnetic beads coated with anti-*Salmonella* antibodies (MBs-pSAb) and modified AuNPs coated with polyclonal anti-*Salmonella* antibody were used as the capture phase (preconcentrating step) and electrochemical labels, respectively. The modified magnetic beads were then captured onto the electrode surface by applying a magnetic field below the printed sensors and analysed electrochemically by DPV.

The addition of other substances such as metal oxides, polymers, electrochemical mediators and complexing agents in the printing formulation can enhance the sensing properties of the inks, while reducing the number of steps in the manufacturing process. Recently, some approaches to screen-print biological inks have been reported, which increase the homogeneity and simplicity of the product fabrication process and provide all-printed commercial biosensors. A water-permeable material is sometimes printed onto the already printed biological products to protect the biolayer from damage during printing of additional layers, while also allowing the aqueous analyte to pass through to reach the transducer surface (Taylor et al., 2014).

To sum up, screen printing is a rapid, simple and robust technique that can be used on its own or in combination with other patterning techniques for the manufacture of commercial biosensors (Gonzalez-Macia et al., 2010; Hayat and Marty, 2014). It

enables the fabrication of miniaturized, versatile devices that can be connected to portable instrumentation for in situ analysis of glucose, DNA, cancer biomarkers, toxins, drugs and other analytes (Montornes et al., 2008; Taleat et al., 2014). Its scalability enables the mass production of low-cost, disposable devices, making this printing approach the most widely used in the biosensor industry.

4.2.1.2 Gravure printing

Gravure printing is a widely used roll-to-roll technique that enables the rapid fabrication of high-resolution printed products, reaching speeds of 20–1000 m/min (Ihalainen et al., 2015). Briefly, the pattern to be printed is engraved as a discrete cavity into a rotary printing cylinder. During the printing process, the engraved cavities are filled with the ink by passing an ink bath and a flexible doctor blade is used to remove the excess ink. A chambered doctor blade system can be used for inks containing highly volatile solvent. The ink on the printing roll is then deposited when the cylinder is brought into contact with the substrate, as is shown in Fig. 4.3A (Gonzalez-Macia et al., 2010; Søndergaard et al., 2013b).

Good ink transfer is ensured by applying a relatively high print pressure (1–5 MPa) and using quite low-viscosity inks (50–200 mPa) (Ihalainen et al., 2015). However, lower viscosity inks (1–10 mPa) have been successfully employed for the gravure printing of biomolecules such as protein and enzymes during the manufacture of printed and flexible biosensors and biochips (Heikkinen et al., 2011; Pavinatto et al., 2015).

The thickness of the printed layer is determined by the gravure cell volume and the pick-out ratio, leading to feature lines ranging from 0.2 to several micrometres (Gonzalez-Macia et al., 2010; Søndergaard et al., 2013b; Cen et al., 2014).

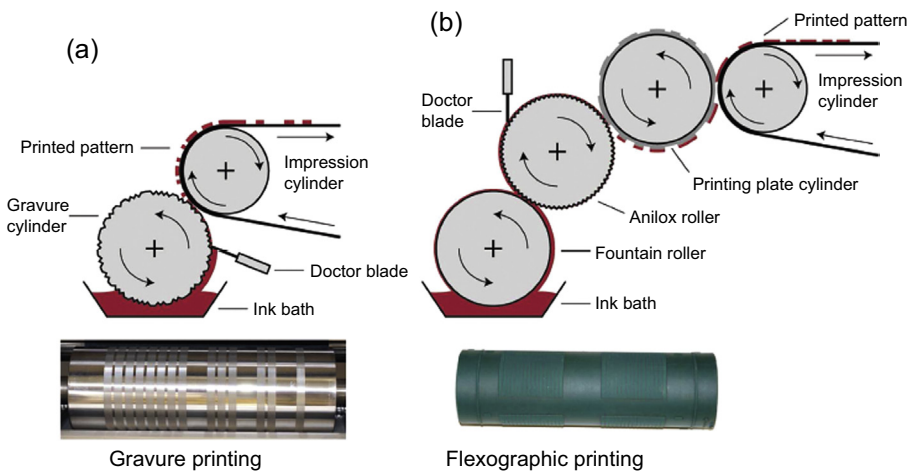

Figure 4.3 Schematic illustration: (a) gravure and (b) flexographic printing
Reprinted from Sndergaard, R.R., Hsel, M., Krebs, F.C., 2013b. Roll-to-roll fabrication of large area functional organic materials. Journal of Polymer Science Part B: Polymer Physics 51 (1), 16–34, with permission from John Wiley and Sons.

Traditionally used for the fabrication of high volumes of catalogues and magazines, this technique has already been applied to industrial-scale applications in printed electronics (Lupo et al., 2013). Functional materials containing graphene, metallic particles (Ag), polymers poly(3,4-ethylenedioxythiophene) polystyrene sulfonate (PEDOT/PSS), polymethyl methacrylate (PMMA) and organic semiconductors have been gravure-printed on various substrates. These include, eg, polyethylene terephthalate (PET), paper, indium tin oxide (ITO)-covered PET for the manufacture of photovoltaic cells (OPV), organic thin-film transistors (OTFT), polymer light-emitting diodes (PLED) and electrochromic (EC) devices (Søndergaard et al., 2013b; Grau et al., 2014; Hernandez-Sosa et al., 2014; Schneider et al., 2014; Secor et al., 2014). Gravure rolls are long lasting, but expensive to produce, making gravure printing cost-effective mainly for very-large print volume applications (Ihalainen et al., 2015).

Gravure printing has demonstrated its potential for use in the rapid fabrication of flexible and low-cost biosensor platforms, although other deposition techniques are preferred for the addition of biologically active layers.

4.2.1.3 Flexographic printing

Flexography is a continuous high-speed roll-to-roll process traditionally used for graphics printing in applications such as packaging. During the printing process, a printing roll coated with the patterned flexography rubber or photopolymer is inked by directly contacting an ink bath or through an intermediate inking roller, creating a uniform film of ink onto the raised motifs (Fig. 4.3B). Like in gravure printing, excess ink is removed by a doctor blade open to air, or chambered in the case of inks with a high content of volatile solvents, ensuring good control of the layer thickness on the printing roll (Søndergaard et al., 2013b). The image is finally transferred by direct contact with the substrate (coated and uncoated paper, board or plastic films), applying smaller pressures than in gravure printing (Gonzalez-Macia et al., 2010; Ihalainen et al., 2015).

Flexographic printing commonly employs low-viscosity (50–500 mPa) and rapid-drying fluid inks, with an emerging focus on more environmentally friendly water-based inks as an alternative to improve the quality of packaging materials and enhance their performance and sustainability (Zvonkina et al., 2014; Żołek-Tryznowska et al., 2015).

Besides food packaging, more recent applications of flexographic printing involve functional materials (PEDOT/PSS, silver-based and dielectric inks) and have been focused on electronics applications such as photovoltaic cells, organic thin-film transistors (OTFTs) and electrochromic displays (Søndergaard et al., 2013a,b). Regarding drug delivery applications, flexographic printing has lately demonstrated the rapid deposition of rasagiline mesylate and tadalafil as model drugs on orodispersible films (ODFs), which disintegrate within seconds when placed onto the tongue and facilitate the oral dosage administration of drugs. An increase in the film homogeneity and lower levels of crystallization of the active pharmaceutical ingredients (APIs) were observed after flexographic printing in comparison with the traditional ODF manufacture technique, solvent casting (Janβen et al., 2013). Few examples exist concerning

the adaptation of flexographic printing for biological applications and most of them are related to the paper industry (Määttänen et al., 2011; Koivula et al., 2013). Philips et al. (2012) have demonstrated the accurate patterning of an array of antibodies onto flexible plastic substrates using flexographic printing. This might be considered as a printed Enzyme-Linked Immunosorbent Assay (ELISA), in which the washing and incubation stages, which normally take place in a microtiter plate, take place on a low-cost polymer film. The ink additives did not seem to negatively affect the activity of the antibodies, showing flexographic printing as a promising alternative for the highly flexible and cost-effective manufacturing of immunoassays as well as biosensors involving DNA, enzymes, aptamers or cells (Phillips et al., 2012). In fact, a few companies already offer materials for flexographic printing suitable for biosensing applications (Genina et al., 2012).

4.2.1.4 Microcontact printing

Another printing method that has been extensively used for the preparation and functionalization of micro- and nanostructured surfaces for a variety of applications is microcontact printing (μCP) (Mrksich and Whitesides, 1995; Wang et al., 2014). This photolithography-assisted technique is utilized to manufacture for instance, biosensor platforms by direct delivery of biological materials (proteins, antibodies and cells) from elastomeric stamps typically composed of patterned polydimethylsiloxane (PDMS) (Gonzalez-Macia et al., 2010; Kaufmann and Ravoo, 2010; Dias et al., 2014). The stamp is generally 'inked' with the solution containing the desired material to be deposited and brought into contact with the substrate (commonly ultraflat metal, silicon or glass). The material is then transferred to the surface only at those regions where the stamp contacts the surface, creating a pattern defined by the pattern on the stamp (Mrksich and Whitesides, 1995). The stamps are generally fabricated by using silicon wafer masters containing the negative image of the desired pattern in a multistep process involving photolithographic masks and photoresists. A broad range of materials – including conducting polymers, metallic nanoparticles, carbon nanotubes, proteins and DNA – have been deposited using μCP (Sung et al., 2009; Kaufmann and Ravoo, 2010; Fuchsberger et al., 2011; Salomon et al., 2012). However, further modifications of the stamp and the substrate are sometimes required to improve the material transfer because it has been suggested that the difference in wettability between the two surfaces plays a critical role in the efficiency of the transfer process (Gonzalez-Macia et al., 2010). For example, poly (4-aminostyrene) has been employed to micropatttern biomolecules and nanomaterials on glass slides and to print metallic structures on a carbon surface. Poly (4-aminostyrene) can be converted to an aryldiazonium salt, which exhibits a pH-dependent hydrophobicity. In general, polyelectrolytes are very promising modifiers to assist in μCP because they are water soluble and increase the wettability of the substrates without further modifications of the PDMS stamp (Wang et al., 2014). Other alternatives for protein deposition have been reported by Ricoult et al. (2014). The use of hydrophilic PDMS stamps together with the humidification of the stamp–substrate interface has been shown to enhance protein diffusion and enable large-area patterning with nanometer resolution (Ricoult et al., 2014). A biosensor

for the early-stage detection of human interleukin-10 (IL-10), an antiinflammatory cytokine related to multiorgan dysfunction in end-stage heart failure (ESHF) patients, was fabricated by a combined method involving chemical vapour deposition and μCP. A hafnium oxide (HfO_2) substrate was first functionalized using an aldehyde-silane and the antihuman 1L-10 monoclonal antibody was subsequently deposited by μCP creating 10 × 10 μm patterns. The antibody–antigen interaction was characterized by fluorescence and electrochemical impedance spectroscopy (EIS), with a linear range of 0.1–20 pg/mL and a sensitivity of 0.49 $(ng/mL)^{-1}$ (Lee et al., 2012). More recently, polymeric biomaterials deposited on a thermoresponsive polymer by μCP have been used for the immobilization of live cells (a human cancer cell line, mouse embryonic stem cells and human mesenchymal stem cells) at 37°C and the subsequent release of cell–biomaterial complexes at room temperature. This method was simple and inexpensive compared to the conventional methods and it showed the potential for future applications in drug delivery, tissue engineering and cell tracking (Wang et al., 2015).

Despite numerous examples in the literature, μCP is a contact-based printing technique involving relatively high pressure on a stamp to transfer the material to the selected biosensor substrate, which is not always compatible with biological materials. Moreover, highly accurate alignment between stamp and substrate is required for reiterative micropatterning on the same surface and new stamps are to be fabricated for different patterns, which slow down the manufacturing process (Dias et al., 2014). Therefore, many applications using μCP for biomaterial patterning can be found at the research lab scale. However, no truly mass production application has been reported so far, to the best of the authors' knowledge.

4.2.2 Noncontact methods

4.2.2.1 Inkjet printing

Inkjet printing has become one of the most versatile and reproducible dispensing techniques for rapid prototyping available in the market. It allows the accurate deposition of very small volumes of liquid droplets (typically, 1–30 pL) on a wide range of substrates (glass, plastic, metal) with a precision and reproducibility higher than other techniques such as screen printing, pen dotting or pipetting (Wang et al., 2009). The most commonly used inkjet printers are based on either drop-on-demand thermal or piezoelectric droplet formation methods, although there are also other existing technologies in the market such as electrostatic and acoustic printers (Fig. 4.4). The number of operating nozzles in the printhead (between 1 and 128 for lab-scale inkjet printers) has an impact on the achievable printing speed. This represents one of the limitations of this relatively new technology for individual industrial applications. However, the use of multiple nozzles and printheads can speed up the process which is capable of reaching high-speed mass production (Gonzalez-Macia et al., 2010; Søndergaard et al., 2013b; Teichler et al., 2013). Typical feature sizes of approx. 30–50 μm are typically achievable with the standard inkjet printer models available in the market (Fujifilm Dimatix Inc., MicroFab Technologies Inc. and Microdrop Technologies GmbH for some examples) although features as small as 1 μm have been recently

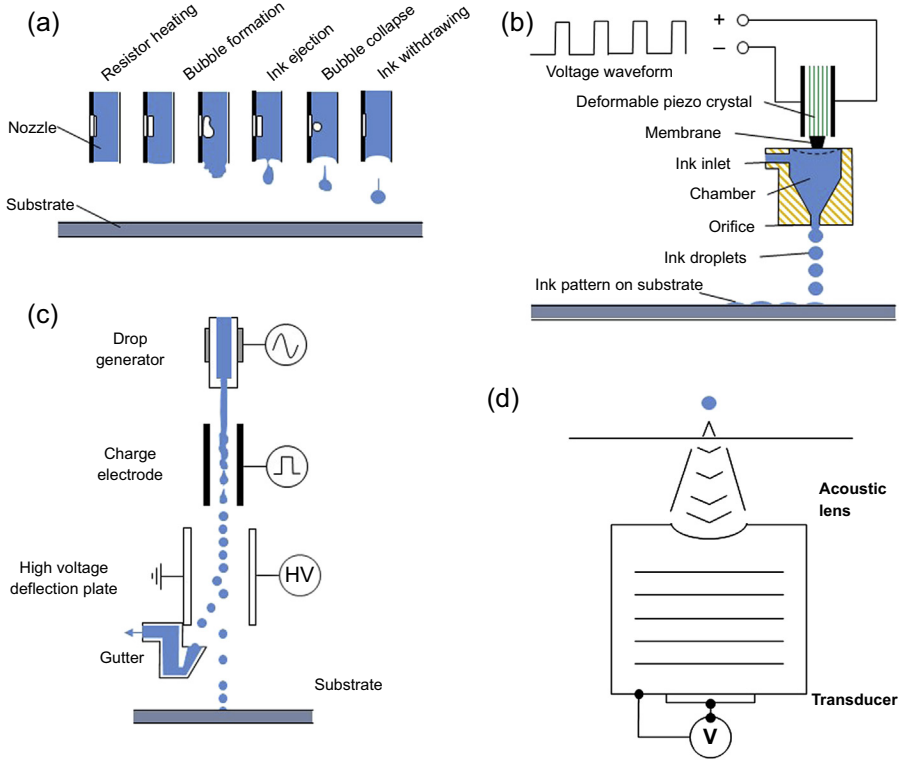

Figure 4.4 Inkjet printing ejection mechanisms: (a) Thermal inkjet printing (*Reprinted from Ballarinet al., 2004, with permission from Elsevier*); (b) piezoelectric inkjet printing (Fuller et al., 2002); (c) electrostatic inkjet printing (Lin and Bai, 2004); (d) acoustic inkjet printing (Parashkov et al., 2005).
Reproduced from Gonzalez-Macia, L., Morrin, A., Smyth, M.R., Killard, A.J., 2010. Advanced printing and deposition methodologies for the fabrication of biosensors and biodevices. Analyst 135 (5), 845–867 with permission of The Royal Society of Chemistry.

demonstrated by employing inkjet technology with subfemtoliter droplet volumes (Sekitani et al., 2008). These resolutions are compatible with the fabrication of multiplexed biosensors.

The preparation of inkjet-printable inks can be challenging due to the restrictive rheological properties required for the inkjet-printing process. To ensure the formation of a continuous film onto the substrate and avoid clogging or dripping at the nozzles, the viscosity and the surface tension of the inks should be in the range of 3–20 cP and 20–70 dyn cm^{-1}, respectively (de Gans and Schubert, 2003; Ngamna et al., 2007). Generally, inks comprise a liquid carrier or solvent containing functional molecules (pigments, dyes, metallic nanoparticles and biological

components) mixed with polymeric binders, wetting agents and other additives necessary to improve the printing resolution and film quality. The amount of solids contained in the ink is restricted by the viscosity limit in inkjet printing, in which the inks are less viscous than in other technologies such as screen and offset printing (Magdassi, 2010; Castrejon-Pita et al., 2013).

Because it is a noncontact technology, no masks or lithography steps are involved and the pattern required can be easily changed through the printer software, which allows rapid changes to the product to be made (Teichler et al., 2013). Another advantage is the minimal waste generated during the process, which becomes crucial when the materials utilized are expensive or can only be synthesized in small quantities (Pataky and Brugger, 2014).

There are many examples in the literature of the application of inkjet printing for biosensor fabrication since the first patent in 1988 (Kuriyama, 1988; Delaney et al., 2009; Pavinatto et al., 2015). Inkjet printing stands as one of the most promising techniques for the rapid prototyping of biosensors because it allows the deposition of not only biological inks but also the rest of the device components (Pataky and Brugger, 2014). An 'all-inkjet-printed' paper-based device for H_2O_2 determination has been recently reported. A nonvolatile hydrophobic ink was first deposited on the paper and subsequently UV cured to create microfluidic channels in the order of 272 μm. The back cover and the rest of the reagents necessary for the colorimetric detection of H_2O_2 were then patterned, again by inkjet printing. The environmentally friendly device showed high selectivity, low level of interferences and a calculated limit of detection of 14.4 μM (Maejima et al., 2013).

The numerous advantages of inkjet printing as a material deposition technique have led to its increased utilization for the fabrication of biosensors since its first application more than 30 years ago. To become a routine technology for industrial biosensor manufacture, however, challenges such as printing speed, implementation of mechanical processes to optimize ink rheology and integration of the printing step with other processes to obtain fully automated analytical systems need still to be overcome (Li et al., 2015).

4.2.2.2 Wax-ink printing

Wax printing is a simple method mainly focused on the fabrication of microfluidic paper-based analytical devices (μPADs), which have generated increased interest in academic research in the last decade (Gubala et al., 2012). μPADs are gaining popularity in POC diagnostics because they are ready-to-use devices that enable high-throughput multiplex detection by means of a simple, rapid and inexpensive procedure (Chan et al., 2013).

Paper is, of course, a low-cost, comparatively environmentally friendly and renewable substrate. It is suitable as a substrate for the fabrication of multiplexed assays with small-volume samples, with a significant potential for use as diagnostics in developing countries (Lu et al., 2009; Costa et al., 2014). Patterning paper was first achieved by Whitesides and co-workers, who initially used PDMS or photoresist to generate hydrophobic boundaries for precise control of the liquid flow

within the device (Martinez et al., 2007; Bruzewicz et al., 2008). More recently, wax printing has emerged as an easy and affordable alternative to the fabrication of μPADs, in which only a commercially available wax printer and a hot plate are required. The printing process begins with the deposition of wax patterns on the surface of paper by the wax printer and the subsequent melting stage to allow the wax into the paper, creating the hydrophobic barriers. Due to the anisotropy of the paper – fibres tend to be more horizontal than vertical – the spread of melted wax through the paper is not uniform, leading to wider barriers than the original pattern which reduces the resolution of the technique (minimum average barrier width of 850 ± 50 μm). Compared to most patterning techniques, features made by wax printing are not of high resolution. However, the technique is rapid, efficient and economical. 100–200 μPADs can be patterned in less than 5 min and can cost as little as US$0.01 per device or less depending on the type of paper selected. Moreover, such devices have demonstrated acceptable analytical performance when compared to other validated techniques such as spectrophotometry (Liana et al., 2012; Bhakta et al., 2014).

Paper-based multizone plates (96- and 384-zone plates), lateral-flow devices and 3D μPADs are some of the devices which have already been manufactured by wax printing (Carrilho et al., 2009). Three-dimensional paper-based microfluidic systems can also be produced by stacking 2D patterned sheets, condensing a greater number of assays into a smaller print area (Martinez et al., 2008). Additional plastic thin layer and retention materials (eg, chitosan) can be incorporated into the device to protect the reagents, avoid fluid evaporation and guarantee the biomaterial preservation within the diagnostic design (Schilling et al., 2012; Wang et al., 2012). Biomedical diagnostics and environmental analysis are some of the potential applications of paper-based devices fabricated by wax printing and many examples can already be found in the literature (Fig. 4.5) (Mentele et al., 2012; Bhakta et al., 2014; Lan et al., 2014; Liang et al., 2015; Noiphung et al., 2015).

A less costly but more manually demanding technique for μPAD fabrication compared to wax printing is wax screen printing. In this method, solid wax is first rubbed through a screen onto paper substrates and subsequently melted onto the paper using a hot plate to generate hydrophobic barriers. Wax screen printing is simple, rapid and inexpensive and does not require the use of a clean room, UV lamps or other sophisticated instrumentation, resulting in a suitable technique for developing countries (Dungchai et al., 2011). Although its application for the simultaneous determination of glucose and iron in serum samples has already been demonstrated, the manual deposition of solid wax in this case may impact the method reproducibility and its application for mass production of commercial biosensor devices.

Wax printing shows the potential to be adapted to a roll-to-roll process, in which the paper substrate would first go through a wax printer, then through a heat source and, finally, through an inkjet printer in which the biomaterials or other reagents for assays could be deposited in the test areas (Carrilho et al., 2009). It is particularly capable for manufacturing large batches (hundreds to thousands) of μPADs that can be used as platforms for diagnostic devices or other analytical applications.

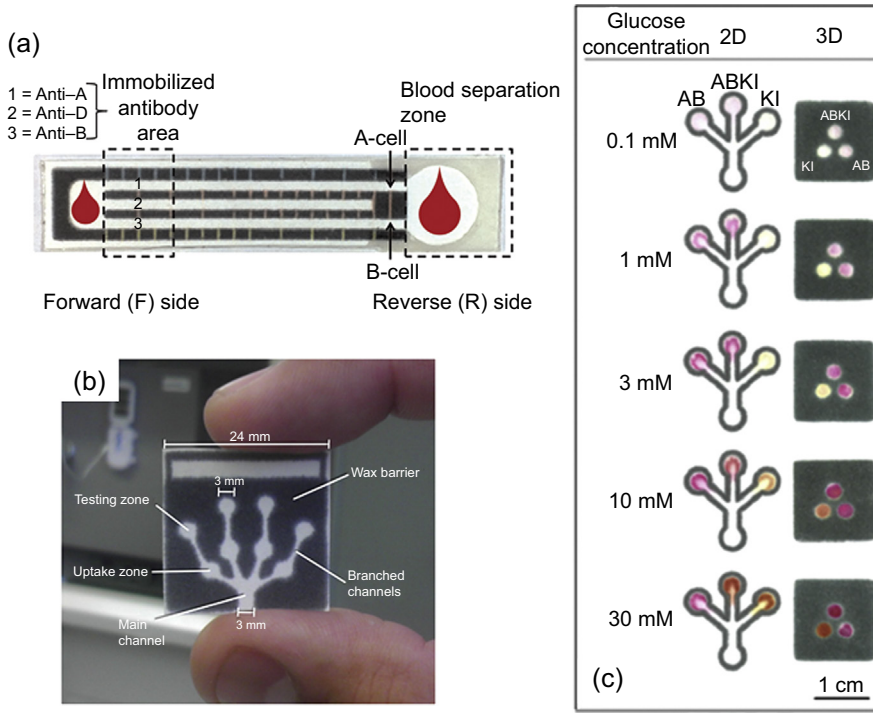

Figure 4.5 Paper-based analytical devices fabricated by wax printing: (a) simultaneous determination of blood typing (ABO) and Rh groups *(Reprinted from Noiphung, J., Talalak, K., Hongwarittorrn, I., Pupinyo, N., Thirabowonkitphithan, P., Laiwattanapaisal, W., 2015. A novel paper-based assay for the simultaneous determination of Rh typing and forward and reverse ABO blood groups. Biosensors and Bioelectronics 67 (0), 485–489 with permission from Elsevier)*; (b) nitrite detection in saliva for periodontitis monitoring *(Reprinted from Bhakta, S.A., Borba, R., Taba Jr., M., Garcia, C.D., Carrilho, E., 2014. Determination of nitrite in saliva using microfluidic paper-based analytical devices. Analytica Chimica Acta 809 (0), 117–122, with permission from Elsevier)*; (c) 2D and 3D lateral-flow glucose sensors and the colorimetric results obtained when tested with 0.1–30 mM glucose solutions *(© Institute of Physics (IOP) Publishing. Reproduced with permission. All rights reserved Costa, M.N., Veigas, B., Jacob, J.M., Santos, D.S., Gomes, J., Baptista, P.V., Martins, R., Incio, J., Fortunato, E., 2014. A low cost, safe, disposable, rapid and self-sustainable paper-based platform for diagnostic testing: lab-on-paper. Nanotechnology 25 (9), 094006).*

4.2.2.3 Three-dimensional printing

Although first reported in the late 1980s, it has been during the last decade that 3D printing has experienced exponential growth in its application, mainly due to advances in computer engineering, reduction of instrumentation and process costs and expansion of its application range (Vaccarezza and Papa, 2015). Three-dimensional printing is a rapid prototyping technique that enables the layer-by-layer fabrication of objects using 3D images of a physical model stored in a

computer-aided design (CAD) drawing file (Pataky and Brugger, 2014). During the additive manufacturing process, successive layers of materials are first deposited and subsequently cured following various approaches depending on the type of printing method employed. In stereolithography (SLA), a photosensitive resin (epoxy or acrylic based) is cured immediately after it is deposited by direct/laser or mask-based writing, whereas in inkjet printing, a printed liquid binder attaches layers of powdered solid particles (50–100 μm) evenly allocated on the support stage to produce the 3D prototypes. Similarly, Selective Laser Sintering (SLS) also employs powder-based materials to generate a 3D model, in which the polymer powders are sintered by a high-power laser instead of liquid binders. This variation allows the use of a wide range of materials such as polycarbonate, polyvinyl chloride, nylon, polyesters and metal and ceramic powders. Fused Deposition Modelling (FDM) is one of the most extensively used layer-by-layer techniques for 3D manufacturing, in which thermoplastic materials are extruded through a nozzle tip, heated to a semimolten state and then deposited onto the substrate, leaving behind a 3D object when the material has solidified. Laminated Object Manufacturing (LOM) also builds 3D structures. However, the fabrication process may be more correctly associated with Polymer Laminate technology (Section 4.4.3). The design is edited by a laser or razor on layers of sheet materials (paper, plastic or metal), which are subsequently piled up together by adhesives or welding to generate the 3D stack (Bogue, 2013; Gross et al., 2014).

Existing applications of 3D printing include biomedicine (artificial tissue scaffolds and biomedical models), industry (creation and analysis of prototypes of intended final products), architecture and education (Dimitrov et al., 2006; Xing and Xiaojiang, 2010; Pignataro, 2014; Zhao et al., 2014; Kamei et al., 2015; Zhu et al., 2015). In the biosensing area, this technique allows the fabrication of 3D devices from biocompatible materials and their potential incorporation into POC systems and implantable therapeutics (Dias et al., 2014; Thomas et al., 2014). In this regard, the one-step fabrication of transparent 3D microfluidic devices for on-chip optical detection has been recently reported. A commercial 3D printer showed the potential of print time and device cost as low as 12 min and US$1, respectively. Feature sizes as low as 250 μm for microfluidic channels were achieved and the application of a 3D microfluidic structure for the continuous monitoring of nitrate in tap water was also demonstrated (Shallan et al., 2014). Moreover, 3D printing can be employed for the fabrication of templates for soft lithography with no mask requirements and with which positive structure sizes of 200 μm have already been achieved. This has the potential to decrease both the time and the cost of soft lithography manufacturing process.

One of the disadvantages of 3D printing is the high cost of the instruments required for high-resolution fabrication. Most of the printers that allow for print resolution below 100 μm cost around US$20,000, whereas printers of US$250,000 are required to achieve resolutions as low as 16 μm (Gross et al., 2014; Shallan et al., 2014). Therefore, 3D printing has shown the potential to generate POC platforms, although its future growth as a tool in the diagnostics industry will be determined by the factors of resolution, processing time, cost and final print size.

4.3 Thin-film deposition

Traditionally, thin-film deposition techniques have been related to the semiconductor industry and not to the biomedical sector. This is mainly due to the extreme conditions generally required for the material deposition process and their lack of compatibility with biological systems. However, the interdisciplinary characteristics of biosensors and POC devices has opened an entirely new range of applications for these techniques, mainly orientated towards the fabrication of platforms that can be employed as both the transducer for the sensing process and the support for the immobilization of the recognition molecule (Lin and Yan, 2012; Ceylan Koydemir et al., 2013).

One of the most widely used techniques for the fabrication of thin films is chemical vapour deposition (CVD). Briefly, the substrates to be coated are heated in a chamber and exposed to a precursor gas or gases, which undergo chemical reactions on the hot surfaces, resulting in the deposition of a thin film on the substrates. This process has been mainly used in semiconductor manufacturing (Warwick and Binions, 2014; Zheng et al., 2014), and it is particularly useful for insoluble and infusible materials (Alf et al., 2010). Although the generally abrasive environment in the reaction chamber makes this technique unsuitable for biological materials, it can be of significant value for the preparation of customized surfaces to regulate protein surface adsorption for applications such as biosensors and other biomedical devices. There are three main methods for CVD: CVD of poly(*p*-xylylenes) (or parylene), plasma-enhanced CVD (PECVD), and initiated CVD (iCVD)/photo-initiated CVD (piCVD). Unlike CVD of poly(*p*-xylylenes), the feasibility for scale-up of PECVD and iCVD/piCVD has already been demonstrated as the monomers utilized in both processes are commercially available and the latter has shown the ability to operate in semicontinuous roll-to-roll mode (Yang et al., 2012).

Unlike CVD, the technique of thermal evaporation involves the direct evaporation of the materials to be deposited without further chemical reaction taking place on the substrate. This technique has already been applied to the fabrication of sensing platforms for glucose (Umar et al., 2007), urea (Slaugther, 2012), H_2O_2 (Li et al., 2010) and phenol (Zhao and Jiang, 2010).

Sputtering is a physical vapour-deposition method in which a high-energy argon ion plasma stream bombards the target material in a vacuum environment, resulting in some particles being ejected and sputtered onto the substrate, creating a thin film. The temperatures required are lower than for evaporation methods, making this technique particularly useful for depositing materials with a high melting point or a mixture of materials. This is a rapid and scalable process that is widely used in the semiconductor industry, screen displays and photovoltaics, and can be adapted for the fabrication of POC platforms (Singh et al., 2007; Nazarpour, 2013).

Spray deposition techniques enable the formation of ceramic or metallic films onto a substrate by spraying the materials in a powder configuration. Among them, plasma-assisted spraying has received special attention in healthcare as it enables the deposition of bioceramics and surface modification of metallic implants (Nazarpour, 2013).

A simpler approach to thin-film formation is spin coating. During this process, the solution containing the desired material is first deposited onto the substrate, which is

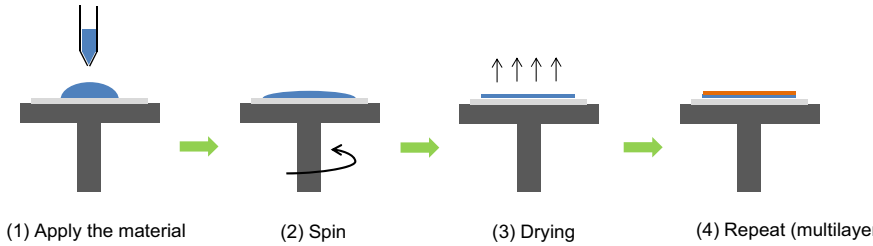

Figure 4.6 Scheme of the spin-coating process.

subsequently accelerated, spreading the solution on the substrate surface and creating a homogeneous film, as shown in Fig. 4.6. The material concentration, the type of solvent and the spin-coating speed define the film thickness (Seker and Elcin, 2015). This technique is appropriate for coatings using polymers and other materials that are readily solubilized, whereas processes with more intense conditions are required if metallic films are to be formed. The mildness of this method in comparison to other thin-film deposition techniques has enabled the deposition of biological molecules, mainly enzymes in hydrogels, as well as other materials for the fabrication of biosensors (De Wael et al., 2012; Mateescu et al., 2012; Burrs et al., 2015).

There are numerous examples in the literature regarding the application of thin-film techniques for the fabrication of biosensors that can be incorporated into POC devices (Jenison et al., 2001; Weng et al., 2004; Mandal et al., 2012). Due to the extreme conditions required, these processes are primarily orientated to the manufacture of the biosensor platforms, whereas the biological samples are mainly deposited using other techniques.

4.4 Other POC prototyping techniques

Many other techniques have been applied to the fabrication of biosensors in the literature, mainly at the laboratory scale. In this section, we focus mainly on methods that are now being, or in the near future will be, applied to the fabrication and mass production of biosensing devices.

4.4.1 Injection moulding

One of the leading technologies in the cost-effective mass production of microfluidic systems for POC devices is injection moulding (IM), mainly due to its speed, low cost and range of process-compatible materials. Briefly, a thermoplastic material is heated to become molten before being forced under pressure inside a mould cavity. Once the temperature is decreased below the glass transition temperature of the polymer, the material solidifies, adopting the cast shape and is then ejected from the cavity (Fiorini and Chiu, 2005). This process generally lasts between a few seconds and few minutes, which reinforces the capabilities of the technique for mass production. Polymers such as polymethyl methacrylate (PMMA), polycarbonate (PC), polysulfone (PSU) and

cyclic olefin copolymer (COC) are commonly used for IM fabrication of microfluidic devices (Becker and Gärtner, 2008). They have demonstrated improved characteristics over other materials such as glass and silicon with regards to manufacturing cost of disposable devices and fabrication processing (Attia et al., 2009). Typical features sizes are in the order of millimetres or greater. However, recent advances have permitted the well-controlled reproduction of nanostructures with features sizes as small as 50 nm (Mazzeo et al., 2007; Hainberger et al., 2010). Although a single mould can make many thousands of parts, mould manufacture can be costly (≥US$10,000), making this technique more extensively used for microfluidics devices in industrial applications than in research laboratories.

There are already a few examples of the application of IM for the fabrication of POC devices (Ritzi-Lehnert et al., 2011; Iwai et al., 2014; Roy et al., 2015). For instance, an integrated microfluidic chip that can replicate all the steps of an ELISA for Human Immunodeficiency Virus (HIV) diagnosis was recently reported. The device, made of transparent polystyrene (PS) and COC, enabled the simultaneous diagnosis of HIV and syphilis using only 1 μL of blood sample. The applicability of the 20-min assay test was evaluated in remote regions in Rwanda, showing sensitivity and specificity analogous to lab-based analysis (Chin et al., 2011). IM was also employed for the fabrication of microstructured platforms integrated in POC devices for the monitoring of blood coagulation (Fig. 4.7). The surfaces were additionally modified with labelled fibrinogen and coagulation activation reagents for the fluorescent determination of microclots in human plasma samples during lateral flow on the chips (Dudek et al., 2011). Other biomedical applications of IM include the fabrication of implants from advanced powdered materials such as titanium and magnesium (Sidambe, 2014; Wolff et al., 2014) and the production of artificial scaffolds for tissue engineering purposes (Mi et al., 2013).

4.4.2 Hot embossing

Hot embossing is a mass production technique in which thermoplastic materials, typically in the form of flat sheets, are patterned against a master (stamp) using heat (Dirckx et al., 2006). Like IM, hot embossing utilizes the ability of thermoplastic materials such as PMMA, PC, COC or polystyrene (PS) to be moulded when heated near their glass transition temperature (T_g). The stamps, generally made of silicon or metal, and the materials are placed into a hydraulic press, and then heat is applied to emboss the plastic against the master. The embossing technique is relatively simple. However, the fabrication of the stamp by means of micromachining and electroplating is a laborious process, and is only worthwhile for the application of the technique to mass production rather than for testing prototypes (Sharma et al., 2011). A variation of hot embossing involves high pressure instead of heat, allowing the process to be performed at room temperature (imprinting) (Fiorini and Chiu, 2005).

One of the characteristics that make hot embossing amenable to industrial applications is its feasibility for roll-to-roll processing. Unlike IM, the tool cost for hot embossing is comparatively low, whereas the substrate is typically more expensive, with prices in the order of US$0.002 to US$0.02 per cm^2 (Mazzeo et al., 2007). Despite the initial investment of time and capital required for this technique due to

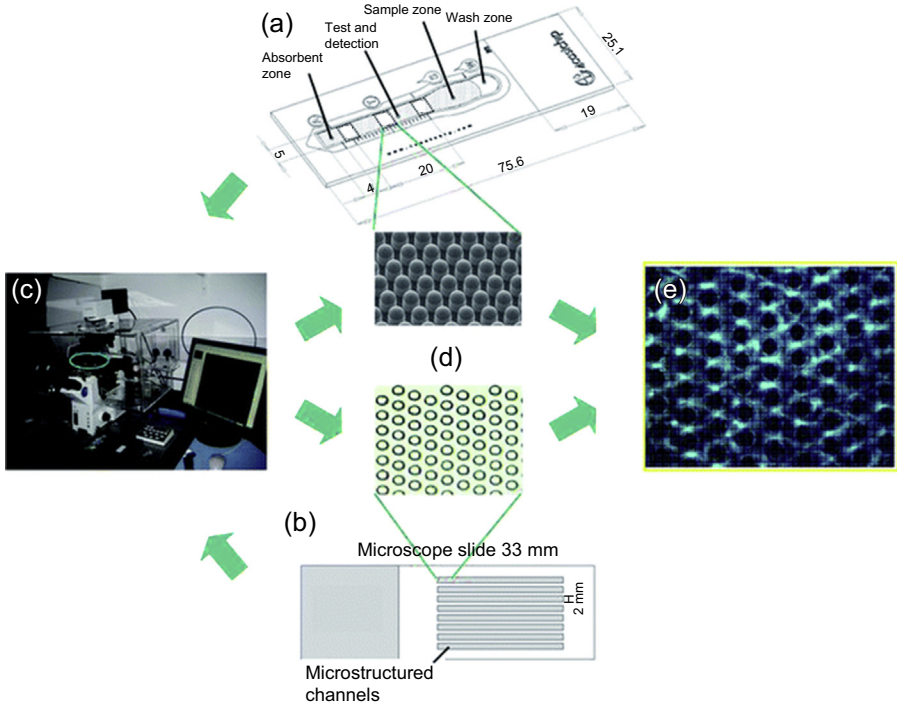

Figure 4.7 Scheme of the POC devices for the monitoring of blood coagulation: (a) polymeric microstructured platform composed of micropillars fabricated by IM; (b) glass substrate with channels containing several size micropillars; (c) fluorescence microscope with CCD camera; (d) image of the microstructured area under study; (e) video image file of the fluorescent output, processed by a LabView interface.
Reproduced from Dudek, M.M., Kent, N.J., Gu, P., Fan, Z.H., Killard, A.J., 2011. Development of a fluorescent method for detecting the onset of coagulation in human plasma on microstructured lateral flow platforms. Analyst 136 (9), 1816–1825, with permission of The Royal Society of Chemistry.

the high start-up costs and long development times of the machines and the stamps, the low cost of thermoplastic materials and the possibility of scaling up immediately decrease the unit cost when going to mass production (Chou et al., 2013). Hot embossing is consequently an excellent method to fabricate small-scale biochip sensors at relatively low cost that can be further integrated in POC devices (Tondra et al., 2009; Mathur et al., 2010; Obuliraj et al., 2014).

4.4.3 Polymer laminate technology/lamination

Polymer laminate technology is a rapid prototyping technique using standard film lamination for the fabrication of microfluidic devices. Polymeric materials (eg, PMMA, PET, PS, polypropylene and copolymers such as Zeonor) in thin sheets or

foils from 25 to 500 μm are first patterned using cutting instruments such as CO_2 laser, metal-blade die cutter or computer-driven vinyl cutter, and subsequently bonded (laminated) together with pressure or thermally sensitive adhesives to form stacked fluidic units (Sun et al., 2009; Gubala et al., 2012).

One of the main advantages of this technique is that all fabrication processes can be performed at room temperature (except from thermal bonding), reducing the risk of damaging reagents previously deposited on the materials. Other characteristics are reduced cost, robust adhesion, the possibility of customizing diagnostic platforms and ease of scale-up (Levine, 2009). Feature sizes in the devices are generally between 100 and 1000 μm although most microfluidic channels are larger than 400 μm due to the limited resolution of most cutting tools (Gubala et al., 2012).

Polymer laminate technology can be employed in the POC industry for the fabrication of device platforms, as has been already demonstrated (Sun et al., 2010; Chin et al., 2012). For example, a biosensor array for the simultaneous monitoring of lactate, glucose, glutamine and glutamate was produced based on a polymer laminate technology microsystem. The 0.15-μL flow-through cell showed independent analyte determination in the clinically relevant range of concentrations with a minimum stability of one week for continuous operation (Moser and Jobst, 2013). In addition, a POC device for self-monitoring the effect of anticoagulant therapy can be found in the literature. The low-cost, disposable device is based on a laminated polymer microfluidic strip fabricated from a combination of hydrophobic and hydrophilic cyclic polyolefins. The miniaturized device also contains the reagents for the fluorescence-based anti-Factor Xa (FXa) assay and was capable of determining heparin, tinzaparin and enoxaparin effect in samples with CVs lower than 10% (Harris et al., 2013). More recently, a multiparametric platform for in vivo determination of glucose, lactate and glutamate together with oxygen has been reported. The biosensor array system was fabricated by a combined process involving thin-film and laminate technology on a flexible polymer, leading to low-cost multisensor strips (Weltin et al., 2014).

4.5 Conclusions

There are a significant number of scalable fabrication technologies that have been applied to the development and mass manufacture of POC biosensor devices. These have come from a wide variety of sources, notably initially through the graphic arts, but also through the microelectronics industry and broader mass manufacturing systems, as well as through many imaginative and innovative academic concepts. This range reflects the challenge of the fabrication of such a diverse array of materials into an equally diverse array of assay and device formats. Certainly, no one technique is ever likely to address all device fabrication requirements. However, with further ingenuity, these systems can and are being integrated into seamless production systems which result in high throughputs and low unit costs, which is to the eventual benefit of the end user, as it brings the ability to detect and monitor disease closer to the patient, in a manner that is increasingly less dependent on their geographical or socioeconomic situation.

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Lab-on-chip (LOC) devices for point of care (POC) applications

5

S. Mukherji, D. Mondal
Indian Institute of Technology Bombay, Mumbai, India

5.1 Introduction

Lab-on-chip devices are aimed at miniaturizing the conventional laboratory instruments for detection of analytes. Although laboratory techniques are very accurate and considered gold standards for measurement, the time required for analysis is quite high and trained personnel are required to operate the instruments. Further, the requirements in terms of volume for the sample as well as reagents may be high. Hence, microfluidic lab-on-chip devices have gained importance in recent years owing to their cost-effectiveness, portability, low sample volume requirement, easy handling, quick response time, etc.

Although microfluidic devices are the miniaturized versions of bench-top instruments, the analogy in principles or physics involved is not always very simple and cannot be linearly scaled down; eg, increased surface area-to-volume ratio and the omnipresence of laminar flow in microfluidic devices [1]. The main issues in any lab-on-chip device are sensitivity and scalability because reduced sample volume results in a reduced number of analyte molecules which makes detection all the more difficult.

Besides the generalized problem of scalability, lab-on-chip (LOC) devices may face other problems particular to their type. For example, optical LOC devices suffer from reduced optical path length, interference from background noise signals, etc. To overcome these difficulties, researchers have developed innovative techniques by using photomultiplier tubes (PMTs), air reflection mirrors, collimation lenses, etc. Electrochemical methods suffer from fouling and degradation of electrodes due to absorption of analytes on the inside walls of the microfluidic chip and metal electrodes. Reusability of these devices is a major concern; however, the time-consuming cleaning process can be avoided by developing disposable LOC devices.

This chapter discusses different categories of LOC devices based on the transduction technique involved ie, optical, electrochemical, thermal, etc. The approach of this chapter is to discuss the types of on-chip devices from the point of view of the working principle, followed by some highlights of recent advances in the development of such devices.

5.2 Optical detection

Optical detection is the most commonly used transduction technique for application in laboratories due to its high sensitivity and immunity from coupled electromagnetic

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interferences. However, the main concerns with the implementation of optical detection in LOC devices are miniaturization, cost-effectiveness, degradation in performance at smaller dimensions, etc. Over the years, researchers have developed different methods techniques to resolve these shortcomings [1–3].

5.2.1 Fluorescence

Fluorescence intensity measurement is one of the most common techniques used for LOC systems owing to its high sensitivity, selectivity, readily available fluorophores, and labeling chemistry. Fluorescence is mainly induced either by laser or by LEDs. Although LEDs are cheaper than lasers, they have high divergence and relatively broad emission spectra. Lasers, on the other hand, have low divergence, can be focused at a point easily, and integrated into a portable system. Uses of microlenses and waveguides in microfluidic systems have improved the detection limit in several applications.

Waveguides used for optical applications are basically a dielectric structure with high refractive index and transmission surrounded by another medium with low refractive index. The light is guided through the waveguide by total internal reflection. The most commonly used materials for waveguides is polydimethylsiloxane (PDMS), polymethylmethacrylate (PMMA), SU8, silicon nitride, etc. These materials are low-cost, optically transparent, nontoxic, easily fabricated using molding, spin coating, and soft lithography techniques, have excellent adhesion and chemical resistance, etc. In addition, coupling of light into these waveguides is easier.

The use of a two-dimensional (2D) PDMS lens in a microfluidic device with channels for both liquid flow and insertion of optical fibers for fluorescence excitation (Fig. 5.1) was reported by Camou et al. [4]. The light was guided using optical fibers into an insertion channel. The insertion channel ended in a PDMS microlens which focused light to a point, depending on the radius of curvature of the lens, inside the fluidic flow channel. The light output side was essentially a mirror image of this process. The two

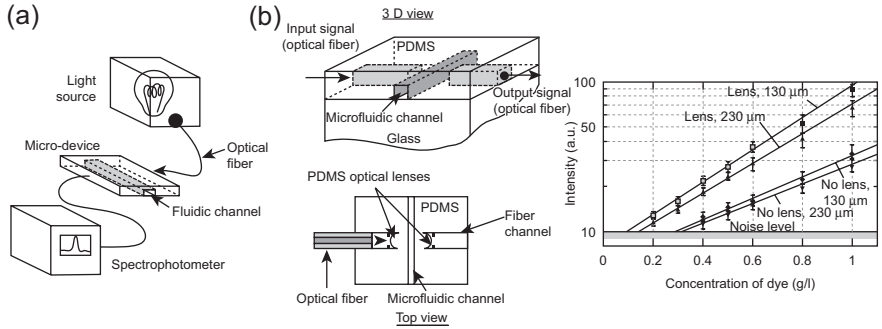

Figure 5.1 (a) Schematic of the experimental setup, (b) cross-sectional view of the device, and (c) fluorescence intensity response of the device for FITC-tagged albumin.
Reproduced from S. Camou, H. Fujita, T. Fujii, PDMS 2D optical lens integrated with microfluidic channels: principle and characterization, Lab on a Chip 3 (2003) 40–45, with permission of The Royal Society of Chemistry.

channels being very close to each other resulted in good efficiency of the fluorescent excitation of dyes. The fluorescence intensity for detection of fluorescein isothiocyanate (FITC)-tagged albumin molecules almost doubled in the case of PDMS lenses as compared to a flat air–PDMS interface.

Light passing through a fluid medium is frequently scattered and absorbed by species other than the analyte of interest. To overcome this, evanescent field-based sensing systems may be utilized, as was done by Hofmann et al. [5]. Rabbit immunoglobulin (IgG) was immobilized on a silicon nitride waveguide and the analyte molecule was Cy5 labeled antirabbit IgG. The interaction took place inside a three-dimensional (3D) PDMS flow cell. Laser light was coupled into the waveguide through a corrugation grating. Fluorescence from the bound analyte was guided through the waveguide and finally coupled back out of the grating to be detected by a photodetector.

Different configurations in terms of placement of the light source and detector have been implemented in fluorescence-based microchips. The examples discussed earlier have the light source and detector placed outside the microchip. This leads to problems in alignment of the source/detector with the waveguides. For better light coupling in miniaturized systems, efforts have been made to incorporate both the source and detector on the chip itself. Light-emitting diodes (LEDs) in general are 3D structures which make integration in a microdevice difficult. In contrast to the normal inorganic LEDs, organic light-emitting diodes (OLEDs) have flat surfaces, thus making integration easier with microfluidic devices. OLEDs also have flexibility in terms of fabrication and can be made in any desired size and shape by photolithographic techniques [6]. Organic photodiodes can also be integrated on silicon substrates for measuring the fluorescence emitted from dye molecules. On-chip thin-film interference filters help in preventing the interference of the excitation light with fluorescence detection [7].

Integration of thin-film organic LED and organic photodiode (OPD) in microfluidic chips have been successfully implemented in [8] by Pais et al. (Fig. 5.2). Limit of detection (LOD) of Rhodamine 6G was 100 nM, whereas that for fluorescein dye was 10 μM.

Other applications of OLEDs integrated with microfluidic devices have been reported for detection of proteins [6], human serum albumin (HSA) [9] with a detection limit of 10 mg/mL. A capillary electrophoresis system integrated with on-chip fluorescence detector on a silicon substrate was proposed by Webster et al. [7] for separation of DNA-restriction fragments using SYBR Green I intercalating dye with detection limit in the order of femtograms.

Apart from OLEDs, use of inorganic thin-film LEDs has also been integrated into microfluidic chips. A portable microassembly of a fluorescence detection system was illustrated by Chediak et al. [10] wherein a CdS thin-film filter and an (In, Ga)N thin-film blue LED was integrated with a disposable PDMS microfluidic device with Si PIN photodetector substrate.

Fluorescent intensity detection, although the most widely used technique, suffers from certain disadvantages due to the use of fluorescent dyes. The dyes are costly, have low shelf life, and are easily affected by variation in chemical properties ie, pH, temperature, etc. These and other factors like quenching due to proximity of surfaces in microfluidic channels, photobleaching, etc., are challenges that need to be overcome for successful implementation of these devices.

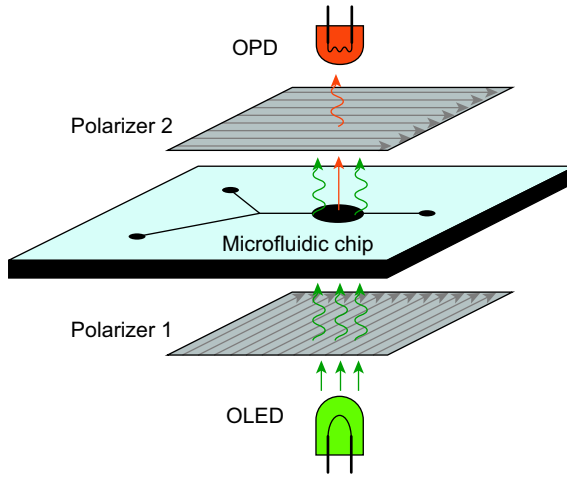

Figure 5.2 Schematic of organic excitation and detection system with microfluidic device for fluorescence detection. Green organic light-emitting diode (OLED) acts as the light source, the polydimethylsiloxane (PDMS) microfluidic chip contains the dye detection volume, and polarizer 2 is used to filter out the excitation light and allows only the emission signal to pass through to the organic photodiode (OPD).
Reproduced from A. Pais, A. Banerjee, D. Klotzkin, I. Papautsky, High-sensitivity, disposable lab-on-a-chip with thin-film organic electronics for fluorescence detection, Lab on a Chip 8 (2008) 794–800, with permission of The Royal Society of Chemistry.

5.2.2 Absorbance

Absorbance-based spectrometric detection is most commonly used in macroscale devices. In absorbance spectroscopy, the attenuation of incident light intensity upon interaction with an analyte is measured to quantify the concentration and composition of a sample. Absorption of light passing through a medium is governed by the Beer–Lambert law (Fig. 5.3) which states that

$$T = \frac{I}{I_0} = e^{-\varepsilon lc}$$

$$A = -\ln\left(\frac{I}{I_0}\right) = \varepsilon lc$$

in which T and A are the transmittance and absorbance of light, I_0 and I are intensities of incident and transmitted light, ε is the absorption coefficient, l is the optical path length, and c is the concentration of the sample.

The sensitivity of absorbance-based transduction is directly proportional to the path length. In flow cell-based bench top instruments, typical path length is about 10 mm. However, in microfluidic devices, in which the optical path length is much smaller, the sensitivity of absorbance-based techniques may largely be affected.

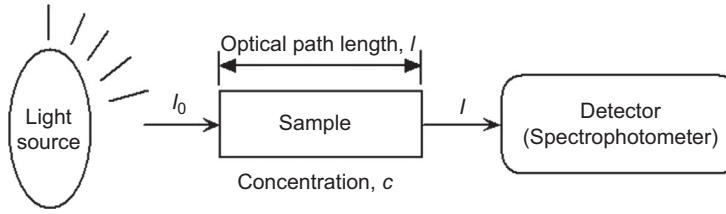

Figure 5.3 Illustration of absorbance spectroscopy and its relation with Beer–Lambert law.

Increasing the optical path length in microscale devices can be achieved by the use of air mirrors [11]. Air mirrors utilize incorporation of air cavities of specific shapes in PDMS substrate near the flow channel such that light coupled with PDMS is reflected at the air–PDMS interface (total internal reflection) and sent back into the fluid (Fig. 5.4). Placing the air mirrors at optimum positions adjacent to the flow cell provides significant increase in optical path length keeping the flow cell volume constant. Llobera et al. [11] used ring multiple internal reflection (RMIR) structure and propagating multiple internal reflection (PMIR) mode to detect fluorescein with LOD of 41 nM and 110 mM, respectively.

As discussed in the previous section, PDMS, SU8, and polymers are routinely used as waveguide materials. Different shapes of polymer waveguides coupled with evanescent wave-based absorbance, localized surface plasmon resonance phenomenon-based absorbance, etc., can be used for enhanced sensitivity of detection. As mentioned earlier, to overcome the issues of nonspecific absorption and scattering, evanescent wave-based absorbance techniques are used. The analyte interacts with the evanescent wave field of the light passing through a waveguide and results in change in the light intensity at the output end of the waveguide. This absorbance of light depends on the refractive index change near the waveguide or optical fiber [12] as well as possible quenching elements (ie, analyte molecules of interest in very close proximity (<1 μm) to the surface of the waveguide).

Prabhakar et al. developed a U-bent SU8 optical waveguide which formed part of the wall of a microchannel for capillary electrophoresis-based separation followed by evanescent wave absorbance-based detection of Methylene Blue dye up to 20 μM [12]. The input and output light were coupled in and out from the waveguide through optical fiber via a fiber-to-waveguide coupler (Fig. 5.5). The same system can be used, without electrophoresis, for sensitive detection of analyte molecules, provided the corresponding capture molecules are immobilized on the waveguide surface. Sensitivity of detection can be improved by combining the effect of evanescent wave-based absorbance with localized surface plasmon resonance (LSPR) properties of Au nanoparticles. This technique was implemented with a C-shaped polymer waveguide on a SU8 substrate coated with Au nanoparticles for sensing refractive index changes near the C-bent [13].

Similar to fluorescence-based systems, absorption-based LOC optical devices can be created by integration of waveguides, detectors, and light sources on a microfluidic chip, collectively known as integrated optofluidics. Balsev et al. developed a microfluidic device integrating liquid dye laser, polymer waveguides, fluid mixing channels,

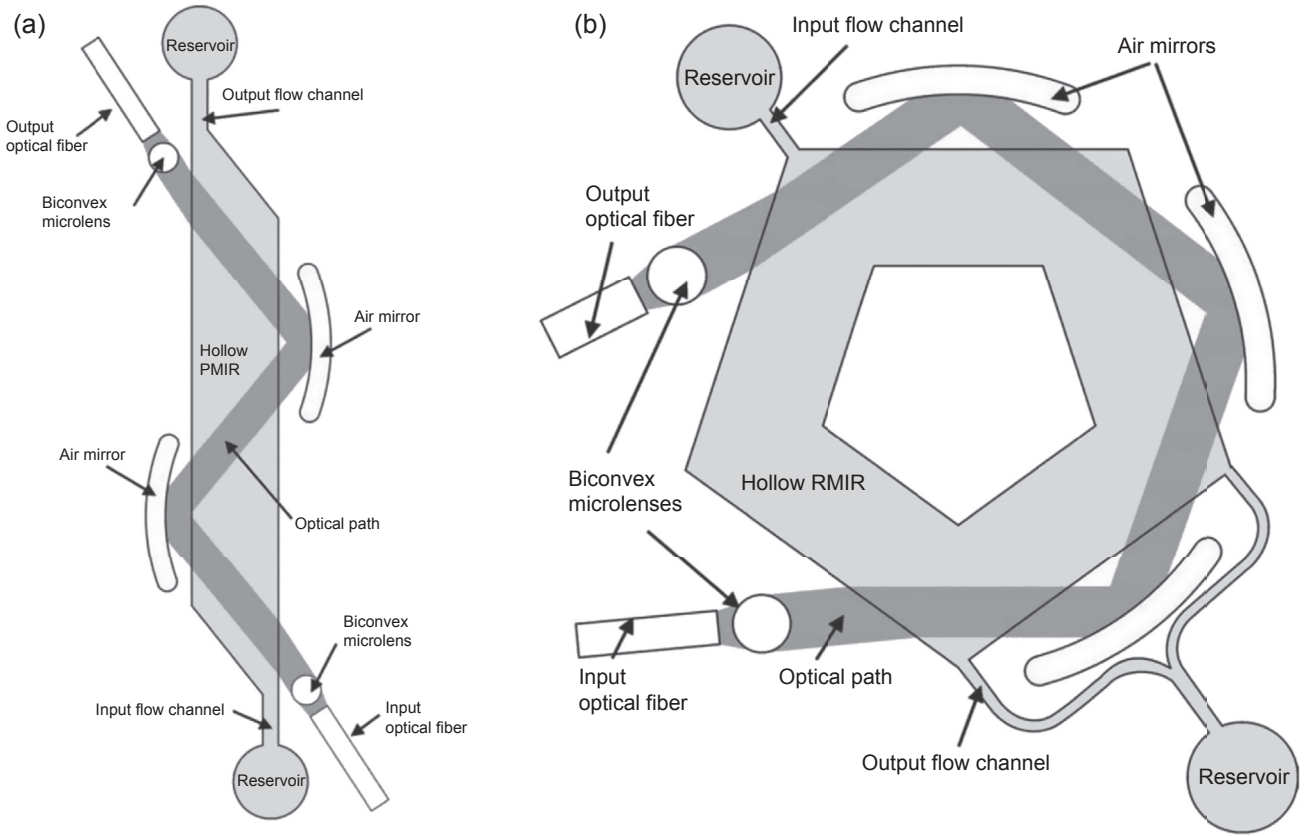

Figure 5.4 Air mirrors adjacent to the flow channel utilize the reflection of light at the air–PDMS interface to increase optical path length without affecting the flow volume. (a) Propagating multiple internal reflection (PMIR) setup and (b) ring multiple internal reflection (RMIR) configuration. Reproduced from A. Llobera, S. Demming, R. Wilkea, S. Büttgenbach, Multiple internal reflection poly(dimethylsiloxane) systems for optical sensing, Lab on a Chip 7 (2007) 1560–1566, with permission of The Royal Society of Chemistry.

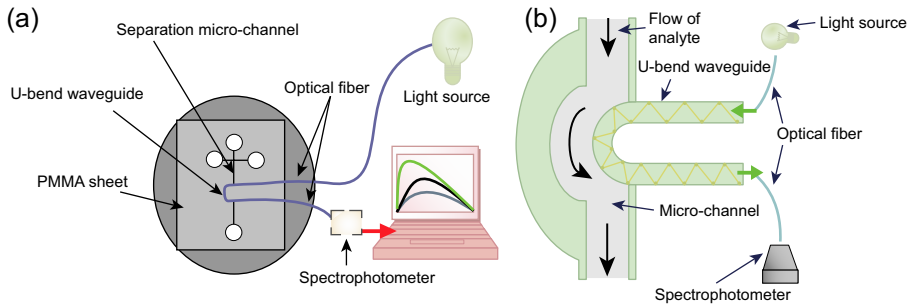

Figure 5.5 (a) Schematic of the experimental setup of evanescent wave absorbance-based detection using polymer waveguides. (b) Closer look into the U-bent polymer waveguide coupled with the light source and detector.
Reproduced from A. Prabhakar, S. Mukherji, Microfabricated polymer chip with integrated U-bend waveguides for evanescent field absorption-based detection, Lab on a Chip 10 (2010) 748–754, with permission of The Royal Society of Chemistry.

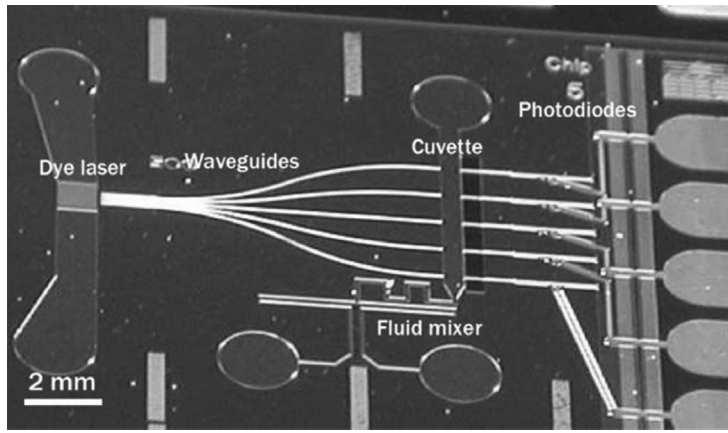

Figure 5.6 Photograph of lab-on-chip (LOC) optical transducer with integrated dye laser, polymer waveguides, cuvette, microfluidic channel, and photodiodes.
Reproduced from S. Balslev, et al., Lab-on-a-chip with integrated optical transducers, Lab on a Chip 6 (2006) 213–217, with permission of The Royal Society of Chemistry.

and measurement cuvette in a SU8 polymer, and embedding photodiodes in silicon substrate (Fig. 5.6) [14]. The light emitted from the dye laser was coupled to five waveguides, each directed toward a different measurement location on a cuvette. The transmitted light from the cuvette was received by five more waveguides placed on the other side and directed toward an array of photodiodes.

Miniaturized optical detection system based on integrated waveguide technology has also been reported by Malic et al. [15]. An array of 10 multimode SU8 waveguides was placed perpendicular to a channel and sandwiched between two PMMA layers. The alignment scheme was implemented by etched V-grooves. Alexa Fluor 633 dye

was used as sample, and the detection limit for fluorescence detection was 10 nM and for absorbance detection was 1 μM. Even though the sensitivity of absorbance-based detection is lower compared to fluorescence method, the simple instrumentation associated with it makes it one of the most widely used techniques for LOC devices.

5.2.3 Chemiluminescence

Chemiluminescence (CL) refers to the phenomenon of emission of light due to chemical reaction. The main attraction for using chemiluminescence is its ability to produce photons without any excitation. Among other advantages are high detectability in low sample volume, simple instrumentation involved, possibility of integration with imaging systems, etc.

Other types of chemiluminescence are bioluminescence (BL, emission of light by a living organism itself or by utilizing the enzymes and photoproteins isolated from the organism) [16], electrochemiluminescence (ECL, luminescence generated due to relaxation of electrochemically generated intermediates to a lower level state during an electron–transfer reaction occurring at the electrode surface) [17], and thermochemiluminescence (TCL, light produced by thermal decomposition of molecule) [17]. A general reaction scheme for chemiluminescence is illustrated as follows [18]:

$$\text{Catalyst} + \text{substrate} \leftrightarrow \text{catalyst–substrate complex}$$

$$\text{Catalyst–substrate complex} \leftrightarrow \text{catalyst} + \text{converted substrate}$$

$$\text{Converted substrate} \rightarrow \text{excited-state product}$$

$$\text{Excited-state product} \rightarrow \text{product} + \text{light}$$

Light is generated by oxidation of the chemiluminescent reagents in presence of enzyme, hydrogen peroxide, ferricyanide, etc. The chemiluminescent reagents that are most commonly used are luminol, *p*-iodophenol (PIP), and peroxyoxalate (PO). Chemiluminescence-based microfluidic chips have been used for detection of biological agents, herbicides [19], glucose [20], metals [21], DNA hybridization [18], and many other applications [22]. Chemiluminescent signal is usually detected using charge-coupled device (CCD) camera (outside the microfluidic device), organic photodetector (integrated within the microfluidic chip) [23,24], or photomultiplier tube (PMT) via optical fiber [21].

The Emneus group in Denmark has reported the sensing of atrazine in surface water using microfluidic enzyme immunoassay and chemiluminescence as the transduction technique [19]. Silicon microchips were functionalized with proteins A and G via a hydrophilic polymer layer. An affinity capture competitive immunoassay was performed using a mixture of enzyme tracer [horseradish peroxidase (HRP)], atrazine sample, and antiatrazine antibody which was subsequently injected on the microchannel, followed by addition of the substrate mixture [luminol/*p*-iodophenol (PIP)/H_2O_2]. Chemiluminescent oxidation of luminol/PIP/H_2O_2 in the presence of HRP enzyme was monitored via PMT.

Use of organic photodiodes (OPDs) for integrated on-chip chemiluminescence detection was demonstrated, among others, by Wang et al. [24]. A peroxyoxalate chemiluminescence (PO-CL) assay was used for detection of antioxidant capacity of various biological extracts. In another study [23], organic thin-film (copper phthalocyanine–fullerene, CuPc–C_{60}) photodiodes were used to monitor PO-CL within a PDMS microfluidic device. H_2O_2 was used as a model compound, and the LOD obtained was approximately 1 mM with a linear range for over three decades.

Although chemiluminescence-based detection has several advantages, certain factors affect its sensitivity ie, instability of luminescence signal over time, loss in resolution due to diffusion of light-emitting molecules in a flow system, sample matrix can affect the light-generating reaction, etc.

5.2.4 Surface plasmon resonance (SPR)

Surface plasmon resonance (SPR) is defined as the resonant oscillation of electrons at the interface of stimulated by incident light. Surface plasmons are surface electromagnetic waves propagating parallel to the metal/dielectric (or metal/vacuum) interface. Because the waves are on the boundary of the metal (Au, Ag mainly; Al, Cu also) and the external medium, these oscillations are highly sensitive to any change of this interface, eg, change in refractive index at a functionalized metal surface upon interaction with an analyte (Fig. 5.7). Light incident on the metal surface through a prism at a specific angle gets total internally reflected and excites the surface plasmons. At resonance angle, the reflectance intensity decreases drastically. Any change occurring at the interface affects this resonance angle, which can be measured for detection purposes.

The benchtop SPR units used in laboratories, most commonly Biacore from GE Healthcare, are bulky and complex. Research is being conducted to miniaturize and

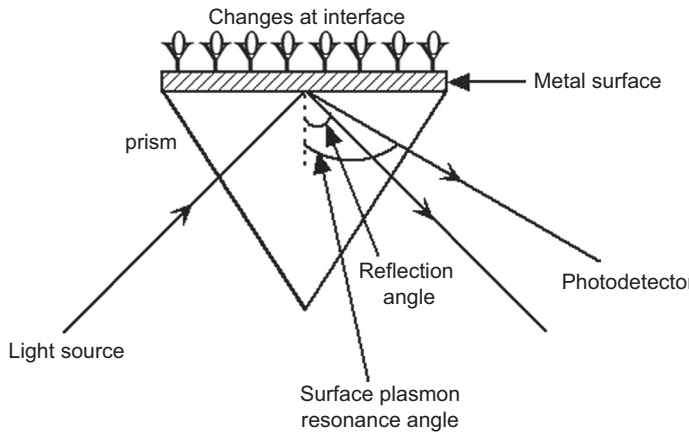

Figure 5.7 Schematic demonstrating surface plasmon resonance (SPR). Change in SPR angle occurs due to interaction at the metal-dielectric interface resulting in refractive index change in vicinity to the interface.

reduce the complexity of SPR sensors by integrating with microfluidic systems. One such output of the ongoing research is the Spreeta, SPR-based biosensor which is a low-cost and commercially available device. It is based on Kretschman's geometry, consists of an aluminum gallium arsenide (AlGaAs) light-emitting diode (LED, 840 nm) with a polarizer, temperature sensor, two photodiode arrays, and reflecting mirror [25]. Light from the LED is incident on the gold-coated thin glass at different angles after passing through a polarizer which allows only the transverse magnetic component. After reflection from the gold-coated glass slide, the light is directed toward the two photodiode arrays with the help of mirror. The device is encased in an optically clear material whereas an opaque coating prevents interference from the external light. This SPR system has been utilized for detection of different analytes with very low concentrations.

Highly sensitive detection of bacteria [25,26] [LOD ~10^2 to –10^3 colony-forming units per milliliter (cfu/mL), toxins [27] (LOD ~200 ng/mL), etc.] using the SPR principle has been reported. The sensor response time varies from 30 s to 20 min maximum. Increase in sensitivity can be achieved using secondary antibodies, magnetic or metal nanoparticles, etc. Soelberg et al. [28] demonstrated detection of staphylococcal enterotoxin B (SEB) as low as 100 pg/mL in both buffer and stool samples by using antibody-coated super-paramagnetic nanobeads in combination with SPR.

SPR imaging (SPRi) technology is one step further from SPR. In this case, the output signal is collected from an area on the chip as opposed to a point. CCD cameras are used for this purpose. The CCD cameras enable real-time monitoring of biomolecular interaction in a microarray format. SPRi is used for immunoassay-based detection of antigens, DNA hybridization, etc. An example of this is the study published by Luo et al. [29], in which a PDMS microfluidic device with an array of gold spots was coupled with SPR imaging for real-time measurements (Fig. 5.8). An assay time of 10 min was required to monitor the immunological reaction between biotin- bovine serum albumin (BSA)/antibiotin (antigen/antibody) with LOD of 0.21 nM. Using gold nanoparticles in sandwich assay format, the sensitivity of the device was increases up to 38 pM, however at the cost of increased response time (60 min).

SPRi has also been coupled with electrowetting-on-dielectric (EWOD) microfluidic technology for detection of DNA hybridization [30]. In this study, the bottom plate of an EWOD chip contained the reservoir, fluid path, and designated detection electrodes. The top electrode coated with gold was used for SPR imaging. Droplet dispensing, splitting, merging, and displacement were achieved using 90 V actuation voltage and 100 Hz switching frequency. Combining EWOD microfluidic device with SPR imaging helped simultaneous immobilization of different DNA probes at designated detection sites. The applied electrostatic field at the interface provided dynamic control of the immobilized probe orientation and density for enhanced hybridization efficiency.

SPR-based detection systems, although highly sensitive, suffer from the complex instrumentation associated with conventional SPR systems. Further, the systems are affected by temperature variations, and the cost of the chip is quite high. Unless these issues are addressed, it is unlikely that SPR systems will be used for point of care diagnostics.

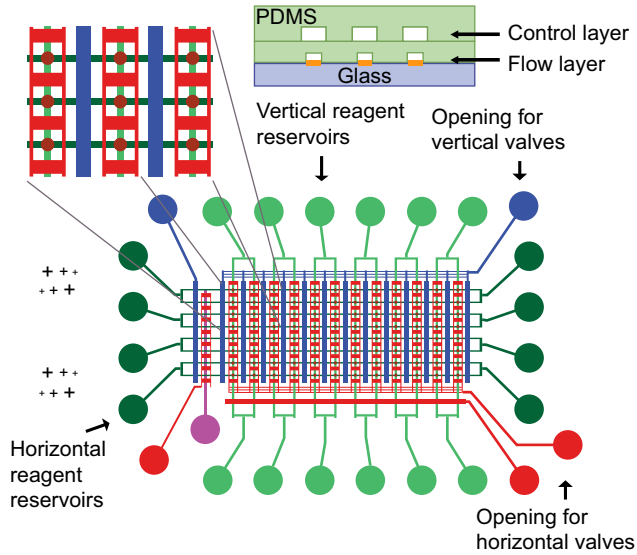

Figure 5.8 Schematic of the microfluidic device. In the top left figure, the valves (red and blue rectangles) in the control layer are aligned with the flow channels (light and dark-green lines) in the flow layer. Gold spots are represented by the brown dots and are located at the intersections of the flow channels.
Reproduced from Y. Luo, F. Yu, R.N. Zare, Microfluidic device for immunoassays based on surface plasmon resonance Imaging, Lab on a Chip 8 (2008) 694–700, with permission of The Royal Society of Chemistry.

5.2.5 *Surface enhanced Raman spectroscopy (SERS)*

In recent years, Surface Enhanced Raman Spectroscopy (SERS) has emerged as a highly sensitive analytical technique for detection of analytes in microscale. SERS refers to the enhancement of Raman scattering by molecules adsorbed on rough metal surfaces or nanostructures. SERS is advantageous in terms of detection of nonfluorescent samples, high sensitivity due to virtually zero background noise, no photobleaching of SERS labels, surface specificity, and detection of multiple analytes due to narrow and tuneable emission spectra [31]. High-precision measurements can be obtained using continuous flow and homogeneous mixing of analytes and nanoparticles which can easily be achieved using microfluidic channels [32].

Raman scattering refers to the inelastic scattering of photon when light is incident on a molecule. A small portion (~10^{-7}) of the exciting photons is scattered with a frequency less than that of the incident photon. This results in a spectrum of low-energy peaks which can be used to identify a molecule. Raman scattering is largely enhanced in LSPR substrate geometries ie, rough surface, nanoscale gaps, etc. Extremely high electric field intensities are present in the region surrounding the nanostructures, thus giving rise to increase in intensity of Raman scattering in that region [3]. This phenomenon is commonly known as SERS. Various researchers have worked on ordered nanostructures to predictably enhance Raman scattering on analysis chips, which can

lead to point of care (POC) devices [33–37]. An example is the study published by Quang et al. [38], who developed an array of micropillars contained within a PDMS fluidic channel (Fig. 5.9). Reproducibility was ensured by homogeneous mixing between the analytes and nanocolloidal silver in a continuous flow format. This system was used for quantitative analysis of hazardous material ie, dipicolinic acid and malachite green, and LOD obtained was 200 and 500 ppb, respectively.

SERS biosensors have also been used in conjunction with reporter molecules which have strong, distinct Raman spectra as labels. Some such molecules are thiophenol (TP), 2-naphthalenethiol (NT) [39], 5,5-dithiobis(succinimidyl-2-nitro-benzoate) (DSNB, a derivative of dithiobis (benzoic acid)) [40], (4-mercaptobenzoic acid, 4MBA) [41], etc. Because the Raman signal is usually quite weak, high-power lasers are required to elicit a measurable signal. However, it is not easy to miniaturize such lasers. Hence, enhancement of the signal by surface modification (ie, SERS) and/or using reporter molecules makes this technique more amenable to use in POC scenarios.

5.2.6 Interferometry

Label-free detection with high sensitivity can be achieved by interferometry-based LOC devices. Interferometric biosensors rely on the technique of splitting coherent light into two paths, one of which is functionalized with biomolecules sensitive to the

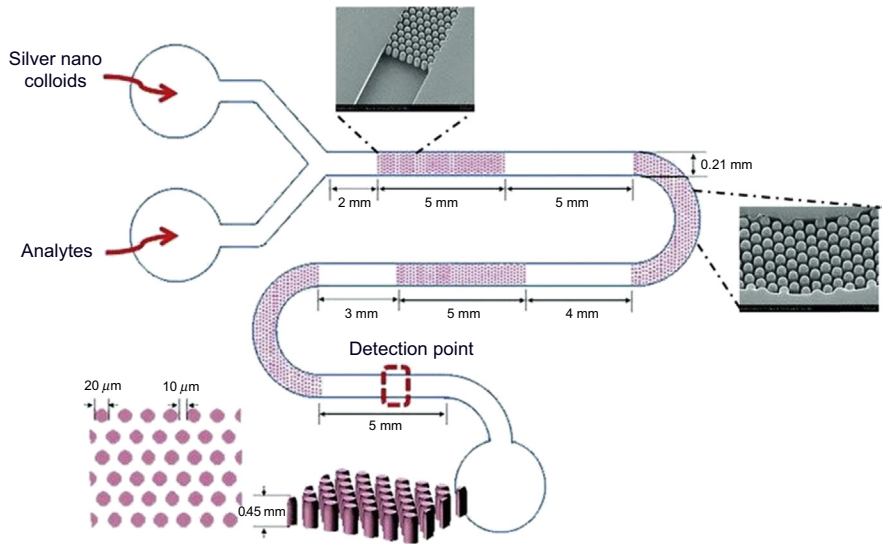

Figure 5.9 Schematic representation of polydimethylmethacrylate (PDMS) microfluidic channel with micropillar array for the Surface Enhanced Raman Spectroscopy (SERS) detection of hazardous materials. Red-dashed rectangle denotes the Raman measurement area. Scanning electron microscope (SEM) image of the micropillars is shown in the bottom left figure.
Reproduced from L. Xuan Quang, et al., A portable surface-enhanced Raman scattering sensor integrated with a lab-on-a-chip for field analysis, Lab on a Chip 8 (2008) 2214–2219, with permission of The Royal Society of Chemistry.

analyte of interest whereas the other path is unmodified ie, reference. Refractive index change occurring due to interaction of the sample with immobilized biorecognition elements results in a phase shift of the light beam in the functionalized channel with respect to the reference channel. This causes a change in interference patterns at the output which can be imaged for studying the binding response (Fig. 5.10). The reference channel is used to account for the common mode interferences ie, temperature variation, nonspecific adsorption, intensity variation, etc.

The two well-known configurations of interferometers are Mach–Zehnder (MZI) and Young (YI) interferometers. Both the interferometers use a Y-junction splitter to split a coherent beam of light into two beams passing through two waveguides, one for sensing and other for reference. However, MZI and YI differ in terms of the interference pattern readout. In MZI, the two channels are recombined by another Y-junction. It has on-chip readout using a single photodetector; however, high stability of light coupling is necessary. YI, on the other hand, has an off-chip readout system and the changes in interference pattern are measured in far field, making it independent of the light intensity and more reliable and easier to use [43].

The bimodal waveguide (BiMW) interferometer is a single-channel waveguide interferometer based on the interference pattern of two waveguide modes ie,

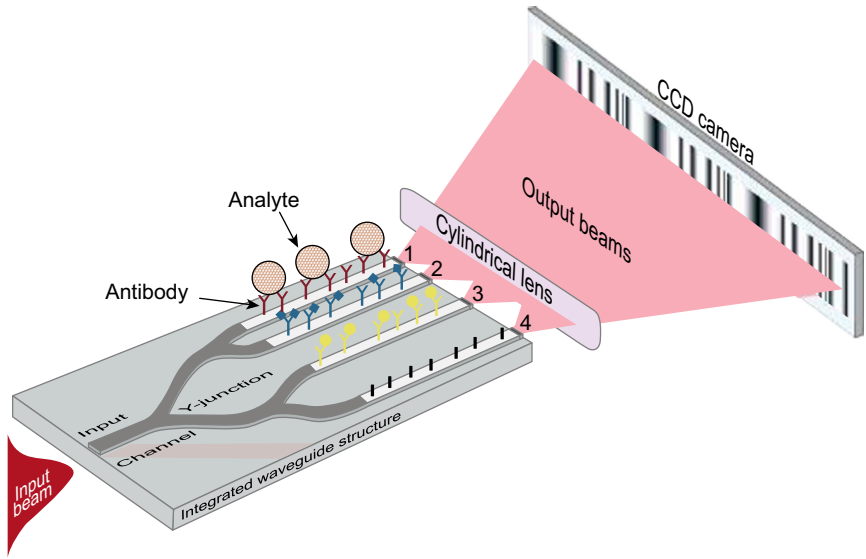

Figure 5.10 Schematic representation of the principle of interferometry. Input light is split into different beams passing through each channel. The unmodified channel serves as reference (Channel 4). Other channels are functionalized by specific reagents (Channels 1, 2, and 3). Upon interaction with the analyte, a change in refractive index occurs in these channels, resulting in change of optical path of the light and the interference pattern at the output changes which is detected using the CCD camera.
Reprinted (adapted) with permission from A. Ymeti, et al., Fast, ultrasensitive virus detection using a young interferometer sensor, Nano Letters 7 (2) (2007) 394–397. Copyright 2007 American Chemical Society.

fundamental and first-order modes of the same polarization. The advantages of BiMW configuration is the ease of mass fabrication because there is no need for Y-shape splitters which is the most complex component of MZI and YI devices. Lechuga's group in Barcelona has developed BiMW interferometers for label-free analysis of various biological analytes ie, BSA [43], human TSH [44], etc.

The Young interferometer chip can be integrated with microfluidic channel for detection of proteins, virus [42,45], etc. The interaction between the analytes and reagents immobilized on the waveguides results in variation of optical path lengths in different channels depending on the concentration of the analyte. The interference patterns generated by overlapping the output light from each channel are detected by a CCD camera. Shift in the interference pattern is determined by Fast Fourier Transform (FFT).

In place of a Y-junction to split light into two paths for a Mach–Zehnder interferometer, Gao et al. [46] used two parallel nanoslits in a thin metal (silver) film. One slit was used to scatter light into two different beams for the reference and functionalized channel and the second slit was used for the generation of the interference pattern. It is amply clear that interferometry is quite compatible with present microfabrication techniques, and if the cost of the devices can be kept within reasonable limits, this technology can prove to be a strong force in the LOC domain. Some attempts toward that have been made by various groups [47–49] including one by Bruck et al. [50], who developed an MZI system with integrated polyimide waveguides on silicon substrate.

5.3 Electrochemical method

One of the most common transduction techniques used in microfluidic LOC systems is electrochemical detection. Advantages of electrochemical detection include easy fabrication, integration, and miniaturization of electrodes within microfluidic devices. Although electrochemical techniques may lag behind optical detection in terms of sensitivity, nevertheless its low cost, ability to work with turbid samples, inertness to ambient light, temperature, etc., prompted researchers to incorporate it in LOC devices for POC applications.

The electrical parameters that are measured are current and voltage (amperometric, potentiometric or voltammetric), impedance, and conductivity. In this section, we discuss electrochemical methods applicable to LOC devices.

5.3.1 Amperometric

In amperometric transduction, the working electrode is maintained at a fixed potential, with respect to a reference, while the current is monitored. Frequently the current is injected through a counter electrode. The applied potential drives the redox reaction of analytes and the oxidation or reduction current provides information about the electron transfer reaction and in turn the concentration of the analytes. Amperometry can be single potential or pulsed potential. In single-potential amperometry, a fixed

voltage is applied. The measured current changes as an electro-active analyte is oxidized at the anode or reduced at the cathode. In pulsed amperometry, a working potential is applied for a short time, followed by higher or lower potentials that are used for cleaning the electrode. The current is measured only while the working potential is applied; sequential current measurements are processed by the detector to produce a smooth output.

The main difficulty in working with amperometry is the need for decoupling of the detection signal to eliminate any interference from the applied electric field, precise electrode positioning, and electrode stability [51]. However, use of decoupler in front of the detector [52,53], or end-column detection [54,55] may resolve these issues.

Amperometry can be used in conjunction with capillary electrophoresis (CE). On chip the CE system uses an electric field for separation of analyte molecules in a narrow channel or capillary on the basis of electrophoretic mobilities of molecules. The CE system usually consists of a sample injection channel, a separation channel, and electrodes for application of electric field, as well as measurement of current signals. There are quite a few instances of such development [56–58]. For example, Wang et al. integrated micromachined capillary electrophoresis with amperometric detection for dopamine detection [59]. The glass chips consist of a separation channel, a sample injection channel, and a thin layer of sputtered Au (acting as working electrode) at the outlet of the separation channel (Fig. 5.11). Dopamine was detected with LOD of 1 μM and linear response from 20 to 200 μM.

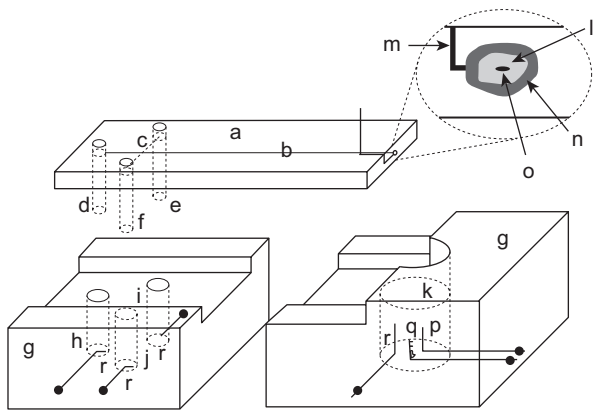

Figure 5.11 Schematic of the capillary electrophoresis (CE) system: (a) Glass microchip, (b) separation channel, (c) injection channel, (d) pipet tip for buffer reservoir, (e) pipet tip for sample reservoir, (f) pipet tip for sample waste reservoir, (g) Plexiglass body, (h) buffer reservoir, (i) sample reservoir, (j) sample waste reservoir, (k) detection reservoir, (l) Au working electrode, (m) working electrode contact, (n) insulator layer, (o) channel outlet, (p) counter electrode, (q) reference electrode, and (r) contacts to high-voltage electrodes.
Reprinted (adapted) with permission from J. Wang, B. Tian, E. Sahlin, Integrated electrophoresis chips/amperometric detection with sputtered gold working electrodes, Analytical Chemistry 71 (1999) 3901–3904. Copyright 1999 American Chemical Society.

5.3.2 Voltammetric detection

Instead of a fixed potential, voltammetric method is based on the application of a potential sweep at the working electrode and measuring the current. There are different types of voltammetry ie, linear sweep, square wave, cyclic, anodic stripping, cathodic stripping, adsorptive stripping, etc. In linear-sweep voltammetry, the potential between the working and reference electrode is varied linearly and current is measured. Oxidation or reduction of species is indicated by a peak/trough in the current signal at the potential at which the species starts getting oxidized/reduced. Square-wave voltammetry also involves linear sweep of the working electrode potential; however, the nature of the sweep waveform is the combination of staircase signal and a square wave. Cyclic voltammetry is the most common one, in which the working-electrode potential is ramped linearly with time till a specific time, after which the potential is ramped back. This to-and-fro ramping can happen multiple times during a measurement.

Generally, a three-electrode system consisting of working, reference, and counter electrodes is used for voltammetric measurements. The working electrode is where the interaction of the analyte and the reagents occur, resulting in a change of current flowing from the working to the counter electrode. The working electrode is modified with desired reagents for detection of analytes. The reference electrode maintains the potential at the working electrode constant during the measurement with no current flowing through it. The reference electrode is typically a nonpolarizable electrode ie, Ag/AgCl whereas the counter electrode fabricated from polarizable material ie, noble metals (usually, Pt).

Voltammetric systems have been incorporated in microchips for detection of heavy metals [60], explosives [61], biomolecules [62,63], etc. Systems like these, using various types of flow cells (such as PDMS) have been used for detection of the effects on anticancer drugs on HeLA cells [62]. In a slightly different format, Naseri et al. reported a plastic three-electrode micro flow cell device with the electrodes fabricated by injection molding from a conducting grade of polystyrene loaded with carbon fibers [64]. The reference electrode was coated with a layer of Ag/AgCl, whereas the counter electrode was unmodified conducting polymer electrode. The working electrode was either conducting polymer coated with Au or bare conducting polymer. Anodic stripping voltammetry were tested for detection of metal ions ie, Cu(II) and Pb(II). Similar systems can be developed for detection of biomolecules at POC as well.

5.3.3 Impedance-based detection

Impedance spectroscopy is a widely used method that can be integrated with microfluidic channels in LOC devices. This technique is based on perturbation of the sample with an alternating current (AC) potential of small amplitude and monitoring the behavior of the system to reach steady state in response to the perturbation. Change in impedance of a sample with concentration of analyte is observed when the analytes get attached to the electrodes, changing the dielectric properties of the surrounding medium [65]. Impedance-based transduction techniques allow rapid, low-level,

label-free detection with high sensitivity, reduced assay time, and easy data interpretation. The impedance response is fitted in an appropriate electrical equivalent circuit, derived from prior knowledge about the physical phenomenon occurring at the electrode–electrolyte interface. Using the equivalent circuit, different parameter variations with change in analyte concentration can be identified for better understanding of the phenomenon.

Electrical impedance is defined as the ratio of incremental voltage to the resulting current. The excitation voltage applied is a modulated AC signal ie, a small direct current (DC) bias is superimposed with an AC signal. Let us assume the following:

$$\text{Applied voltage, } V_{\text{applied}} = V_{\text{DC}} + V_{\text{AC}} \sin(\omega t)$$

$$\text{Resulting current, } I_{\text{resulting}} = I_{\text{DC}} + I_{\text{AC}} \sin(\omega t - \varphi)$$

The complex impedance $Z(\omega)$ will have a magnitude of $V_{\text{AC}}/I_{\text{AC}}$ and a phase angle of φ. The complex impedance should be measured using a frequency sweep; however, this requires more time. Single-frequency excitation, on the other hand, takes less time but accuracy may suffer. For single-frequency measurement, a lock-in amplifier is used. The applied voltage is usually very small in case of impedance biosensors, because the voltage–current relationship is linear for small perturbations [66]. The electrochemical impedance spectroscopy leaves the biomolecular probe layer undisturbed because very low voltage is used for excitation, unlike amperometry or voltammetry in which relatively higher voltages are applied.

Two types of impedances are measured in electrochemical impedance spectroscopy (EIS): faradic and nonfaradic. Faradic impedance is associated with the process which involves transfer of charge across an interface. In faradic impedance measurement, a redox probe is used which is alternately oxidized and reduced due to transfer of electrons to and from the metal electrode resulting from the biological events occurring near the electrode surface. Nonfaradic impedance (mostly capacitive measurements) on the other hand is associated with transient flow of current or displacement current without actual transfer of any electron. In this case, no redox probes are required.

Impedance spectroscopy allows analysis of interfacial changes occurring due to biorecognition events at the electrode surface. Formation of bioreceptor layer and further binding with the target of interest alters the capacitive and resistive properties of the electrode–electrolyte interface. A double-layer capacitance (C_{dl}) arises due to combination of events ie, analyte binding (C_{anal}) to the sensing layer (C_{sens}) on the electrode (C_{elec}) acting in parallel. Sensitivity is determined by the relative capacitance change due to binding of analyte on the sensing layer. Antibody being less polar than water, the effective permittivity after antibody layer formation decreases and so does C_{dl}. Further decrease in C_{dl} is observed after Ag–Ab binding owing to the combined effect of decrease in dielectric permittivity and increase in thickness of the dielectric layer.

Sensitivity depends on proper thickness of the sensing layer. For a very thin sensing layer, underlying the electrode surface will be partially exposed leading to nonspecific interactions from interfering species. For a very thick sensing layer, AC impedance

current is drastically reduced, and so is the capacitance change upon analyte binding. For faradic impedance, the electron transfer resistance (R_{et}) is a combination of resistance due to analyte binding (R_{anal}) to the sensing layer (R_{sens}) on the electrode (R_{elec}). Resistance at conductive electrode-sensing layer interface being negligible, the change in electron transfer resistance is mostly due to analyte binding and the sensing layer underneath. For measurement of R_{et}, presence of redox probe is necessary.

Although the most commonly used electrode configuration is interdigitated (coplanar in nature) due to high surface area for interaction, high sensitivity, easy fabrication, and alignment, top–bottom electrodes are also used in microfluidic devices due to the uniform distribution of electric field lines between the electrodes throughout the entire height of the microchannel (Fig. 5.12).

Researchers have detected various biological analytes on the basis of both faradic and nonfaradic impedance measurements. Bashir et al. [67] developed a dual-purpose silicon-based microfluidic device for concentration of bacterial cells from dilute samples using dielectrophoresis as well as detection of metabolic activity of the cells by electrical impedance measurement for quantifying the sample. In another study [68], Varshney et al. detected bacteria in a PDMS microfluidic channel with an embedded interdigitated gold microelectrode array (IDAM). This was used in conjunction with magnetic nanoparticle–antibody conjugates (MNAC) to obtain an LOD of 1.6×10^2 and 1.2×10^3 cells of *Escherichia coli* O157:H7 cells present in pure culture and ground beef sample, respectively.

Impedance spectroscopy has also been widely used for detection of cancer cells. Impedance response of cancer cells adhered to the surface of microelectrodes being usually higher than that of the normal cells, distinction between normal and cancer cells is possible [69]. Real-time analysis for distinguishing oral cancer cells (CAL-27)

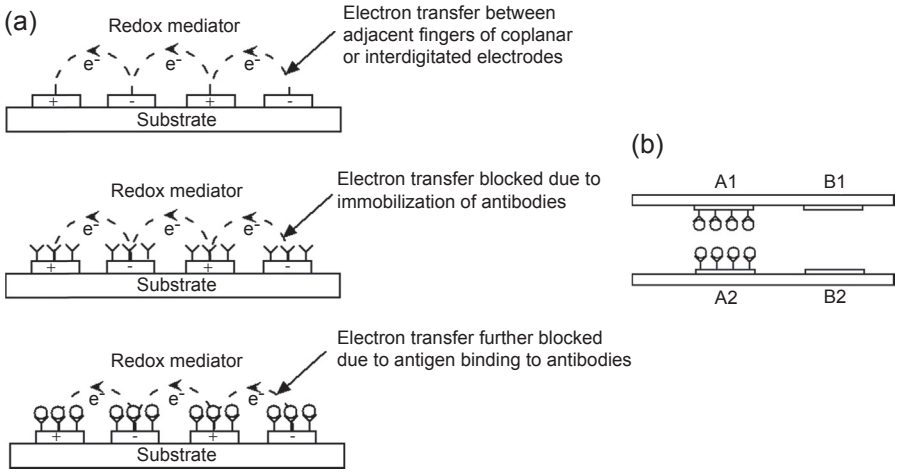

Figure 5.12 (a) Electron transfer event in faradic impedance-based immunoassay. The electron transfer resistance increases with increasing layers on the electrode–electrolyte interface. (b) Top–bottom electrode configuration. First set of electrodes (A1–A2) is functionalized whereas the second set (B1–B2) are the reference electrodes ie, without functionalization.

and normal oral epithelial cells (Het-1A) [70], study of cellular activities ie, cell adhesion, spreading, and proliferation of oral cancer cell line [69] has been reported using faradic impedance spectroscopy on a microchip with IDAM. A similar principle can be applied for detection of the breast cancer cell line [71], highly and poorly metastatic head-and-neck cell line (686LN and 686LN-M4e, respectively) [72], *Babesia bovis*-infected red blood cells [73], etc.

5.3.4 Conductometric detection

Conductometric detection is based on measurement of specific conductance of an analyte and is preferable because it can be applied for detection of both electroactive and electroinactive species. The conductivity electrodes can either be in contact with the solution or insulated using a thin layer. Conductivity detection is mostly associated with capillary electrophoresis (CE).

In contact-mode conductivity measurement, the electrodes are in direct contact with the sample. Although better contact with sample results in good sensitivity and lesser response time, it is accompanied with the increased risk of degradation of electrodes and contamination of sample [51]. Baldock et al. used contact conductivity detection for isotachophoresis separation using different types of conducting polymer as drive and conductivity electrode material. Separation of three anionic dyes, two inorganic anions, and a mixture of eight alkaline earth, transition, and lanthanide metal cations was performed successfully [74]. Contact-mode conductivity measurement has also been applied for detection of alkali metal ions, organic acids [75], circulating tumor cells (CTC) [76], etc.

Contactless conductivity detection, on the other hand, does not suffer from electrode instability. In this case, the electrodes are separated from the buffer or sample by a thin layer of insulation. For achieving good capacitive coupling, the insulation layer needs to be as thin as possible. A contactless movable conductivity detection microchip was developed in [77] for capillary electrophoresis to detect low-energy explosives and nerve-agent degradation. Laugere et al. [78] developed an on-chip four-electrode conductivity detection system. Four contactless metal electrodes covered by silicon carbide were deposited and patterned on glass substrate for on-chip conductivity detection. Electrophoretic separation of three inorganic cations (K^+, Na^+, Li^+) and six organic acids were performed with an LOD of 5 μM for potassium.

Conducting polymers are also used for conductance-based detection. They are organic conjugated compounds with an extended π-orbital system allowing movement of electrons from one end to other. Conducting polymers have unique electrical and optical properties that can be tuned by reaction with redox active agents. Electrical conductivity of these types of polymers depends on their ability to transport charge carriers along the polymer backbone. Interaction with redox active species results in the change in conductivity of the polymers which can be measured either in DC or AC conditions. Commonly used conducting polymers are polyaniline, polyacetylene, polypyrrole, polyfluorine, etc. [79]. The advantages of conducting polymers are their environment stability, easy processibility, compatibility with biomolecules, ability to be coated on the surface of desired dimensions, and modification to bind to biomolecules.

These properties taken together have paved the way for developing conducting polymer-based sensors for detection of bacteria, proteins, trace metal ions, and many more.

Such a conductometric biosensor was developed by Dixit et al. [80] in which polyaniline thin film doped with Fe-Al was deposited on glass substrate. On top of this film, an interdigitated Au electrode array was designed for detection of *E. coli* in liquid samples. The current–voltage characteristics of the film upon exposure to microorganism were measured using source measuring unit. Change in conductivity of the film is due to the interaction of charged microorganism with the polymer film resulting in redox reactions at the polymer film interface.

Improved sensitivity can be obtained by increasing the surface area of reaction ie, by using conducting polymer nanoparticles or nanofibers and then subsequently functionalization for detection of the analyte. Polyaniline nanowire-based conductometric biosensor for detection of immunoglobulin (IgG) and myoglobin was reported by Lee et al. [81].

Conducting polymers are quite inexpensive and offer a unique transduction process, in which biochemical phenomena (like binding processes or enzymatic reactions) occurring on the surface give rise to changes in electrical properties of the material, particularly if they are thin films. In this sense, conducting polymers behave like field effect transistors. The possibility of creating such devices on organic substrates (like plastics) opens up the possibility of very inexpensive devices both in terms of materials as well as manufacturing processes. Thus, incorporation of conducting polymer-based sensors on LOC systems may be beneficial from the point of view of not only sensitivity, but also cost.

5.4 Other detection techniques

5.4.1 Thermal detection

Thermal changes associated with a chemical reaction or phase transitions can be monitored for diagnostic purpose. The basic principle of operation is that any change in phase or physical transformation is accompanied by heat flow which can be measured by calorimetry. Differential scanning calorimetry (DSC) is a technique in which the difference in the amount of heat required for increasing the temperature of a sample and reference is measured as a function of temperature, with both the sample and reference maintained at a same temperature approximately throughout the experiment. Depending on the process, the change may be exothermic or endothermic in nature. When a solid sample melts to a liquid it absorbs heat, whereas during crystallization heat is generated.

Ming Su and his group at Northeastern University have developed nanoparticle-based scanning calorimetry systems for detection of DNA [82], thrombin [83], cardiac biomarkers [84,85], etc. The property of phase-change nanoparticles to absorb heat and generate sharp melting peaks while changing from solid to liquid phase, thus giving rise to heat signatures, can be utilized for detection of proteins (Fig. 5.13) [84,85]. Multiple protein detection can be achieved by using different types of nanoparticles, each having different heat signature corresponding to a specific protein.

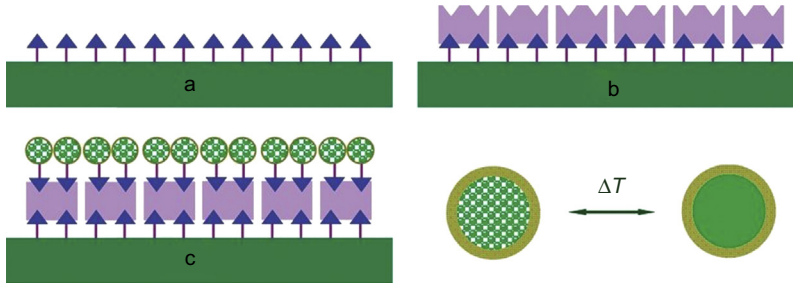

Figure 5.13 Thermal addressing of nanoparticles for antigen sensing. (a) Antibodies are immobilized on the substrate. (b) Interaction between antibody and specific antigen occurs. (c) Secondary antibody-tagged nanoparticles bind to the antigen forming a sandwich immunoassay. The thermal property of nanoparticles is measured instead of antigen–antibody interaction-related changes for detection and quantification of the antigen.
Reprinted (adapted) with permission from L. Ma, C. Wang, Y. Hong, M. Zhang, M. Su, Thermally addressed immunosorbent assay for multiplexed protein detections using phase change nanoparticles, Analytical Chemistry 82 (2010) 1186–1190. Copyright 2010 American Chemical Society.

The melting peak and fusion enthalpy of phase-change nanoparticles during thermal analysis provide information about the type and concentration of biomarkers, respectively. The different types of nanoparticles used are metal (indium, tin, aluminum, cadmium, copper, magnesium, silver, bismuth, gadolinium, and palladium), bimetal alloy (lead–tin, or any combination from the aforementioned metals) or eutectic alloys [86]. Use of nanoparticles helped increase the sensitivity to picomolar or femtomolar range.

5.4.2 Acoustic wave-based detection

Acoustic wave-based sensors utilize the mechanical acoustic waves as the transduction mechanism. Acoustic wave in LOC is favorable due to its noninvasive nature, compatibility with soft lithography micromachining and ability to work for almost any type of microparticle. Mainly, three types of acoustic devices are available: bulk acoustic wave (BAW), surface acoustic wave (SAW), and acoustic plate mode (APM) [87]. In BAW, the acoustic waves are guided through the whole volume of the substrate. SAW devices involve propagation of acoustic waves along a single surface on the substrate, whereas APM devices consist of waves guided by reflection from multiple surfaces. For LOC applications incorporating microfluidics, generation of BAW is difficult because it requires very good acoustic reflection properties of the channel material which is unlikely in PDMS and other commonly used materials. In addition, integration of bulk acoustic transducers in a microsystem is an issue.

SAWs, on the other hand, are routinely used to focus, separate, align, and manipulate fluids in a microchannel [88] and can be generated by microelectrodes using piezoelectric substrates (Fig. 5.14). On-chip manipulation of particles (cells or beads) using SAW is reported by many authors [89,90]. Several biosensing applications of acoustic wave-based sensors are reported in [91–94].

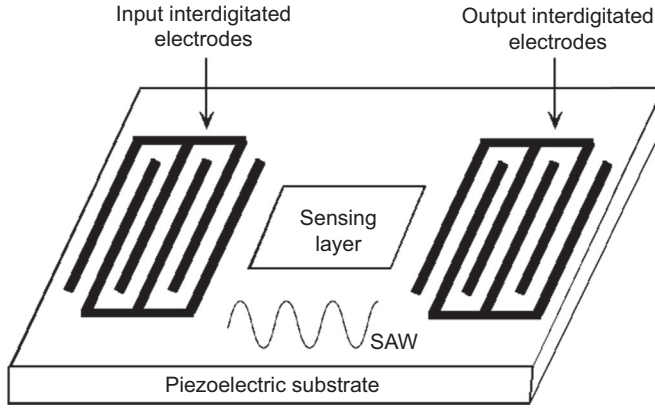

Figure 5.14 Surface acoustic waves generated using two interdigitated electrodes and a piezoelectric substrate.

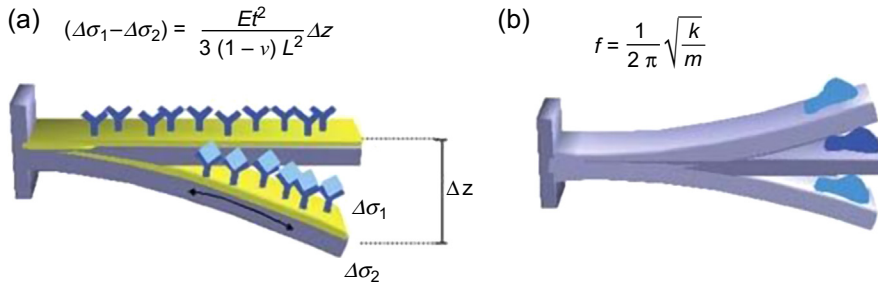

Figure 5.15 Different modes of operation of microcantilever: (a) Static or deflection mode. Change in surface stress between the top and bottom sides, $(\Delta\sigma_1 - \Delta\sigma_2)$ is directly proportional to the cantilever-free displacement, Δz, the cantilever thickness, t, and inversely proportional to the square of the cantilever length, L. Youngs modulus and Poisson coefficient are represented by E and ν, respectively. (b) Dynamic or resonance mode. Resonance frequency, f, is directly proportional to the square root of stiffness constant, k, and inverse of the mass, m. Reproduced from M. Alvarez, L.M. Lechuga, Microcantilever-based platforms as biosensing tools, Analyst 135 (2010) 827–836, with permission of The Royal Society of Chemistry.

Microcantilevers are often used in biosensing applications [95]. Microcantilevers work on the principle of change in bending (static mode) or resonance frequency (dynamic mode) upon interaction with analytes (Fig. 5.15). In static mode, cantilever bending arises because of change in surface stress due to molecular reaction taking place on only one of the cantilever surfaces. The factors affecting the change in surface stress are due to interactions from electrostatic, hydration, steric, and van der Waals forces, changes in the surface hydrophobicity, or conformational changes of the adsorbed molecules, etc. On the other hand, in the dynamic mode functionalization of only one cantilever surface is not necessary because the change in resonance

frequency is governed by the total mass adsorbed on both sides. Microcantilever, while operating in dynamic mode, is extremely sensitive to mass changes ie, in the attogram range [95].

Microcantilevers are usually fabricated from polysilicon, silicon nitride, silicon dioxide, polymers, SU8, etc. Using proper functionalization chemistry, microcantilevers can be applied for detection of DNA hybridization [96], antigen–antibody interaction, pesticides, bacteria, etc.

Signal transduction from microcantilevers is based on optical, piezoelectric, and piezoresistive methods. Optical transduction is based on measurement of displacement of reflected light from the surface of microcantilever with the help of a position-sensitive photodetector [95]. However, this is not suitable for POC devices, because a larger path length might be required, which precludes portable instrumentation. Piezoelectric and piezoresistive detection involves integration of a piezoresistive (polysilicon) or piezoelectric material, respectively, on the microcantilever. In this case, the instrumentation can obviously be miniaturized. However, the requirement for a mechanically stable environment, difficulty in functionalization of a large number of cantilevers at the same time, fabrication losses, etc. are obstacles to commercialization of devices based on microcantilevers as POC products.

5.5 Miscellaneous

5.5.1 Paper microfluidic devices

The concept of paper microfluidics was first invented by Whitesides group of Harvard University. The main advantages of paper-based microfluidic devices are low cost, easy usage, and ecofriendly disposal. Paper is an ideal substrate for LOC–POC devices because it has an inherent sampler in the form of its wicking capability. A hydrophilic paper substrate was patterned to selectively modify certain areas to be hydrophobic to govern or restrict the flow of sample through the hydrophilic areas/channels only. The patterning technologies include photolithography, wax printing, plotting, plasma etching, inkjet etching, etc. The reagents required for detection are mostly enzymes or small molecular dyes. The hydrophilic detection regions can be spotted with specific reagents for detection of glucose, protein, etc. [97].

Colorimetry combined with microfluidic paper-based analytical devices (μPADs) have been reported most commonly for detection of analytes ie, uric acid [98], metals [99], etc. Colorimetric detection provides qualitative analysis; however, quantitative information from these assays was obtained by measuring the amount of color change in such assays [98–100]. Colorimetry-based analysis of urine samples was reported by Martinez et al. [97] (Fig. 5.16) in which three hydrophilic regions were patterned on chromatographic paper by using photoresist. Detection of protein and glucose in an artificial urine sample was achieved using colorimetric analysis. μPADs based on electrochemical detection have been used for sensing heavy metal ions, glucose [101]. The electrodes that are mostly used are screen-printed carbon and Ag/AgCl electrodes as the working and reference electrodes, respectively.

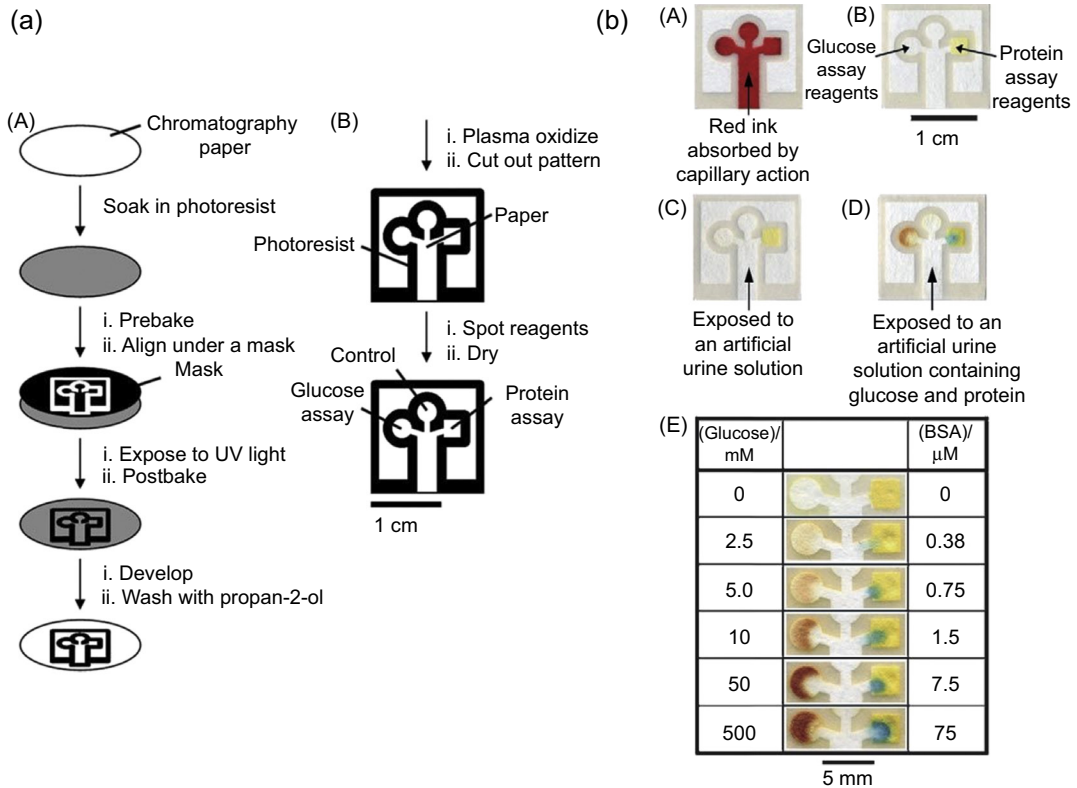

Figure 5.16 (a) (A) Fabrication procedure for patterning chromatographic paper substrate, (B) Modification of the patterned paper with specific reagents for use as biological assay. (b) Analysis of proteins and glucose in urine samples: (A) Patterned paper after absorbing Waterman red ink via capillary action, (B) complete assay after spotting the reagents. Circular region to the left is for glucose sensing, whereas the square region on the right is for protein sensing. (C) Negative control for glucose (left) and protein (right) by using an artificial urine solution, (D) positive assay for glucose (left) and protein (right) using a solution containing 550 mM glucose and 75 μM BSA in an artificial urine solution. The control well was spotted with the potassium iodide solution, but not with the enzyme solution. (E) Glucose and protein detection assays by using varying concentrations of glucose and BSA. Reproduced from A.W. Martinez, S.T. Phillips, M.J. Butte, G.M. Whitesides, Patterned paper as a platform for inexpensive, low-volume, portable bioassays, Angewandte Chemie International Edition 46 (2007) 1318–1320, with permission from John Wiley and Sons.

Apart from 2D devices, the Whitesides group has fabricated 3D μPADs by stacking alternating layers of patterned paper and double-sided adhesive tape patterned with holes for detection of glucose and protein [102]. In addition, paper-based invasion assays for isolating cancer cells [103], urinalysis (assays for glucose, protein, ketone, nitrate) [104], etc., have been developed. A 3D μPAD based on the art of origami was reported by Ge et al. [105] for blood plasma separation using sandwich chemiluminescence immunoassay (Fig. 5.17).

μPADs act as potential candidates for POC devices because they provide low cost and rapid detection along with ease of fabrication and disposal. Wicking property of paper allows the use of small sample volume for analysis purposes. However, issues like sensitivity, reproducibility, and quantitative measurement are still challenges to be

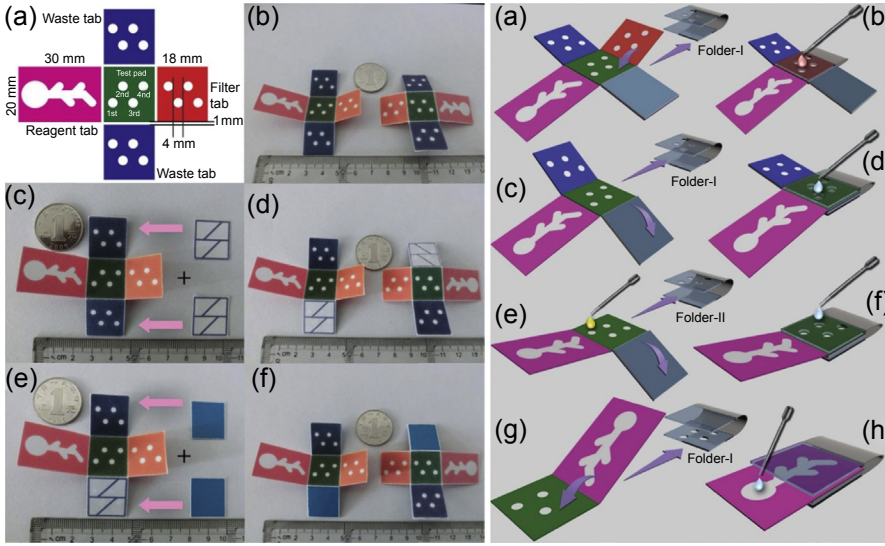

Figure 5.17 (Left figure) (a) Schematic of the 3D origami-based device; (b) front and back surface of the device; (c and d) binding a baked thin wax-patterned blotting paper to each waste tab followed by (e and f) binding an unbaked thick-wax patterned blotting paper onto each waste tab. (Right figure) (a) The filter tab folded above the test pad; (b) blood samples added to each paper microzone on the folded filter tab with the help of Folder-I; (c) filter tab torn off and one waste tab folded below the test pad; (d) washing buffer added into each immunozone on the test pad to rinse the immunozones using Folder-I; (e) used waste tab was torn off and a solution of silver nanoparticles (AgNPs)-luminol/Ab2 added into each immunozone on the test pad; (f) rest waste tab folded below the reversed test pad and the device washed again using Folder-II; (g) used waste tab torn off and the reagent tab folded above the test pad; (h) H_2O_2 solution added into the reagent cell to trigger the CL reactions using a new Folder-I.

Reproduced from L. Ge, S. Wang, X. Song, S. Ge, J. Yu, 3D origami-based multifunction-integrated immunodevice: low-cost and multiplexed sandwich chemiluminescence immunoassay on microfluidic paper-based analytical device, Lab on a Chip 12 (2012) 3150–3158, with permission of The Royal Society of Chemistry.

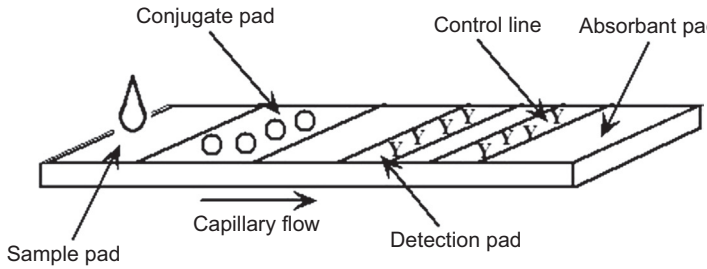

Figure 5.18 Lateral flow immunoassay (LFIA) strip with its components.

overcome, so that the potentials of paper devices can be utilized fully in the development of disposable POC diagnostic devices.

5.5.1.1 Lateral flow immunoassay (LFIA)

Lateral flow immunoassay (LFIA) is a special type of microfluidic test strip in which flow of liquid is governed by capillary forces. The chemicals required for detection are preimmobilized on the test strip and the detection is usually colorimetric, so that the results are visible using the naked eye. Because colorimetry provides qualitative results, electrochemical and other optical transductions have been used for quantitative detection of analytes.

In general, a typical LFIA format consists of surface layer that guides the sample from the application pad through the conjugate release pad along the strip to the detection zone and finally to the absorbent pad (Fig. 5.18) [106]. The pads overlap onto one another and are mounted on a backing card using a pressure-sensitive adhesive.

The materials used for LFIA strips are nitrocellulose, nylon, polyethersulfone, polyethylene, fused silica, etc. A conjugate pad is where the analyte of interest forms a complex with the preimmobilized particles. Depending on the assay format, different types of particles may be immobilized in the conjugation pad ie, colloidal gold, colored or fluorescent particles, biomolecules, etc. In the detection pad, other specific biorecognition elements are immobilized and the interaction with the analyte is sensed using colorimetric, electrochemical, or another detection technique. The purpose of the absorbent pad is to wick the excess fluid so that higher sample volume can be used for better sensitivity.

Yager and his group at the University of Washington have developed 2D paper networks for implementing Y or T sensor and H filter (Fig. 5.19) [107] chemical signal amplification [108], controlled reagent transport by modification of the channel structure, width and position of source [109], malaria antigen detection [110], etc.

Among the advantages of LFIA are rapid, low-cost assay, no washing step involved, disposable, requirement of low sample volume, etc. However, LFIA suffers from certain disadvantages ie, restriction of sample volume compromises the sensitivity, qualitative, or semiquantitative results. Although qualitative, LFIA still provides fast and cost-effective solutions for detection of different analytes, depending on the application.

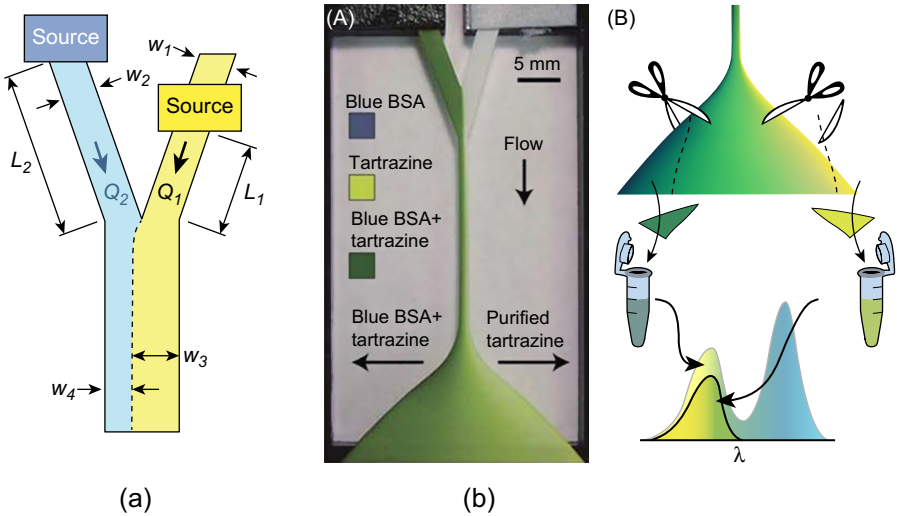

Figure 5.19 (a) Schematic of the paper-based Y or T sensor, an alternative to the existing microfluidic Y device. The interface between the two fluids remains constant as long as there is sufficient source fluid in the source pad and ample unfilled capacity in the absorbent pad to continue flow in the device. (b) Size-based separation in a paper H filter. (A) Image of a paper H filter with a mixture of blue-black BSA and tartrazine in the left sample pad and deionized (DI) H_2O in the right. (B) Schematic of the extraction process.
Reproduced from J.L. Osborn, et al., Microfluidics without pumps: reinventing the T-sensor and H-filter in paper networks, Lab on a Chip 10 (2010) 2659–2665, with permission of The Royal Society of Chemistry.

5.6 Conclusion

Microfluidics-based LOC devices are the future of POC diagnostics. With the advanced fabrication and integration technologies available, it is possible to develop small, robust, sensitive, handheld devices which can be used by a layman. In this chapter, a brief idea about the different types of LOC devices has been discussed. Various research groups have developed techniques to overcome the difficulties faced while miniaturizing a device. However, research is ongoing for the increased sensitivity of the different types of LOC devices.

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Intelligent tattoos, patches, and other wearable biosensors

6

X. Zhu[1], *W. Liu*[2], *S. Shuang*[3], *M. Nair*[1], *C.-Z. Li*[1]
[1]Florida International University, Miami, FL, United States; [2]University of Miami, Miami, FL, United States; [3]Shanxi University, Taiyuan, People's Republic of China

6.1 Introduction

6.1.1 Wearable biosensors

Wearable biosensors (WBSs) are noninvasive biological sensors integrated into various wearables, such as watches, clothes, bandages, glasses, contact lenses, rings, and so on. WBSs are widely used to measure and track a broad range of physical parameters of human bodies, which includes, but is not limited to, heart rate, blood pressure, body temperature, respiration rate, and body motion. With the advance in the microfabrication techniques and the development of new physical sensors in the last decade, WBS has demonstrated highly improved comfortableness to wear and has become widely acceptable.

We are currently witnessing the fastest-ever growth of marketed WBSs because of their capability for health monitoring in a real-time manner. Multiple biosensor-incorporated smart-device accessories record the physical activities and physiological traits of the human body. One can track his/her accumulative and real-time indexes. Corresponding adjustment for exercises can be made periodically to maintain or improve health status. When connected with telemedical systems, WBSs allow health care providers to monitor physiological traits of patients after therapeutics or treatments (demonstrated in Fig. 6.1). As an example, heart rate is a critical indicator for cardiovascular functions after myocardial infarction. Its long-term monitoring helps the health care provider remotely evaluate the treatment results and the effectiveness of recovery plans, and response to any potential life-threatening changes immediately as well.

Vital signals of wearers are collected by the wearable biosensors. Through the mobile telemedical terminal (such as a smartphone), information coupled with location is transmitted to health care providers, family members, and optional emergency services. The health care provider evaluates the health status of the wearers and provides health maintenance and improvement of feedbacks.

6.1.2 Intelligent tattoos and patches

This chapter focuses on the intelligent tattoos and patches, a subclass of WBSs that attaches to the epidermis and is able to sense the physiological traits on or under the skin physically, chemically, or electrochemically. Sweat and other biosamples on skin are enriched in physiologically significant ions and other excreted metabolites. These

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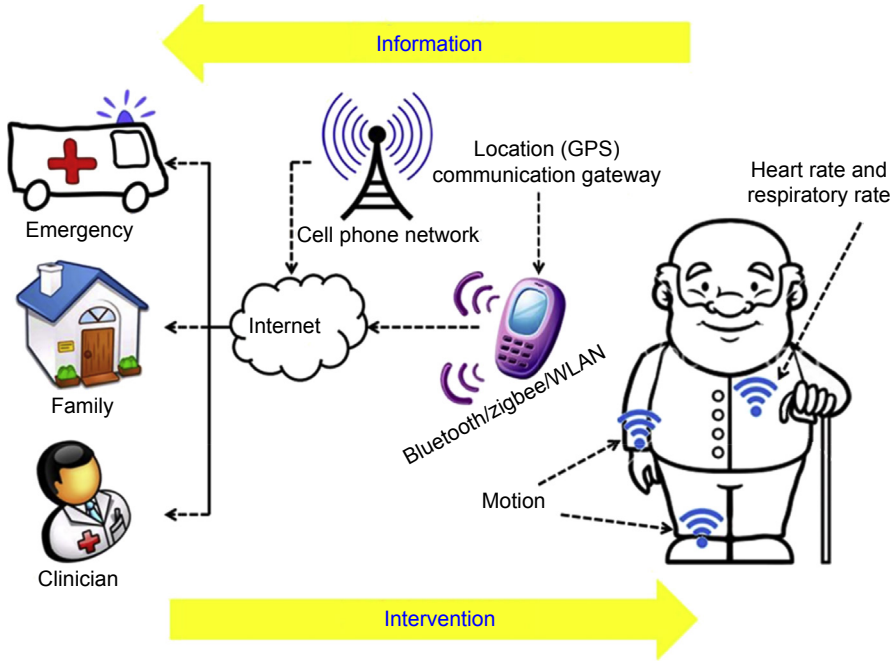

Figure 6.1 A typical telemedical network with the incorporation of wearable biosensors.

biomarkers will reflect complementary information about the health status by wearable devices other than regular physical biosensors. Electrochemical biosensors can be integrated onto flexible textile materials via multiple fabrication methods. Combined with screen-printing technologies the integration of flexible electrodes with textile-based materials gives high comfort to wearers without compromises in functionalities. Alternatively, skin-compatible and screen-printable electrodes are patterned on epidermis to directly sense the local environment of the on-body surface. Although the idea of intelligent patches and tattoos is newly born and still largely limited to laboratory demonstrations, innovations in fabrication techniques and electrodes have sprouted in recent years.

To get an insight into the technical details involved in wearable tattoos, patches, and other biosensors, in the remainder of this chapter we will first briefly address the mechanisms underlying various detection methods. Following that will be a discussion about the fabrication methods available so far, from the most basic to the highly integrated. The applications of this subclass of WBSs in general health, disease control, and sports are presented. We will end with concluding remarks and future perspectives.

6.2 Detection mechanism

6.2.1 Electrochemical

The dominant detection mechanism for analytes in biofluid sample is the electrochemical assessment. Either the amperometry or the potentiometry method has been widely

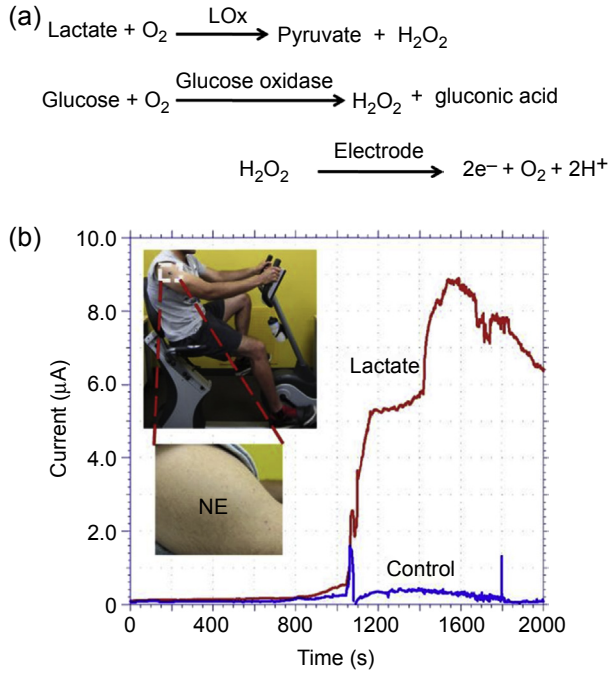

Figure 6.2 Wearable biosensors for epidermal glucose and lactate assessments. (a) The electrochemical reactions involved in glucose and lactate measurements. (b) Real-time noninvasive lactate sensing in human perspiration during exercise events using a flexible printed temporary-transfer tattoo electrochemical biosensor that conforms to the wearer's skin *(Adapted with permission from Jia, W., Bandodkar, A.J., Valdes-Ramirez, G., Windmiller, J.R., Yang, Z., Ramirez, J., Chan, G., Wang, J., 2013. Electrochemical tattoo biosensors for real-time noninvasive lactate monitoring in human perspiration. Anal. Chem. 85, 6553–6560; Copyright (2013) American Chemical Society.)*.

adopted for wearable biosensor development for sweat, saliva, and tears (Bandodkar and Wang, 2014). Theoretically, every agent in body excretion could be measured electrochemically. In reality, to achieve high signals, the most common electrochemical method used in both research and commercialized products is to record amperometry profiles of analytes with the presence of corresponding catalytic bioenzymes coupled to electrodes. For instance, the diabetic marker glucose and physical exercise indicator lactate can be successfully monitored with their electron transfer reaction catalyzed by glucose oxidase and lactate oxidase (Fig. 6.2(a)). Fig. 6.2(b) presents a noninvasive lactate-sensing platform for human epidermis during exercise (Jia et al., 2013).

6.2.2 Colorimetric

Colorimetric sensors are designed based on the color-changing property of the color indicator molecules in response to chemical or physical reactions. The readout could be as simple as visualization by the wearer or through the usage of portable readers. The

best well-known colorimetric molecular indicators are pH dyes. Their switch between protonated or the unprotonated state gives various colors that can be employed as single or pattern measurement for biofluid samples. A microfluidic sensor for colorimetric pH measurement is described in Section 6.3.3.

6.2.3 Optical

One of the most powerful detection approaches to noninvasively quantify the analytes or physiological events under the skin is optical devices. Optical devices are based on the measurement of absorbance, the reflectance of visible lights when encountering the interface of analytes. Photoplethysmography sensors use a light-based technology to sense the rate of blood flow as controlled by the heart's pumping action, which is a direct reflection of the heart rate. A light-emitting diode (LED) irradiates through the skin. A photodiode in the sensor receives the light reflected by the skin and underskin tissues and converts the light to an electrical signal. The reflection by the relatively solid underskin tissues is relatively steady, whereas the light reflected from the artery is a function of blood flow along with time. The signal from the cyclic blood flow becomes evident after the subtraction of the portion from other components.

6.3 Fabrications

6.3.1 Overview

The ability to detect/measure certain analytes presented in a biosample on the skin surface in real time presents a major challenge to WBS developers. Nevertheless, intelligent patches or tattoos, as a newly introduced idea, has been undergoing extensive laboratory practice currently for its advantages over the conventional WBS by providing valuable physiological traits in skin excretion. The fabrication of WBSs in the forms of patches or tattoos is a systematic process from the preparation of sensor substrates to integrations of all the necessary components (electrodes, microfluidic channels, power supplies, electronic module, wireless transmitter, etc.). Although through the practices in conventional WBSs, techniques for most modular design and production have been readily developed, some improvement or modification needs to be performed to facilitate the direct epidermal contacting nature of patches and tattoos.

6.3.2 Screen-printed electrodes

Screen-printed electrochemical sensors have been widely adopted by glucose monitoring (Wang, 2008), medical diagnostics (Carpini et al., 2004; Centi et al., 2010), environmental monitoring (Wang and Tian, 1993b; Zaouak et al., 2010), and analytical applications (Wang and Tian, 1993a) for over 30 years. As the core component in these sensors, screen-printed electrodes have evolved to a highly conserved multiple layer design (Fig. 6.3(a)). Printing the conductive ink directly on top of textile or other

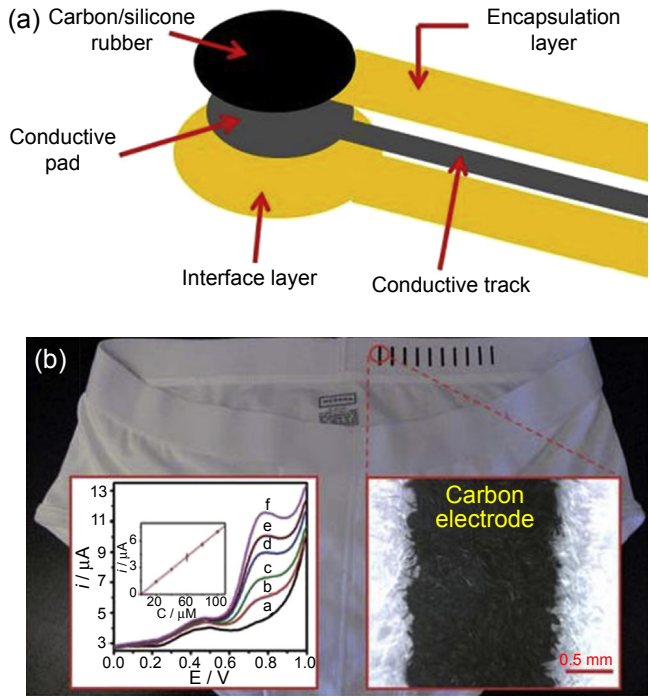

Figure 6.3 Screen-printed electrodes for textile incorporation. (a) Multiple layer construction of screen-printed electrodes. (b) A prototype of amperometric sensors with carbon-based ink printed on the elastic waist of underwear *(Yang, Y.L., Chuang, M.C., Lou, S.L., Wang, J., 2010. Thick-film textile-based amperometric sensors and biosensors. Analyst 135, 1230–1234)*.

solid substrate usually leads to reduced conductivity due to the surface permeability and roughness. As a result, smoothening the substrate surface by an interface layer between the substrate and conductive layer is necessary. The most frequently used interface paste is polyurethane which could be cured by exposure to therm or UV postprinting. A diversity of conductive ink is available on the market. Silver ink is printed as conductive track, whereas the working electrodes are mostly printed using graphite inks. Other materials such gold or platinum are also used for the conductive layer. Individual ink has its unique electron transfer properties and is utilized for different analytical purposes. Blend with additives is used to improve adherence and dispensability. Notably, despite the disadvantage in price, gold is widely used in screen-printed electrodes because of the high strength of the gold–thiol bond for surface modification with biomolecules. On top of the conductive track is the encapsulation layer to prevent any direct contact with skin and provide mechanical strength for the thick and narrow conductive track. The conductive pad interacts with the biofluid directly or through an absorbance pad for concentration. In some case in which the electrical transduction between the electrode and epidermis is required, an extra carbon-loaded silicone rubber paste is dispensed on top of the conductive layer to improve the electrical connection.

Besides the typical screen-printed electrode preparation, efforts to print conductive inks directly onto textiles have also been made. A prototype of amperometric sensors with carbon-based ink printed on the elastic waist of underwear was described by Yang and Wang (Fig. 6.3(b)) (Yang et al., 2010). Mechanical stress studies, relevant to the wearer's daily activity, have indicated that textile-based printed sensors survive large deformations. Both bending and stretching of the textile substrate have a minimal detrimental effect on electrochemical properties. However, in another study, various inks begin to degrade and display lower conductivity after 25 wash cycles indicating destructive impact of repeated soaking on textile-based screen-printed inks (Karaguzel et al., 2009). Notably, both mechanical stress and repeating soaking-caused decay of electrochemical property are heavily dependent on the combination of ink, textile, and printing protocol. Thus optimizations of textile–ink combinations and their corresponding printing protocol are in demand.

6.3.3 Microfluidics

Biofluids on epidermis are intrinsically small in volume and slow in excretion, which requires the biosensor being capable of processing and analyzing reduced sample size. Because of this, microfluidics is becoming a common fixture in many biofluid-directed biosensors. At the same time, microfluidics presents its advantage over traditional liquid-handling techniques in minimizing the scale of assembled WBSs.

The typical fabrication of microfluidics involves a method called photolithography by using either positive or negative photoresist (demonstrated in Fig. 6.4(a)). SU-8 is a commonly used epoxy-based negative photoresist. Negative refers to a photoresist whereby the parts exposed to UV become consolidated, whereas the remainder of the film remains soluble and can be washed away during development. The multiple-layer/step fabrication starts with a UV-exposed SU-8 starter layer on silicon wafer. The next layer is applied on top of the previous one and exposed to masked UV activation. After repeats of multiple layers of SU-8 and UV exposures, the mast mold with unique geometry of the microfluidic channels (reverse phase) is achieved by removing the noncross-linked and soluble parts of SU-8 layers. Large-scale replication of the microfluidics can now be produced by casting mixture of silicon-based polymer polydimethylsiloxane (PDMS) and curing agent against the mast mold. After curing at corresponding conditions to the agent used, the PDMS mold can be released from the mast mold. The microfluidic channels can be covered by a silicon layer, aligned to another PDMS mold, or embedded beneath other modules for a complete sensor. In a report by a Japanese group (He et al., 2010), spatiotemporally focused femtosecond laser pulses are used to produce three-dimensional (3-D) microfluidic channels with a circular cross section in fused silica (Fig. 6.4(b)). After the laser irradiation, the sample is subjected to a 150-min etching in a solution of 10% HF diluted with water in an ultrasonic bath. The microchannels are formed after all the areas modified by the femtosecond laser are completely removed.

The microfluidic device can be a WBS on its own without any complexity of electronic, radio, or power module. The work by Benito-Lopez and Diamond in Ireland

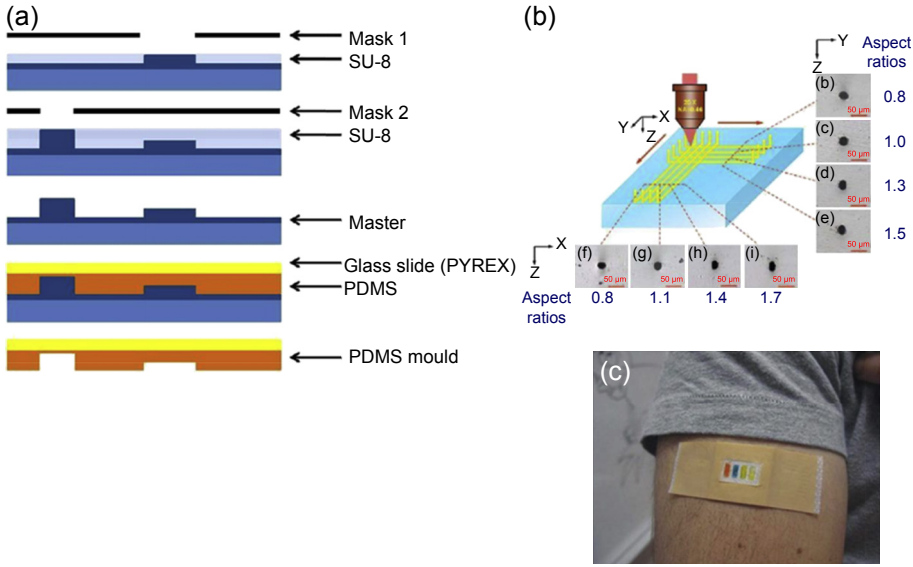

Figure 6.4 Microfluidics in wearable biosensors. (a) The photolithography method to produce microfluidic channels by using negative photoresist SU-8. (b) Fused silica embedded microfluidic channels fabricated by spatiotemporally focusing the femtosecond laser beam *(He, F., Xu, H., Cheng, Y., Ni, J., Xiong, H., Xu, Z., Sugioka, K., Midorikawa, K., 2010. Fabrication of microfluidic channels with a circular cross section using spatiotemporally focused femtosecond laser pulses. Opt. Lett. 35, 1106–1108).* (c) A powerless epidermal pH sensor based on microfluidics *(Benito-Lopez, F., Coyle, S., Byrne, R., O'toole, C., Barry, C., Diamond, D., 2010. Simple Barcode System Based on Ionogels for Real Time pH-Sweat Monitoring, pp. 291–296).*

presents a WBS capable of measuring sweat pH in a real-time manner (Fig. 6.4(c)). This on-body sensor consumes no power and does not require any electronics for signaling acquisition or communication. pH indicator dyes are immobilized within an ionogel polymer matrix and demonstrate different patterns of color in response to sweat at the microfluidic channels (Benito-Lopez et al., 2010).

6.3.4 Fabrication of conductive textile

Another proposal for the integration of conductive wires with textile is direct incorporation of metal wires into fabric yarns instead of screen-printing technique. Researchers at Swiss Federal Institute of Technology (ETH) Zurich developed a hybrid fabric named PETEX (Fig. 6.5(a)). It consists of woven polyester monofilament yarn (PET) with diameter 42 μm and copper alloy wires with diameter 50 ± 8 μM (AWG46, Acheson Industries Ltd.). Each copper wire is coated with a polyurethane varnish as electrical insulation. The copper wire grid in the textile features a spacing of 570 μm (mesh count in warp and in weft is 17.5 cm^{-1}). PETEX features a thickness of 90 μm, a mesh opening of 95 ± 10 μm, an opening area of 44%, and a weight of 74 g/m^2. The

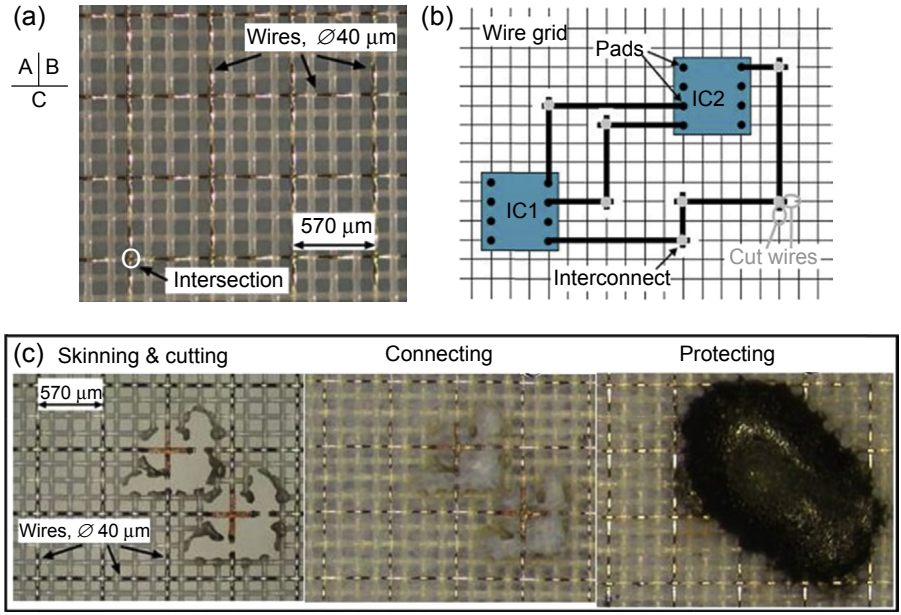

Figure 6.5 Incorporation of conductive wires into textiles for wearable biosensors (http://dx.doi.org/10.3929/ethz-a-005135763). (a) PETEX hybrid fabric. (b) Electrical circuits based on PETEX fabric. (c) The establishment of connection between crossing wires involves three steps, coating removal and wire cut, applying of conductive adhesive, and epoxy resin deposition.
Locher, I., 2006. Technologies for System-on-textile Integration (Dissertation).

fabric is capable of preserving textile properties and copper wire density (Locher, 2006; http://dx.doi.org/10.3929/ethz-a-005135763). Fig. 6.5(b) shows textile embedded electrical circuits based on PETEX fabric. One of the most important techniques for the establishment of electrical circuits is the connection of insulated crossing wires. It starts with coating removal at the defined intersections by laser ablation. Cutting of certain wires with high-fluence laser light needs to be carried out to avoid short circuits with the rest of the routing. After that, a drop of conductive adhesive is dispensed on the ablated spot to connect the two crossing wires. To prevent any electrical contacts with skin, epoxy resin protection is deposited as encapsulation (Fig. 6.5(c)) (Locher, 2006; http://dx.doi.org/10.3929/ethz-a-005135763).

6.3.5 *Stamp transfer electrodes for nonplanar and oversized surfaces*

The Wang group at the department of nanoengineering of University of California San Diego has developed a novel stamp-transfer method for the fabrication of printed electrochemical sensors to a wide variety of rigid and flexible substrates (Windmiller et al., 2012). For the implementation of this technique, a series of elastomeric stamps casted with custom-designed surface reliefs containing the electrode

Figure 6.6 Stamp-transfer electrodes for nonplanar surface. (a) Fabrication of the stamp-transfer electrodes. (b) Examples of surfaces that adopt stamp-transfer electrodes. Windmiller, J.R., Bandodkar, A.J., Parkhomovsky, S., Wang, J., 2012. Stamp transfer electrodes for electrochemical sensing on non-planar and oversized surfaces. Analyst 137, 1570–1575.

pattern were produced. Stamp pads were saturated with diluted silver-based carbon and insulator inks. Stamps loaded with corresponding inks were sequentially pressed on the substrate surface to produce a complete electrode pattern as described in Fig. 6.6(a). The group has successfully demonstrated the preparation of stamp-transferred electrodes onto large structures, and 3-D objects, such as gloves, paper cup, balls, and leaves (Fig. 6.6(b)).

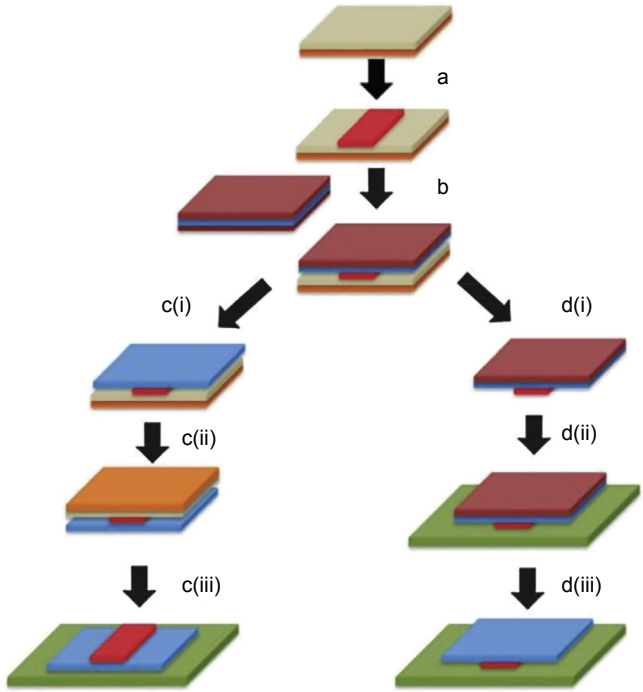

Figure 6.7 Printable temporary-transfer tattoos. Windmiller J.R., Bandodkar, A.J., Valdés-Ramírez G., Parkhomovsky S., Martinez A.G., Wang J. 2012. Electrochemical sensing based on printable temporary transfer tattoos, Chem. Commun. 48, 6794-6796.

6.3.6 Epidermal tattoo/patches as wearable biosensors

6.3.6.1 Printable temporary transfer tattoos

The Wang group also practiced a temporary transfer on-skin tattoo method for the realization of noninvasive epidermal WBS. The fabrication of these tattoos is quite straightforward and described as follows (Fig. 6.7) (Tellkamp et al., 1988). On the release agent-coated (olive) base paper (orange) is screen printed with carbon fiber containing inks to form the electrode pattern. An adhesive sheet (blue) with protective coating (maroon) is applied on top of the printed electrodes. For external environment-targeted biosensors, the protective sheet is removed. The rest of the complex is flipped and applied to the skin. With the release agent-coated base being removed, the electrodes become exposed and ready to sense the analytes in the external environment. For the measurement requiring direct contacts with skin, the release agent-coated base is removed first. The sensor pattern is applied to the skin followed by protective-sheet removal. The presence of carbon fiber within the inks was shown to enhance tolerance against severe mechanical deformation during epidermal wear. Following this protocol, various designs of electrodes can be implemented.

6.3.6.2 *Toward stretch resistant, skin friendly, and multiple functional intelligent tattoos/patches*

The work led by the Rogers group at University of Illinois at Urbana–Champaign (UIUC) shows us the potential to integrate multiple functional sensors into one piece of epidermal tattoos or patch that is both skin friendly and compatible with mechanical stretches (Kim et al., 2011). Fig. 6.8(a) shows a complex sensor system comprising

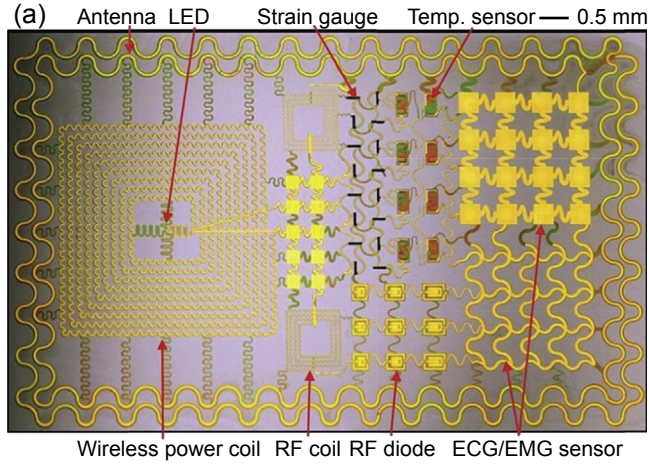

Figure 6.8 Multifunctional electronics-integrated epidermal patches/tattoos. (a) A demonstration platform that attaches to the epidermis through van der Waals forces alone for multifunctional electronics with physical properties, including antenna, LED, temperature sensor, wireless power coil, etc. (b) The WBS can be concealed under a commercial temporary-transfer tattoo as an alternative support to polyester/PVA.
Kim, D.H., Lu, N., Ma, R., Kim, Y.S., Kim, R.H., Wang, S., Wu, J., Won, S.M., Tao, H., Islam, A., Yu, K.J., Kim, T.I., Chowdhury, R., Ying, M., Xu, L., Li, M., Chung, H.J., Keum, H., Mccormick, M., Liu, P., Zhang, Y.W., Omenetto, F.G., Huang, Y., Coleman, T., Rogers, J.A., 2011. Epidermal electronics. Science 333, 838–843.

multifunctional sensors (such as temperature, strain, and electrophysiological), microscale LEDs, wireless power coils, devices for radio frequency communications, and other circuit elements assembled on the surface of a thin (~30 mm), gas-permeable elastomeric sheet based on a modified polyester [Baden Aniline and Soda Factory (BASF), Ludwigshafen, Germany] with low Youngs modulus (~60 kPa). This polymer sheet further lays on top of a polyvinyl alcohol (PVA) film that is water soluble. The PVA film provides temporary supports during manual application of the whole sensor on the skin and can be water removed. Interestingly, the polyester or PVA layer can be replaced by any commercial temporary transfer tattoos. And Fig. 6.8(b) is an example of the sensor layout being concealed under a market-available tattoo. This system is capable of measuring electrical activity produced by the heart, brain, and skeletal muscles. The collected data contain sufficient information for an unusual type of computer-game controller.

Notably, the above sensor system is able to survive a considerable extent of bending and stretch due to its open-mesh structures and noncoplanar interconnects. Further investigation was carried out to employ microfluidics for more profound mechanical wear resistance (Xu et al., 2014). Silicone elastomer (Ecoflex, Smooth-On, Easton, Pennsylvania)-constituted substrate and superstrate are casted with designed layout (Fig. 6.9(a) as an example) for a matrix of microfluidics that encloses constituent devices and associated interconnect network. Each component is immobilized to the substrate support through small cylindrical posts. The inner space between substrate and superstrate is filled with fluid of high molecular-weight silicone oligomer (Sylgard 184, without curing agent) via microinjection. The elements other than the posts are suspended in the high-density fluid. As a result, the device is mechanically isolated and is able to survive extensive mechanical wear, such as stretch and twist, as demonstrated in Fig. 6.9(b) and (c).

6.4 Application

6.4.1 General medicine

Biofluids on excreted on epidermis contain a comprehensive library of agents the relative quantity for which correlates with the health condition of the human body. Noninvasive WBSs are being developed for the measurement of those epidermis residual analytes. Electrolytes, such sodium, chloride, calcium, etc., in the sweat are good indications of electrolyte imbalance, physical stress, osteoporosis, and bone mineral loss (Bergeron, 2003; Wood, 1991; Heaney, 1992; Klesges et al., 1996). Ethanol is present in sweat after an alcoholic drink and can be measured by wearable biosensors (Gamella et al., 2014). This sweat-based WBS is able to detect ethanol just 5 min after ingestion of the alcoholic drink with higher sensitivity compared to the conventional breathalyzers. Other than the blood, sweat is an alternative biological matrix useful to detect drugs of abuse intake. It is produced by eccrine and apocrine glands originating in the skin dermis and terminating in secretory canals that flow into the skin surface and hair follicles. Wearable patches are being developed to monitor/measure the intake of cocaine metabolites, opiates, cannabis, and amphetamines in both direct and indirect ways (De Giovanni and Fucci, 2013).

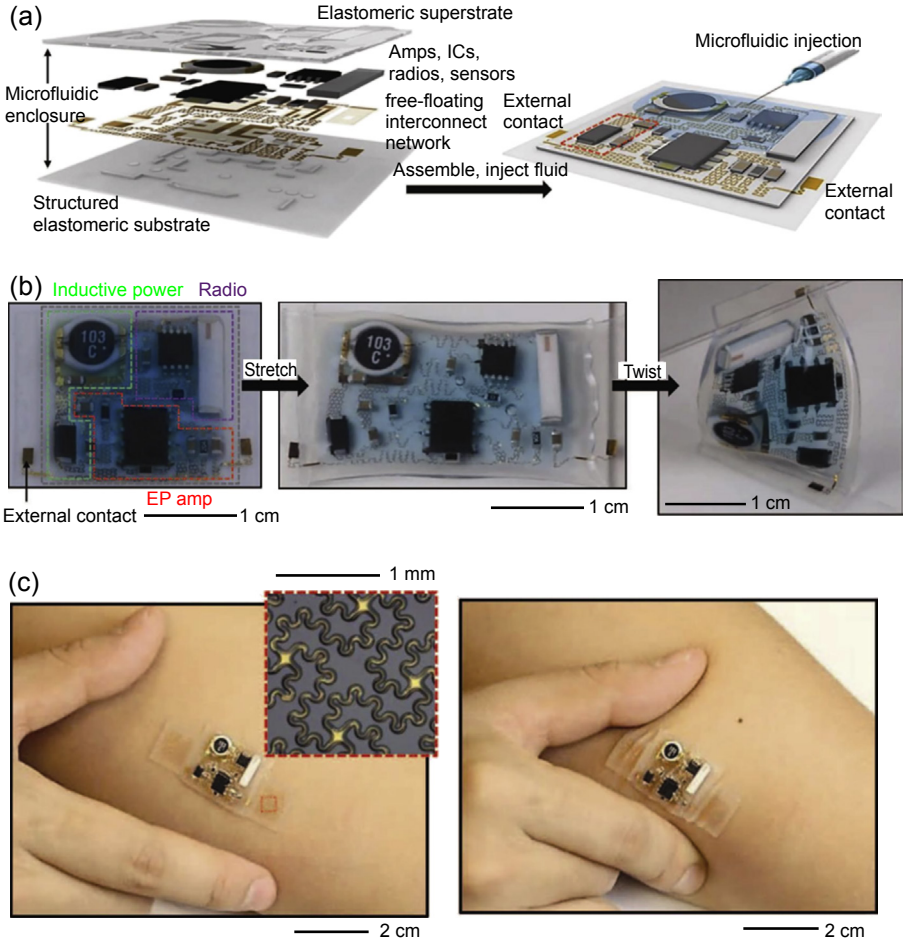

Figure 6.9 A soft, stretchable electronic system that integrates strain-isolated device components and a free-floating interconnect network in a thin elastomeric microfluidic enclosure. (a) Exploded-view schematic illustration of the key components (left). Mechanical strain isolation is a function of the microfluidic system after enclosure and microinjection (right). (b) A demonstration system undergoing extensive stretch and twist. (c) A forearm-attached device in undeformed state (left) and undergoing compression and twist (right). Xu, S., Zhang, Y., Jia, L., Mathewson, K.E., Jang, K.I., Kim, J., Fu, H., Huang, X., Chava, P., Wang, R., Bhole, S., Wang, L., Na, Y.J., Guan, Y., Flavin, M., Han, Z., Huang, Y., Rogers, J.A., 2014. Soft microfluidic assemblies of sensors, circuits, and radios for the skin. Science 344, 70–74.

Another class of WBSs measures the physical vital traits such as heart rate, respiration, body temperature, blood pressure, and so on. With the advances in fabrication techniques, these WBSs are highly capable of continuous and real-time measurement and monitoring. Integrated with telemedical systems, these devices allow the health care providers to track the health status and provide health advice to their patients. Fast response as emergency service could be triggered for any life-threatening changes in patient's vital traits.

Posttherapeutic treatment followups on the recovery effectiveness are extremely critical for surgery and diseases like cancer and cardiovascular disorders. For patients receiving surgery for myocardial infarction, heart rate is a critical indicator for heart functions. Physicians can rely on the continuous monitoring to evaluate the recovery of patients. Sometimes, bad recovery is due to the patients not strictly following treatment/recovery plans. Researchers are developing WBSs that can monitor the monitoring adherence to therapies. As an example, a pair of wearable and ingestible sensors is developed to monitor the intake of medicine (Belknap et al., 2013). This system electronically confirms unique ingestions and record the date/time of the ingestion without involvement of any objective report from patients.

The most successfully commercialized WBS is the so-called activity tracker. The device available on the market comes with different forms as watches, rings, wrist bands, phones, or other wearable accessories. The core component of activity trackers is the triple axis accelerometry that records the movement of a body-attached device in forward–backward, left–right, and upward–downward axes. Algorithms are developed to analyze the signals and convert to physical steps, distance, and energy consumptions. Wearers can simply refer to the results from the activity tracker to adjust for their exercise plans. The popularization of activity trackers is believed to help the wearer to maintain a healthy lifestyle.

6.4.2 Sports medicine

The application of wearable biosensors in sports is extremely valuable in several aspects. First, WBSs with small size easily attach to the athletes are able to measure the physiological traits that are closely relevant to sport performance. These data provide information about the physiological status, and eventually performance after analysis, of the athletes throughout the activity and record the completion of exercises. Second, for sports that require high body-movement controls like high and long jumps, wearable motion sensors are used to correct nonstandard postures and movements and thus improve the athletic performance. Third, WBSs can be developed for X-sports to monitor the parameters in external environment, such as temperature, pressure, humidity, altitude, and so on. As an example, a WBS integrated into swim suits is being developed to determine the presence of environmental pollutants and security threats in the marine environment.

6.4.3 Diseases

6.4.3.1 Cystic fibrosis

Cystic fibrosis is a disease caused by genetic variation in the gene encoding the cystic fibrosis conductance regulator protein that mainly affects the lungs and digestive system. The whole world has an estimated affected population of 70,000. Over the last 50 years, the sweat test has been the "gold standard" for diagnosing cystic fibrosis (CF). One remark of CF patients is abnormal sodium and chloride excretion in the sweat. Sweat-based intelligent patches are under development by several groups around the world (Mu et al., 2015).

6.4.3.2 Diabetes

The number of Americans with diabetes has continued to rise during the last 20 years. According to the reports from the US Centers for Disease Control and Prevention, over 12% of the US adult population is estimated to have the disease. One critical component of therapeutics for diabetes is the monitor of blood glucose levels by patients. Most POC testing devices available on the market for glucose are noncontinuous and invasive. However, the blood glucose level fluctuates and end-point measurement may not accurately represent the effective level in certain time windows. As a result, the development of frequent or even continuous monitoring of blood glucose would be especially beneficial for patients who need to take insulin injection in accordance with the test value. Several wearable continuous glucose monitoring biosensors have been commercialized, including the GlucoDays by Menarini Diagnostics Co., Dexcom G4 by Dexcom Co., etc. The ultimate goal is to engineer a device that is wearable and capable of continuous glucose measuring and automated insulin injection. The miniMed 530G with Enlite is a system that combines both functions and constitutes an artificial pancreas.

6.4.3.3 Cardiovascular diseases

Cardiovascular diseases have become one of the leading causes of death worldwide. According to WHO reports, by 2030 more than 23 million people will die annually from cardiovascular diseases (http://www.who.int/cardiovascular_diseases/en/). Modern wearable sensors for patients with cardiovascular diseases are able to measure physiological parameters like ECG, heart rate, blood pressure in a real-time manner.

6.4.3.4 Cancer

Cancer is a leading cause of death worldwide and responsible for 8.2 million deaths in 2012 according to WHO reports (http://www.who.int/cancer/en/). Although the field of WBS development for cancer is almost blank, biofluids excreted on epidermis contain multiple biomarkers for cancers (Malon et al., 2014). Interleukin-8 (IL-8) (St John et al., 2004; Tan et al., 2008; Wong, 2006), tumor necrosis factor alpha, and salivary transferrin (Jou et al., 2010) in saliva have been reported to correlate with oral cancer occurrence. Salivary zinc finger protein 510 peptide (Jou et al., 2011) was identified as oral squamous cell carcinoma. On the other hand, salivary soluble CD44 Ag can be used as a biomarker for head and neck squamous cell carcinoma (Franzmann et al., 2007). It has been shown that salivary vascular endothelial growth factor (VEGF), endothelial growth factor (EGF), and carcinoembryonic Ag elevate with breast cancer (Brooks et al., 2008). Predictably, immunoreaction-based WBSs could be developed by sensing those biomarkers in saliva.

6.4.3.5 Parkinson's disease

Parkinson's disease is a neurodegenerative disorder with symptoms majorly in motor disorder/fluctuation. The typical motor fluctuations include hand tremor, muscular rigidity, and bradykinesia. Extensive effort has been made to employ motion sensors

(represented by triple-axis accelerometer) for continuous measurement and record of motion patterns of patients. Successful development of these devices helps patients to collect data objectively at home, as traveling is not suitable for their conditions, and improves the accuracy of diagnosis. Most importantly, the objective record of motion pattern over the whole period of motor fluctuation (usually lasts for several hours), when utilized for postmedication monitoring, facilitates the progress in medicine titration.

6.5 Conclusions and perspectives

Wearable biosensors for fitness and lifestyle (inertial or motion sensor) has significantly changed the way wearers seek for general physiological health. With the properties of ease of use and highly fashionable integration, this class of WBSs spreads worldwide. However, despite the highly conserved measuring technique/module, a marked drawback of the current motion devices is inconsistent results output from different manufacturers and for different wearers. Improvement of measurement accuracy by optimizing the algorithm for differentiated motion types (walk, climb, cycling, swim, etc.) is in demand.

Integrated within wearable devices, biopotential sensors, and optical sensors are most efficient in carrying out the measurement of physiological events under the skin. Both types of sensors are adopted for heart rate monitoring for cardiovascular disease and general health purposes requiring low energy consumption and providing high accuracy. Another potential usage of the biopotential sensor is mental stress monitoring and control.

Electrochemical measurement of analytes conveys extra information about the physiological traits that exceed the limits of physical sensors. The applications in therapeutics, represented by the wearable glucose metering system, are capable of continuous monitoring and automated treatments. Researchers and engineers are exploring the feasibility of epidermal tattoos and patches for the detection and measurement of analytes in sweat and saliva. Extensive effort has been made for the integration of wearable electrodes, microfluidics technologies, and new textiles into wearable tattoos and patches. However, there are still multiple aspects, such as sensor stability against mechanical wear, power requirements, and incorporation of wireless modules, that need to be addressed. As directly contacting the skin, tests of the biocompatibility and comfort are essential and their evaluation needs standardization.

Acknowledgment

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Wireless biosensors for POC medical applications

7

M.S. Arefin, J.-M. Redouté, M.R. Yuce
Monash University, Melbourne, VIC, Australia

7.1 Introduction

Point of care (POC) biosensor systems which do not rely on the use of laboratory staff or facilities have the potential to improve patient health care [1]. POC system contains different types of wearable or implantable biosensors, readout or interface circuits, power management circuits, and wireless telemetry blocks. A typical POC system is depicted in Fig. 7.1. The system contains several biosensors with analog readout circuits, a digital microcontroller, a radio transmitter, and a battery. The sensors convert the physiological parameters to electrical quantities with readout circuits. The digital microcontroller circuit manages and processes all the sensor data. The data are then transmitted using a wireless transmitter to an external device for remote monitoring and recording.

Wireless POC systems provide the means to continuously measure and monitor biological parameters related to diseases, biomolecules, and pathogenic bacteria and viruses. Diseases like diabetes, ischemia, Alzheimer's, and epilepsy require continuous monitoring and real-time drug delivery system or stimulation. There are many biosensors reported for measuring and detecting biological parameters. These biosensors can be grouped into two categories of POC biosensors, namely, implantable biosensors and wearable biosensors.

Implantable biosensors are continuously becoming more popular and economically viable. In the past, the main contribution in this field was to restore malfunctioning or missing biological structures by artificial prosthetic devices such as an artificial heart and pacemaker for cardiac patients, a neurostimulator for patients with epilepsy and Alzheimer's, stents for damaged arteries, and knee implants for rheumatoid arthritis, osteoarthritis, or traumatic injuries. These biosensors offer improved quality of life

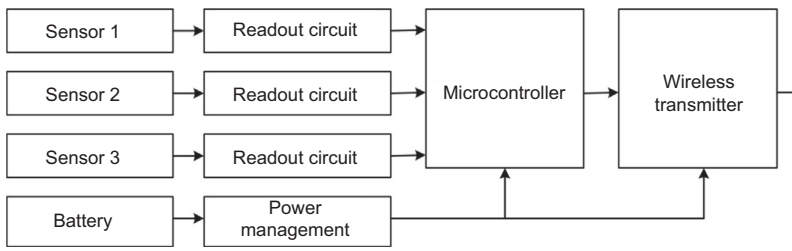

Figure 7.1 A wireless point of care system with integrated biosensors and readout circuits.

Medical Biosensors for Point of Care (POC) Applications. http://dx.doi.org/10.1016/B978-0-08-100072-4.00007-1

for patients. As an extension to this field, miniaturized implantable biosensors are also directed toward the diagnosis of diseases with higher measurement sensitivity and selectivity of physiological parameters [2]. These biosensors provide continuous diagnostics for patients with chronic diseases. The integration of wireless capability with these sensors enables health condition monitoring in real-time as well as real-time drug delivery systems. The continuous glucose-monitoring system, developed by Medtronic, can measure glucose level more than 200 times a day, and the insulin delivery system provides an alternative to multiple daily injections [3,4]. The bladder pill is developed to monitor and log the internal bladder pressure: it is inserted into the bladder cavity through a minimally invasive procedure [5]. The pressure sensor and readout circuit are packaged in a flexible pill-shaped form, having a length of 40 mm and diameter of 5 mm. The continuous measurement of the physiological parameters in the gastrointestinal (GI) tract is often necessary for detecting and monitoring abnormalities [6]. Traditionally, the diagnosis of the GI abnormalities is performed by inserting a tube containing sensors through the mouth [6]. This time-consuming and uncomfortable method for patients has motivated researchers to develop wireless capsules by integrating miniature sensors including pH, pressure, and temperature onto a single platform. The integration of biosensors and integrated circuit (IC) technology with a wireless link forms the basis for future multisensor microsystems and leads to real-time and continuous monitoring of the GI tract.

Extensive research has been performed in the field of wearable biosensors for continuously monitoring health, activity, mobility, and biopotentials. The major monitoring of vital signs are the electrocardiogram (ECG) for measuring cardiac electrical activity and heart sounds, the electromyogram (EMG) for measuring muscular electrical activity, the electroencephalogram (EEG) for measuring the electrical activity of the brain, respiration rate, mobility for limb movement, skin temperature, electrical conductivity of skin, and blood glucose [7]. The biosensors for monitoring these vital signs are embedded in the user's outfit. For example, a wrist-worn device has been developed to monitor ECG, blood pressure, blood oxygen saturation, acceleration, and temperature by combining all sensors, signal processing, and cellular communication [8]. The communication protocol has been designed to support cellular message service to transmit a small number of values, a virtual circuit switched communication channel for long ECG data, and an internet-based channel to reduce the number of direct lines needed at the telemedicine center. A portable physiological signal recorder is also designed and implemented to measure ECG, bioimpedance, and activity using Ag/AgCl gel-paste electrodes and three-axis acceleration sensors [7,9]. A textile-based wearable system has also been developed to monitor ECG, photoplethysmography (PPG), heart rate, blood pressure, body temperature, and skin conductance [10]. The data are sampled and digitized at 250 samples/s and transmitted wirelessly using 2.4 GHz industrial, scientific, and medical (ISM) band to a remote monitoring station [7,10].

Wireless biosensors for implantable or wearable sensors will be discussed in this chapter. The biosensors incorporating electrical measurements such as electrochemical transduction mechanism [potentiometric, amperometric, and field-effect transistor (FET)] and electrical transduction mechanism (resistance, capacitance, and inductance)

will be described in the second section. The interface circuit techniques to convert these electrical properties of the biosensor into voltage, frequency, or phase will also be reviewed briefly. A design example including a capacitive pH-sensor device that detects pH variations will be studied in this section. In the third section, wireless telemetry systems will be described. The applications of the wireless link for implantable or wearable sensors, such as implanted blood-glucose sensor, wireless capsule, and wireless wearable device will be explored in the fourth section. In the fifth section, we will conclude the chapter with some future trends involving biocompatible coatings, suitable packaging, and flexible IC.

7.2 Electrical measurements using biosensors

7.2.1 Biosensing techniques

Microelectromechanical systems (MEMS)-based biosensors are widely used in various biomedical and lab-on-a-chip (LOC) applications, such as chemical and biochemical sensing [11], protein and DNA biosensor array [12], drug screening [13], disease and pathogens diagnosis [13,14], and implantable devices [15,16]. The biosensors transform the changes in chemical and biological analytes including dissolved ions, pH, gases, body fluids, drugs, microbes, human cells, proteins, and nucleic acids into optical, electrochemical, or electrical equivalent of the changes using biologically sensitive elements. A biosensor system example is depicted in Fig. 7.2. The biosensors are usually composed of a biologically sensitive element or compound and a transducer. The biologically sensitive elements interact with the chemical and biological analytes, and the transducer transforms the interaction with the chemical and biological analytes into a measurable signal (optical, electrochemical, or electrical).

Biosensors are classified into two major categories: label-based and label-free sensing. Label-based sensing requires the labeling of target molecules with fluorescent dyes, chemiluminescence, or radioactive labeling using enzyme-linked immunoabsorbent assay [17–19]. These processes collect and attach the target molecules from heterogeneous samples, and hence the specificity of the biosensor is characterized by the

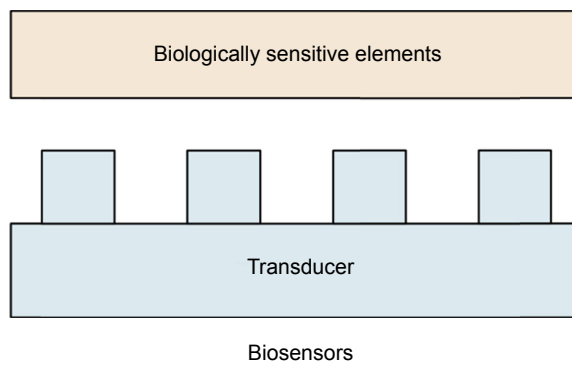

Figure 7.2 A biosensor system consisting of biologically sensitive elements and transducers.

sensing material. These labeling processes may interfere with the binding site, alter surface characteristics, and require a longer time [18]. These limitations may hinder the implementation of label-based sensing for POC applications. On the other hand, label-free sensing is based on the changes of inherent properties of test molecules [18,20]. In this method, the detection is typically performed on the chemical modification of the sensor's surface using the antibody, antigen, and μRNA [18,21,22]. Therefore, the selectivity and sensitivity are dependent on the quality of the surface-modification methods [21]. These methods aid in the development of biosensors for rapid detection with a higher sensitivity and selectivity. Hence, it is practical to implement the POC biosensors using label-free sensing mechanisms.

Apart from the classification based on sensing mechanisms, biosensors are classified into three categories considering the transduction mechanism used. They are optical, electrochemical, or electrical. The optical transduction mechanism includes fluorescence, chemiluminescence, interferometry, and surface plasmon resonance. These techniques involve either the production of light from chemical reactions or the change in refractive index at the interface of biosensing materials.

Electrochemical transduction mechanisms operate according to redox reactions involving the production of electrons or protons that can contribute to current or voltage. This method depends on the Nernstian response of redox reactions at solid–liquid interfaces and can achieve a maximum Nernstian sensitivity. This method is suitable when biochemical reactions between the biomolecules and biologically sensitive elements generate electrical charge. For example, glass electrode-based pH sensors detect pH levels in a sensing half-cell by measuring the voltage difference between the sensing electrode of the sensing half-cell and a reference electrode having a fixed concentration of HCl or a buffered chloride solution. The voltage difference is generated due to the exchange of sodium ions in the reference half-cell for H^+-ions in the sensing half-cell through a glass membrane [23]. There are different types of electrochemical biosensors: potentiometric, amperometric, and FETs. Potentiometric biosensors are based on the potential or voltage difference between the sensing and reference electrodes (Fig. 7.3(a)). This principle is widely used for pH-sensor and gas-sensor applications. Amperometric biosensors rely on the change in current between sensing and reference electrodes (Fig. 7.3(b)). For biosensors using FETs, the solutions are in contact with the gate of the transistor and the redox reaction produces the voltage at the gate that changes the current through the transistor (Fig. 7.3(c)). For the measurement of pH, ion-sensitive FETs (ISFETs) are widely used. An ISFET measures the current through a conduction channel between the source and the drain originating from pH-dependent threshold voltages due to sensing-gate liquid-surface potentials [24–27]. Different sensing materials at the sensing gate region, such as Si_3N_4, Al_2O_3, Pr_2O_3, InN, RuO_2, RuN, and Ta_2O_5 are used to achieve the theoretical upper limit of Nernstian sensitivity of 59.16 mV/pH [25–27]. The techniques utilizing electrochemical transduction are summarized with some design examples in Table 7.1.

The transducers producing electrical equivalents of the changes in passive components are suitable for miniaturized systems, which translate the changes in chemical and biological analytes into resistive, piezoelectric, capacitive, or inductive variations. Such methods are suitable for detecting biomolecules without biochemical reactions

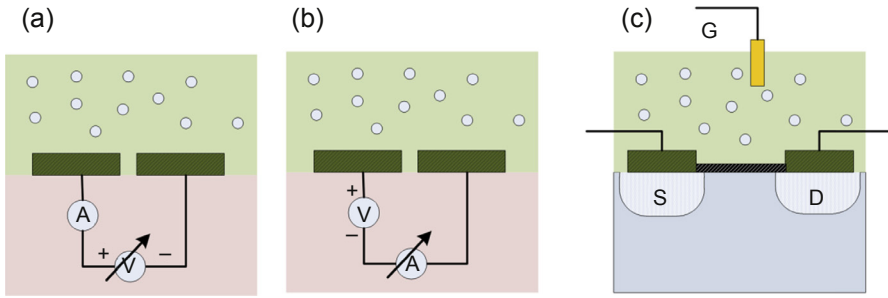

Figure 7.3 Electrochemical transduction techniques: (a) potentiometric technique, (b) amperometric technique, (c) field-effect transistor (FET) technique.

Table 7.1 Examples of electrochemical transduction techniques

Technique	Output	Membrane	Application	References
Potentiometric	Voltage	Alkaline phosphatase	DNA	[28]
	Frequency	N-(2-ferrocene-ethyl) maleimide	DNA	[29]
	Voltage	Si_3N_4 and parylene	Potassium ferricyanide	[30]
	Voltage	Iridium oxide	pH of urine	[31]
Amperometric	Frequency	Polyimide	Protein	[32]
	Current	SU-8	Protein	[12]
	Current	Platinum/nitrogen-doped graphene	Glucose	[33]
	Current	Glucose oxidase	Glucose	[34]
ISFET	Voltage	Indium nitride (InN)	pH	[25]
	Voltage	Si_3N_4	Cell population	[35]
	Current	Tantalum oxide	DNA/Protein	[36]
	Voltage	Si_3N_4	DNA	[37]

when interacting with biologically sensitive elements and hence do not produce electrical charge. For measuring passive electrical components, different interface circuits are required to convert the changes in biological analytes into measurable voltages or currents. The interface circuits (ie, readout circuits) for these sensors are discussed in the next section.

The resistive-type sensors measure strain-related mechanical properties such as strain, force, acceleration, and pressure-utilizing piezoresistive properties of sensitive film resistors [2,14,38,39]. These sensors are also incorporated as biosensors for detecting biomolecules. The electrode configuration is the same as in electrochemical transducers, but it measures the change of resistance when biomolecules interact with the biologically sensitive elements [2]. The piezoresistive microcantilever sensors are also extensively studied for biosensing. The piezoresistive sensors,

which are covered by a biologically sensitive layer, are bent due to strain induced upon binding of biomolecules with a sensitive layer. The bending of piezoresistive sensors produces a change in resistance [14,38]. Piezoelectric sensors are similar to piezoresistive sensors in which the strain-induced mechanical bending or force produces electrical charge.

The capacitive sensors convert the variations of physical, chemical, and biological quantities into capacitive changes utilizing two electrodes or plates separated by a dielectric medium. For simple capacitors composed of two parallel plates of an area A each, separated by a gap G, and filled with dielectric medium with relative permittivity of ε_r, the capacitance C is expressed as $\varepsilon_0\varepsilon_r A/G$. Here, ε_0 is the permittivity of free space (8.854×10^{-12} F/m). Therefore, a change in the capacitance of the sensing device will occur due to the change of dielectric medium, gap, or area. The capacitive biosensors designed to detect the changes of dielectric medium are very common [40]. The electrodes are configured as parallel plates, coplanar electrodes, and interdigitated electrodes (Fig. 7.4). The parallel-plate configuration requires two metal-printed substrates and the dielectric medium is in between the substrates. The coplanar electrode configuration provides simple fabrication steps compared to the parallel-plate configuration as the electrodes are on the same side of a single substrate. The inter-digitated electrodes configuration is achieved by replicating several coplanar electrode configurations. The capacitive biosensors provide low thermal noise and thermal drift levels, low static power dissipation, and low self-heating [41].

The inductive sensors measure the changes of inductance due to the alteration of magnetic property of the medium. The biomolecules are usually not magnetically sensitive, and therefore, the target molecules are labeled and attached by magnetic particles to have the ability to modify the medium under exposure [42]. Some design examples of these electrical passive transduction techniques are given in Table 7.2.

In addition to sensing techniques, it is also necessary to have a range of selective and sensitive detection of analytes for different applications. The selectivity for specific analytes is one of the most critical requirements for biosensors as it yields reliable

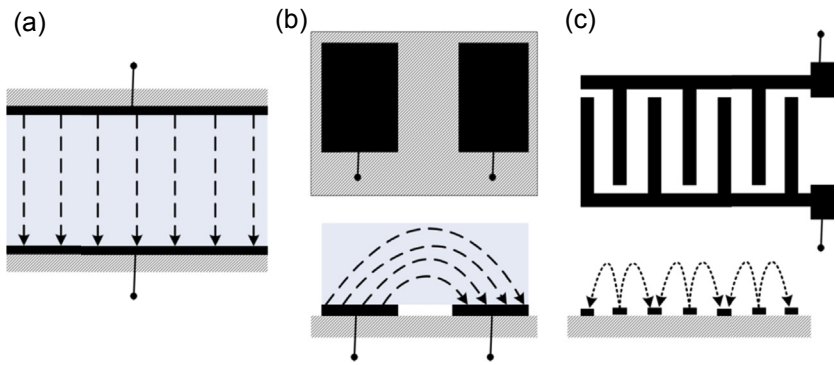

Figure 7.4 The electrode configuration for the capacitive biosensors: (a) parallel plates, (b) coplanar electrodes, (c) interdigitated electrodes.

Table 7.2 The electrical passive transduction techniques

Technique	Sensor output	Circuit output	Configuration	Application	References
Resistive	Resistance	Voltage	Piezoresistive microcantilever	DNA detection	[38]
	Resistance	Frequency	Coplanar electrodes	*E. coli* bacteria	[39]
	Resistance	Voltage	Piezoresistive microcantilever	*Herpes simplex* -1 virus *E. coli* bacteria	[14]
	Resistance	Current	Polymer	Glucose	[43]
Capacitive	Capacitance	Voltage	Interdigitated electrodes	*E. coli* bacteria	[44]
	Capacitance	Digital (8-bit)	Interdigitated electrodes	Dichloro-methane Acetone Methanol Deionized water	[45]
	Capacitance	Voltage	Parallel plates	DNA	[46]
	Capacitance	Frequency	Interdigitated electrodes	pH of gastric acid	[47,48]
Inductive	Inductance	Frequency	Spiral inductor	DNA	[42]

sensing signals in the presence of interfering analytes. The biosensors are developed using biologically sensitive elements such as antibodies and ligands for biological analytes, or metal–organic framework materials for chemical analytes. In addition to the sensing, the sensors are also integrated in systems for immobilizing, analyzing, and identifying chemical and biological analytes.

7.2.2 Readout circuits

Interface circuits for MEMS sensors play an important role for the development of reliable, low-cost, and low-power sensor systems. These signal-conditioning circuits convert the changes of sensor resistance, capacitance, or inductance into equivalent measurable quantities of voltage or current [49,50].

The change in resistance can be measured using a Wheatstone bridge circuit that produces an output voltage proportional to the resistance changes. Another simple way to measure resistance changes as voltage changes is by applying a constant current source through the resistor sensor [14]. The resistance changes of the sensor can also be converted to frequency variations using a voltage-controlled oscillator circuit [51].

There are several readout circuit configurations interfacing with capacitive or inductive sensor systems. The two major circuit configurations are the amplifier-based circuits [49,50,52,53] and modulation-based circuits [15,42,48,51,54–66]. The amplifier-based circuits can be generalized into three categories of alternating current (ac)-bridge [49,53], transimpedance [67], and switched-capacitor (SC) configuration [49,50,52]. In most cases, a single sensing capacitor (C_S) is connected in series with a fixed-reference capacitor (C_R). Capacitor C_R is equal to the rest or initial capacitance of C_S. The C_R and C_S are driven by a differential (180° phase shift) square waves or digital pulses for ac-bridge and switched-capacitor configurations. In transimpedance configurations, an ac-signal (sine wave) is applied. These circuits measure voltage change proportional to the sensor capacitance change ($\Delta C = C_S - C_R$), and the voltage change is amplified using an amplifier circuit. In SC configurations, the amplifier circuit also integrates additional charge from a feedback capacitor at a certain sampling frequency with the sensor capacitance change (ΔC) to minimize the input parasitic capacitance effect. The resolution of SC circuit improves for higher sampling frequencies [49]. The amplified voltage is passed through a low-pass filter to filter out high-frequency components. Various noise sources, such as $1/f$ noise (flicker noise), thermal noise (white noise), substrate–noise coupling (coupled signal from one node to another via the substrate), and parasitic capacitances minimize the sensor readout range and resolution of these circuits [49]. These circuits require lower parasitic capacitance and higher amplitude of input differential signals for the improved range and resolution.

Modulation-based readout circuits have gained popularity for interfacing with capacitive and inductive MEMS sensors to reduce the noise levels of $1/f$ noise and direct current (dc) offset in the circuit. Modulation-based circuits are based on sigma–delta ($\Sigma\Delta$) converter [56,62,65], successive approximation register (SAR) analog-to-digital converter (ADC) [56,66], chopper modulation [54,55,58], pulse-width modulation (PWM) [57,61,63], and frequency modulation (FM) configurations [42,48,51,64,68].

Readout circuits using $\Sigma\Delta$ converters require much faster analog circuits as the sampling rate should much higher than the effective bandwidth, and a digital decimation filter is also needed which adds additional complexity. Moreover, the voltage dependence nonlinearity effect of capacitors results in a lower signal-to-noise ratio (SNR) [60]. Though several nonlinearity compensation techniques have been adopted to adjust the operating point of the CMOS transistors, it is still difficult to maintain the linearity of capacitors with lower supply and bias voltages [60].

An SAR ADC provides a digital output by comparing the analog sensor signal with a sequentially generated analog signal using digital-to-analog (DAC) converter using binary search algorithm [56,66]. This technique consumes lower power and provides high resolution and accuracy.

The chopper modulation approach is an amplitude modulation technique, which uses square wave as a carrier signal. It works by shifting the sensor signals to higher frequencies to suppress the $1/f$ noise. The demodulation of modulated sensor signal is required to recover the original signal [51,58]. Offset and noise compensation should be performed for better performance [51,58].

PWM-based interface circuits exploit semidigital approach in which the capacitance changes are encoded in the time domain and modulated as the period or pulse

width of a digital signal [15,57,61,63]. This technique requires a fast digital counter to convert the time or pulse-width variation into a digital output [59].

The FM configuration that uses oscillator-based reactance sensors detects the frequency shifts introduced by the capacitance or inductance changes of the sensor front-ends [42,48,51,64,68]. These configurations present the advantage of having lower phase and flicker noises at higher frequencies [51,64]. The most common FM circuit for interfacing with capacitive sensors is a relaxation oscillator. A ring oscillator [68,69]-based FM circuit is used in the interface of capacitive sensors, such as a capacitive pressure sensor [68] and a humidity sensor [69]. For the interface of inductive sensors, a Colpitts oscillator topology is adopted to measure nanoscale displacements as a frequency shift in [70]. An integrated complementary cross-coupled oscillator with on-chip LC resonator is investigated in [42,64] for magnetically labeled biosensors and is able to provide highly stable and lower-phase noise output frequency. A low-power FM-demodulation circuit that converts frequency to voltage is also required to measure a voltage quantity. For the implementation of a frequency-to-voltage converter (FVC) block, there are mainly two approaches, including counter-based circuits [68] and integrator-based circuits [71,72]. The counter-based circuits use counters and digital-to-analog converters (DAC) requiring a very high reference-clock frequency compared to the measured signals for high accuracy. The need for a high-frequency clock limits the usage for high-frequency measuring signals [71]. The integrator-based circuits include different approaches such as integrating and holding [72] and switched capacitor [71,72]. Because most of the literature incorporated an FVC block in the design of phase-locked loops (PLLs) or frequency-locked loops (FLLs) as a feedback path for the demodulation of FM signals, it involves higher power requirements. A stand-alone FVC circuit that does not require another oscillator in the feedback loop is also presented to minimize the overall power consumption [73]. The circuit is designed with a negative feedback system, which is based on an integration-based switched-capacitor charge pump circuit. The circuit also provides the control over the sensitivity, dynamic range, and nominal point for the measurement.

7.2.3 Design example of pH sensor and readout circuit

The pH scale measures the acidity or basicity of a substance based on the concentration of hydrogen ions. Due to the pH-dependent nature of several chemical and biological processes, it is frequently necessary to measure the pH for the characteristics of chemical and biological substances. Growing interest in personalized health monitoring has also attracted significant attention to pH measurements in medical science. Local pH measurements in the human body are of significant importance as the changes in pH level in blood, tissues, and other body liquids are an indicator of certain diseases. Therefore, it can provide a timely diagnosis of disease. The pH levels for a healthy tissue lie in the range of 7.2 to 7.6 [74], whereas for the tumor cells, the pH decreases to the acidic side of the neutral level, down to 6.8 [75].

Acidity measurements throughout the digestive system also provide valuable information. The digestive fluid in the stomach, also known as gastric acid, has a usual pH

level of 1 to 2 which is believed to function as a protective mechanism against ingested pathogens [76]. A discrepancy in the pH level of the gastric acid secretion indicates certain disease states [6]. High acidity is generally observed in patients with ulcer, whereas lower level of acid can be an indication of atrophic gastritis and stomach cancer [77]. Conventional methods of measuring the pH of gastric acid are to stimulate gastric secretion by injecting gastric content through a tube placed in the stomach. These methods are time-consuming and uncomfortable [6,78]. Several alternate approaches including endoscopic methods, serum pepsinogens assay, scintigraphic techniques, impedance tomography, alkaline tide, urinary analysis, and breath analysis have been investigated for the measurement of gastric acid [6]. Though these approaches have their own limitations, it is not possible to integrate these approaches with wireless capsules (ie, electronic pills) due to their large-size requirement. Moreover, the wireless capsules can be used as a catheter-free pH detection [79]. They have demonstrated pH measurements with higher sensitivity and resolutions within pH levels of 1.0 to 4.0 [80,81].

Over the years, pH measurement techniques have been evolved from traditional methods, such as pH test strips, potentiometric [31,82–85], and glass electrodes-based [23], to more sophisticated methods including conductometric [86], magnetoelastic [87], optical fiber [88], microcantilever-based systems [89–91], ion-sensitive field-effect transistors (ISFET) [24–27,92,93], and capacitive [47,48]. The glass electrodes-based methods are electrochemical transduction methods that detect pH in a sensing half-cell by measuring the voltage difference between the sensing electrode of the sensing half-cell and a reference electrode having a fixed concentration of HCl or a buffered chloride solution. The voltage difference is generated due to the exchange of sodium ions in the reference half-cell for H^+-ions in the sensing half-cell through a glass membrane [23]. Although the glass electrode method is the standard measuring method for pH due to ideal Nernstian response independent of redox interferences, it shows sluggish response, causes inconsistent results in HF or alkaline solutions, requires conducting sensing materials, and presents unstable responses for miniaturized systems [23]. Potentiometric electrodes can measure the redox reaction potential difference between a reference electrode and pH-sensing metal-oxide electrode, such as PtO_2, IrO_2, TiO_2, SnO_2, Ta_2O_5, RuO_2, RhO_2, and OsO_2 [31,75,82–84]. This method depends on the Nernstian response of redox reactions at solid–liquid interface and can achieve the maximum Nernstian sensitivity of 59.16 mV/pH.

A pH measurement technique based on capacitance changes resulting from permittivity changes as a function of pH is presented in [47,48]. In contrast to the above techniques, this technique can be implemented using interdigitated electrode (IDE)-based sensors, which is suitable to integrate into a miniaturized electronic pill system due to simple structural design and inexpensive fabrication process. The design of IDEs consists of two comb-like electrodes structure on a wafer and the sensing material on top of the electrodes. The amount of H^+-ions, depending on pH values on the sensing materials, change the dielectric permittivity for the fringe electric fields from electrodes. This provides an overall capacitance change for pH change.

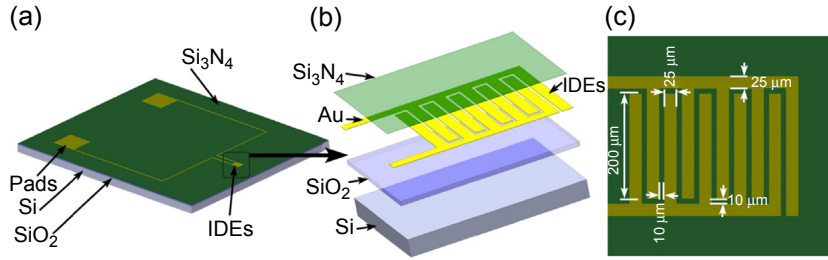

Figure 7.5 (a) Schematic illustration of the microelectromechanical systems (MEMS) fringe-field capacitive pH sensor. (b) Schematic of the multilayered pH sensor fabricated on silicon wafer. (b) Schematic of the IDEs.
M.S. Arefin, M.B. Coskun, T. Alan, J.-M. Redoute, A. Neild, M.R. Yuce, A microfabricated fringing field capacitive pH sensor with an integrated readout circuit, Applied Physics Letters, 104 (2014) 223503.

The MEMS pH sensor detects pH levels by measuring pH-induced permittivity changes in the interdigitated electrodes (IDEs) (Fig. 7.5) [47,48]. In Fig. 7.5(a), the schematic of the sensor is illustrated in three dimensions. The IDEs and pads are fabricated on a silicon (Si) substrate covered with a 500 nm silicon oxide layer. As represented in the exploded view of the sensor in Fig. 7.5(b), the top surfaces of IDEs are passivated by 5 nm thickness of silicon nitride (Si_3N_4) layer to provide a sensing surface for pH buffer solutions as well as to limit Faradaic currents between the electrodes. The IDEs, having each electrode width of 25 µm and length of 200 µm, and interelectrode spacing of 10 µm, are illustrated in Fig. 7.5(c).

In the solution bulk, an electric double-layer capacitance and a diffuse-layer capacitance is formed due to the free H^+-ions in the solution [94,95]. An electric double-layer of H^+-ions forms at the nitride–solution interface that depends on the concentration of H^+-ions of the solutions [94]. The surface charges on the nitride layer act as a source or sink for the H^+-ions in the solutions. In the diffuse layer, the dielectric properties of the solution are modified predominantly by electronic and orientational polarizations under the influence of the high-frequency electric fields. The electronic polarization distorts the electron clouds with the nucleus, whereas the orientational polarization accounts for the reorientation and redistribution of electrical dipoles [95]. Depending on the H^+-ion concentrations, the combined effects of these polarizations alter the relative permittivity (ε_{pH}) of the solutions. The higher concentration of H^+-ions attenuates the external electric fields due to the higher local electric field around the ions and, thus, orients the dipolar water molecules in its vicinity. Hence, lower pH values decrease ε_{pH}. Moreover, the changes in H^+-ions concentrations yield the changes in conductivity (σ_{pH}) of the solution that produce frequency (ω)- dependent complex permittivity $\varepsilon^*_{pH} = \varepsilon_{pH} + j\sigma_{pH}/\omega\varepsilon_0$. Therefore, the change of frequency-dependent dielectric constant for different pH levels is reflected as a change in the capacitance of the sensor.

The capacitance of such a multilayered structure, as shown in Fig. 7.5, having low layer thickness is very difficult to study either by modeling or by finite element method analysis [96]. However, if there is a monotonic increase in permittivity of the layers in the direction of the electrodes' plane to outer medium, the total capacitance of the sensor can be evaluated with the contribution of each layer in series as [96]:

$$C_{\text{sen}} = \frac{N}{2}\left(\frac{C_{\text{ox}}C_{\text{si}}}{C_{\text{ox}}+C_{\text{si}}} + \frac{C_{\text{pH}}C_{\text{n}}}{C_{\text{pH}}+C_{\text{n}}}\right) \tag{7.1}$$

in which C_{sen} is the total sensor capacitance, N is the number of electrodes, and C_{ox}, C_{si}, C_{pH}, and C_{n} are the capacitance contributions from silicon dioxide layer, silicon wafer, pH-buffer solutions, and the silicon nitride layer, respectively. Here, C_{pH} includes the effect of double-layer and diffused-layer capacitance. The sensor under the high-frequency electric fields exhibits an equivalent circuit of the series-connected inductor and sensor capacitance, C_{sen}. The inductance originates from the wired connection of IDEs to pads. At lower frequencies, the capacitance from IDEs is dominant. As the frequency increases, the inductive reactance becomes dominant and cannot provide reactance change for pH change. Therefore, the frequency of operation for the sensor is required to optimize for higher capacitance change.

The capacitive sensor transforms the pH variation to a capacitance change (ΔC). A differential cross-coupled voltage-controlled oscillator (VCO), as shown in Fig. 7.6, is implemented to convert the capacitive variations (ΔC) into frequency variation (ΔF). The differential cross-coupled VCO consists of negative transconductances as well as an inversion-MOS (I-MOS) varactor (C_0) [97] and inductor (L_0) acting as an LC-tank

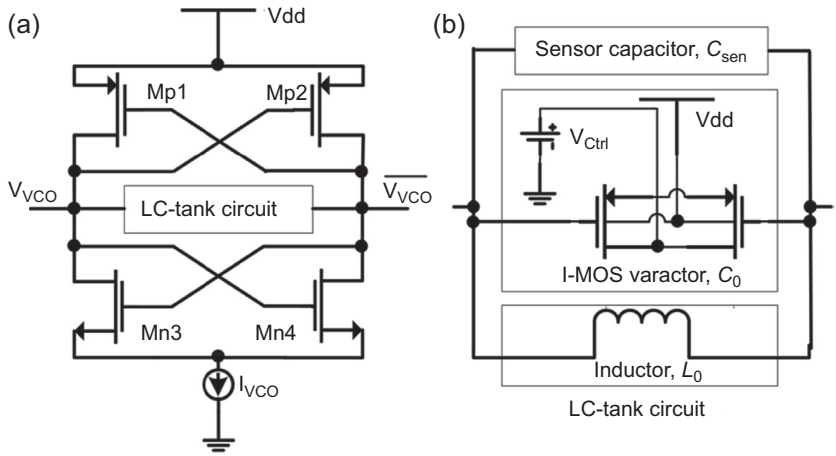

Figure 7.6 (a) Schematic of cross-coupled differential VCO circuit with LC-tank circuit. (b) The LC-tank circuit with the capacitive pH sensor in parallel.
M.S. Arefin, M.B. Coskun, T. Alan, J.-M. Redoute, A. Neild, M.R. Yuce, A microfabricated fringing field capacitive pH sensor with an integrated readout circuit, Applied Physics Letters, 104 (2014) 223503.

of the VCO. A MEMS sensor, C_{sen} is coupled parallel to LC-tank of the VCO to modulate the output frequency. The VCO oscillation frequency can be approximated as $F_{vco} = 1\Big/\left(2\pi\sqrt{L_0\left(C_0 + C_{sen}\right)}\right)$. If the nominal frequency of the oscillator is F_0 for $C_{sen} = 0$ pF, the frequency of the oscillator for the MEMS capacitor, C_{sen} is given by $F_{vco} = F_0\left(1 - C_{sen}/2C_0\right)$ [48,64].

The capacitance of the sensor for the variation of pH obtained from a Vector Network Analyzer (VNA) at 100 MHz is shown in Fig. 7.7. From the figure, the capacitance at pH 1.0 is 7.84 pF that increases by approximately five times and reaches 37.41 pF at pH 5.0. For lower pH, higher concentration of H^+-ions produces lower diffuse-layer capacitance due to higher local electric fields that orient the water molecules in its directions. At very high frequencies, the inductive reactance becomes dominant over the capacitive reactance, hence, the operating frequency of the sensor is kept below 100 MHz.

The sensor is connected to the VCO and the resonant frequency shifts are obtained using a spectrum analyzer. The frequency output of the VCO to corresponding pH values for pH 1.0 to 4.0 is 30.96 MHz that corresponds to a total 31.6% change (Fig. 7.8). Because the molar concentrations of H^+-ions are higher in a strongly acidic region, the sensitivity is very high at low pH levels. The sensitivity for pH 1.0 to 3.0 is 14.335 MHz/pH. The rate of change of resonant frequency decreases for pH 3.0 to 5.0 due to decrease in the molar concentration of H^+-ions. The sensitivity is 1.32 MHz/pH for pH 3.0 to 4.0 and 0.35 MHz/pH for pH 4.0 to 5.0. The magnitudes of the changes for pH 3.0 to 5.0 are still detectable. Similarly, OH^--ion concentrations are higher for a strongly basic region that leads to a larger change as the pH increases, as shown in Fig. 7.8 (inset). Total frequency shift within pH 10 to12 is 4.317 MHz.

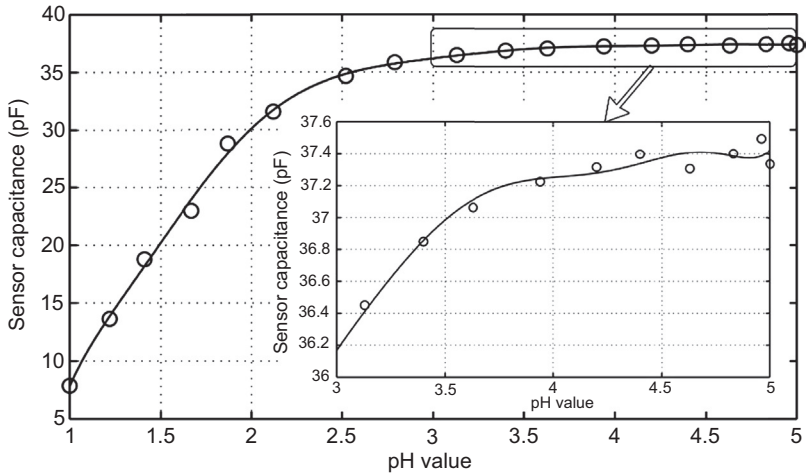

Figure 7.7 The capacitance of the sensor for pH ranging from 1.0 to 5.0 obtained from Vector Network Analyzer (VNA) at 100 MHz. In the inset, the capacitance of the sensor for pH ranges from 3.0 to 5.0.
M.S. Arefin, M.B. Coskun, T. Alan, J.-M. Redoute, A. Neild, M.R. Yuce, A microfabricated fringing field capacitive pH sensor with an integrated readout circuit, Applied Physics Letters, 104 (2014) 223503.

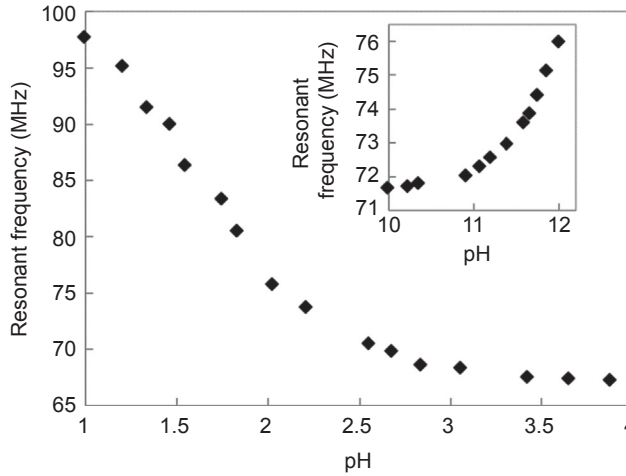

Figure 7.8 The resonance frequency of a voltage-controlled oscillator (VCO) for strong acidic buffer solutions (pH 1–4) and strong basic buffer solutions (pH 10–12) in the inset. M.S. Arefin, M.B. Coskun, T. Alan, A. Neild, J.-M. Redoute, M.R. Yuce, A MEMS capacitive pH sensor for high acidic and basic solutions, in: Proceedings of the IEEE Sensors Conference, 2014, pp. 1792–1794.

In this design example, a capacitive technique has been exploited to measure strong acidic and basic mediums. Such sensors can be integrated within the wireless capsule to measure pH of gastric acid. This technique provides higher sensitivity and low noise readout system while maintaining simple and low-cost fabrication process.

7.3 Wireless telemetry systems

Power consumption is limited in wireless biosensor systems. To prolong the lifetime of biosensors, it is necessary to reduce the power consumption. However, this adds an additional challenge to the digital signal processor (DSP) for supporting radio-frequency (RF) transmissions. Self-powered wireless biosensors are preferred in applications in which sensors are implanted, because, once implemented, it is impractical to access the sensor to recharge the battery. The block diagram of a conventional wireless telemetry system is illustrated in Fig. 7.9. The transmitter circuit is composed of an oscillator, modulator, and a power-amplifier circuit. The modulator processes the measurement data at the operating frequency of the oscillator. The power of the modulated signal is amplified using a power-amplifier (PA) circuit. Finally, the modulated signal is transmitted through antenna via a matching network. The received signal from the antenna and matching network circuit is amplified using a low-noise amplifier (LNA) circuit. The signal is demodulated and filtered to acquire the received data.

For implantable devices, available transmission frequencies compose the medical implant communication services (MICS) band between 402 and 405 MHz, ultrahigh frequency (UHF) 433 MHz or lower-frequency bands including very-high frequency

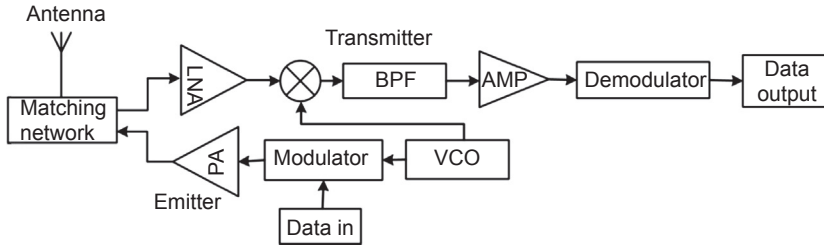

Figure 7.9 Block diagram of a conventional wireless telemetry system.

(VHF) (174–216 MHz) range [40,81,98]. Most of the designs prefer low-frequency transmission due to the inherent high efficiency of transmission through skin layers. As the carrier frequency increases, the attenuation of a wireless signal increases exponentially due to the surrounding tissue [99,100]. This leads to a higher required transmission power. However, lower-frequency transmission requires a larger antenna and occupied space in a wireless capsule. Therefore, there is a trade-off between antenna size and carrier frequency [100].

A simplex telemetry system is employed for the transmission using straightforward modulation schemes such as amplitude modulation, on–off keying, amplitude-shift keying, and phase-shift keying [101–103]. Because the physiological signals vary slowly, such modulation schemes are preferable for miniaturized and low-power wireless biosensors.

For wearable devices, there are several communication technologies to transmit and receive data including MICS, Bluetooth, ultrawide band (UWB), ZigBee, and wireless local area networks (WLAN) [104–111]. Bluetooth operates in the 2.4 GHz ISM bands communicating within seven other devices in a piconet [108]. UWB operates in the 0–960 MHz and 3.1–10 GHz bands [111]. Zigbee operates on a single channel in the 868 MHz, 915 MHz, and 2.4 GHz bands [108]. WLAN follows the Institute of Electrical and Electronics Engineers (IEEE) 802.11 standard describing the physical and Media Access Control (MAC) layer protocol [104,108].

7.4 Applications

Recent progresses in micro- and nanotechnology have led to the design of implantable, swallowable, wearable, or portable wireless biosensors to continuously monitor and detect important physiological parameters. The miniaturization and integration of biosensors, readout circuits, embedded microcontrollers, and wireless transceivers on a single chip have opened the way for new possibilities in medical applications.

7.4.1 Wireless implantable glucose biosensors

Implantable glucose sensors with interface circuits hold a great potential for the continuous measurement and monitoring of blood glucose in patients with diabetes. The sensor can be implanted under the skin [4,33,34,43,112].

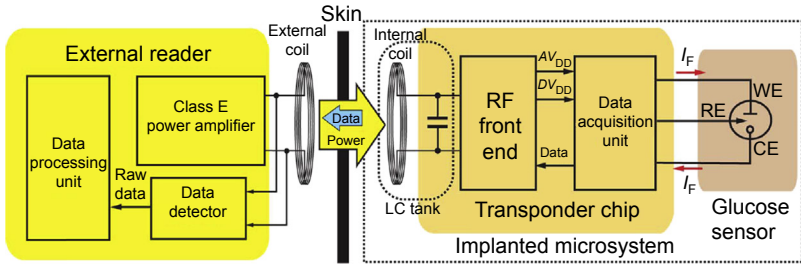

Figure 7.10 Block diagram of the implantable microsystem for continuous glucose monitoring.
M.M. Ahmadi, G.A. Jullien, A wireless-implantable microsystem for continuous blood glucose monitoring, IEEE Transactions on Biomedical Circuits and Systems 3 (2009) 169–180.

The functional block diagram of an implantable microsystem for blood glucose monitoring designed by Ahmadi and Jullien is shown in Fig. 7.10 [34]. The glucose sensor is an amperometric electrochemical biosensor generating a current from the electrochemical reaction between glucose and a glucose oxidase layer on working electrode (WE). The use of iridium-oxide nanoparticles helps for the transfer of the electrons from the glucose oxidase to WE. The reference electrode (RE) eliminates the potential arising from the solution medium. The counter electrode (CE) acts as a reference half-cell to supply the required current for the electrochemical reaction, whereas the WE act as a sensing half-cell to produce the current. The external reader inductively transfers power to the implantable microsystem and receives the transmitted measurement data of blood glucose concentration from the microsystem. The data transmission is performed for every 10 min using a load-shift keying modulation scheme. The interface circuit of the microsystem consists of an RF front-end circuit for receiving RF signals, rectifying, and generating the supply voltage, and a data acquisition circuit for converting the current from glucose sensor to pulse.

The cross-sectional view of the glucose biosensor is illustrated in Fig. 7.11. The titanium–nickel–gold–titanium metallization is essential for the WE, CE, interconnect traces, and bonding pads. The glucose oxidase on gold acts as a biologically sensitive layer. The silver metal layer at RE acts as an Ag/AgCl electrode, which generates current from the solution medium. The integrated interface circuit and the wireless transmitter are bonded on this wafer. The off-chip components and inductive coil for energy transmission are connected on this wafer. The dimension of the microsystem is 8 mm × 4 mm and its thickness is 1 mm.

7.4.2 Wireless capsules

Wireless capsule devices are used in the GI tract to measure physiological parameters. They can monitor motility of the GI tract as a pressure change and transmit the data using low frequencies [40,81]. A summary of previously reported wireless capsules is listed in Table 7.3.

A typical block diagram of wireless capsule system is depicted in Fig. 7.12. The system contains several sensors with analog readout circuits, digital microcontroller

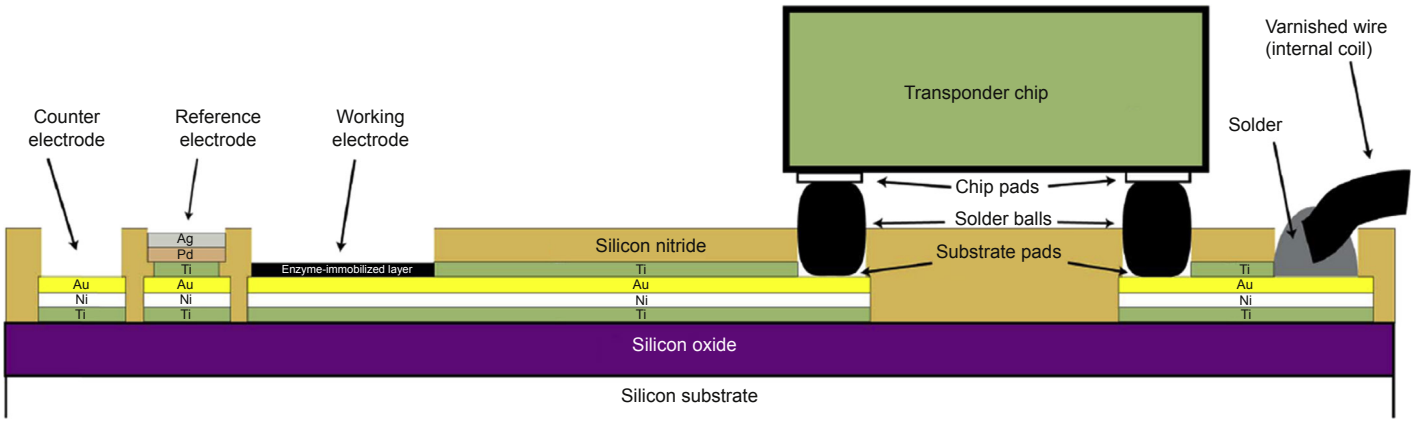

Figure 7.11 Cross-sectional view of the implantable microsystem and glucose sensor for continuous blood glucose monitoring.
M.M. Ahmadi, G.A. Jullien, A wireless-implantable microsystem for continuous blood glucose monitoring, IEEE Transactions on Biomedical Circuits and Systems 3 (2009) 169–180.

Table 7.3 Summary of wireless capsule devices

Capsule name	Capsule dimensions	Sensors	Range	Wireless transmitter	Power source	References
Gutnic	28 mm × 9 mm	pH Pressure Temperature	– – –	–	Gold and Iron electrode battery	[114,115]
Weyrad electronics Ltd.	10 mm × 15 mm	Pressure	0–24.13 kPa	–	Mercury cell battery	[116]
Heidelberg pH capsule	20 mm × 8 mm	pH	1–7	–	Saline activated battery	[117]
Rigel research Ltd.	8.8 mm × 6 mm	Pressure	0–40 kPa	250–570 kHz	Mercury battery	[118]
CorTemp	8.8 mm × 6 mm	Temperature	–	–	Silver oxide battery	[119,120]
Integrated Diagnostics for Environmental and Analytical Systems (IDEAS) (prototype)	55 mm × 16 mm	pH Temperature Conductivity Dissolved oxygen	4–10 0–70°C 0.05–10 mS/cm 0–8.2 mg/L	38.342 MHz (frequency shift keying, FSK)	Silver oxide battery	[101–103]
Bravo pH system	26 mm × 6.3 mm	pH	1.68–7.0	433 MHz	–	[121,122]
Smart pill	22 mm × 9.6 mm	pH Pressure Temperature	– – –	–	Battery	[123]
LIAP	36 mm × 12 mm	pH Temperature	1–10 10–50°C	433.92 MHz (on–off keying, OOK)	Silver oxide battery	[113]

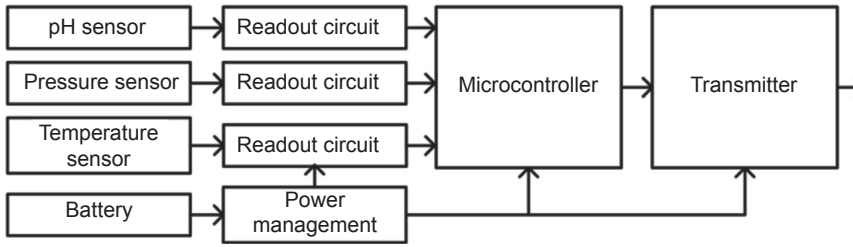

Figure 7.12 The block diagram of a wireless capsule system with biosensors, readout circuits, and transmitter.

circuits, a radio transmitter, and a battery. The sensors convert the physiological parameters to electrical parameters. The controller circuits manage and process all the sensor data. The data from the GI tract is transmitted to an external device for monitoring and recording. The main feature of the system is the integrated multisensor of a pH, pressure, and temperature sensor for real-time signal monitoring of the GI tract abnormalities.

The architecture of a wireless capsule system named the lab-in-a-pill (LIAP) is presented in Fig. 7.13 [113]. It consists of pH and temperature sensors and a custom-made application-specific integrated readout circuit. The pH sensor is a microfabricated ISFET with Ag/AgCl reference electrode. The temperature sensor is an n-channel silicon diode. The system consumes 15.5 mW. The circuit has a power saving feature to operate it for 42 h.

A wireless capsule can provide an invasive method for the diagnosis of the GI tract. The sensor systems for a wireless capsule are composed of mechanical sensors for pressure and position measurement, chemical sensors for pH, conductivity, and dissolved oxygen measurement, and biosensors for bleeding and pathogens detection. In addition to the sensor systems, interface circuits for the sensors also play an important role for the development of low-noise and low-power sensor systems. As a result, wireless capsule requires the development of a reliable, miniaturized, and integrated sensor system with high sensitivity and resolution as well as a low noise, low cost, and a low-power interface circuit system.

7.4.3 Wireless wearable devices

A list of various wireless wearable devices is given in Table 7.4. Different wireless technologies have been used in different wearable devices. A prototype of advanced care and alert portable telemedical monitor (AMON) wrist-worn unit is shown in Fig. 7.14 [8]. The system has two major parts: a wrist-worn unit and a stationary unit at the telemedicine center. The wrist-worn unit measures physiological parameters and transmits through a Global System for Mobile communication (GSM) network to the stationary unit for data collection and processing by trained medical personnel [8]. The stationary unit is composed of a JAVA server platform and a workstation connected to the GSM transceiver.

The functional block diagram of the AMON wrist-worn monitoring unit is shown in Fig. 7.15. The system consists of several sensors, analog readout and

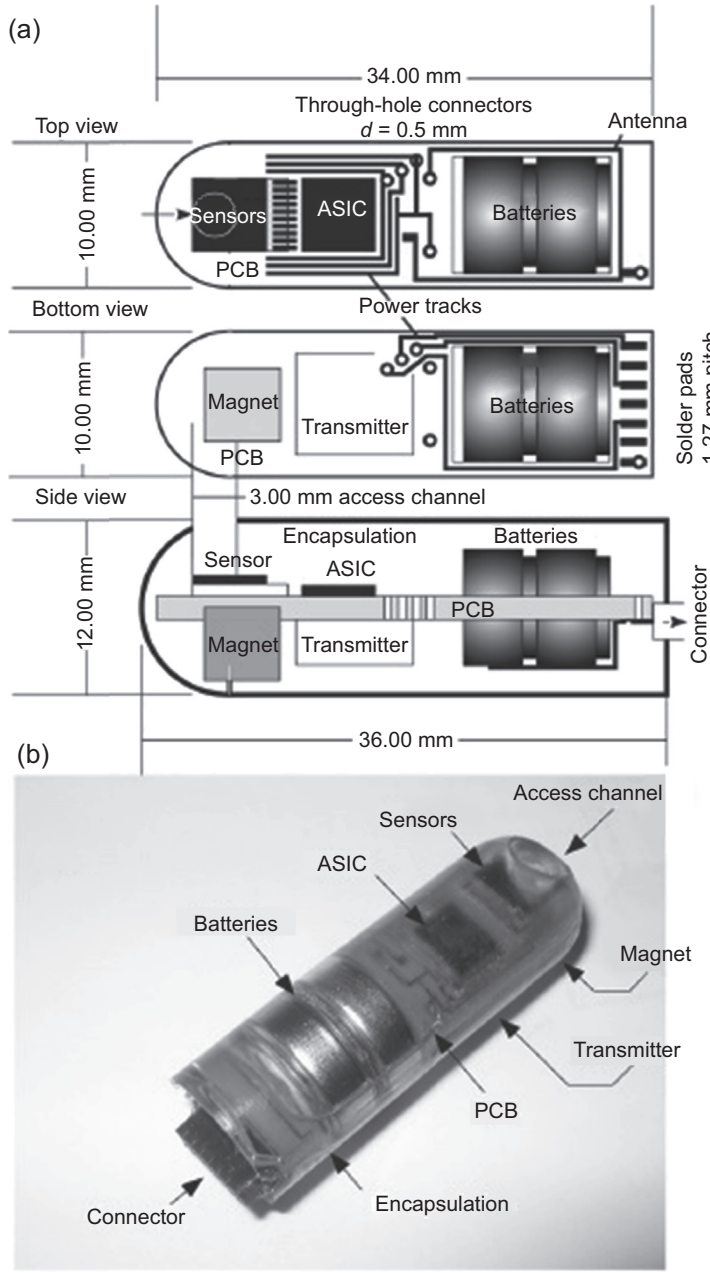

Figure 7.13 The architecture of a wireless capsule containing temperature and pH sensors at the front, followed by application-specific integrated circuit (ASIC) and batteries.
E.A. Johannessen, L. Wang, C. Wyse, D.R. Cumming, J.M. Cooper, Biocompatibility of a lab-on-a-pill sensor in artificial gastrointestinal environments, IEEE Transactions on Biomedical Engineering 53 (2006) 2333–2340.

Table 7.4 List of few wireless wearable devices

Prototype name	Sensors	Wireless Connectivity	Applications	References
AMON	Skin temperature Blood pressure, ECG Blood oxygen Acceleration	GSM	Cardiac/Respiratory patients	[8]
LiveNet	ECG EMG Skin conductance Acceleration	2.4 GHz	Ambulatory health monitoring	[124]
PDA palm-type	ECG PCG Body temperature	Bluetooth	Patient's body condition	[125]
μ-Healthcare system	ECG Blood pressure	IEEE 802.15.4 CDMA	Physiological signal monitoring	[126]
Intrepid	Galvanic skin response Benign positional vertigo Body temperature EMG	Not given	Psychiatric disorder/ anxiety patients	[127]
Not given	ECG Bioimpedance Activity	1 MHz, OOK	Physiological signal monitoring	[9]
Not given	ECG Heart rate Body temperature	Bluetooth	Physiological signal monitoring	[128]

signal conditioning circuits, microcontroller, DSP, communication module, and a display unit. The passive sensors monitor and measure skin temperature, blood pressure, ECG, blood oxygen saturation, and acceleration. The analog readout and signal conditioning circuits are operated for measuring temperature, blood pressure, and ECG. The DSP conditions the ECG and blood pressure signals. The communication module uses a Siemens TC35 Cellular system to connect to the GSM network.

7.5 Conclusion and future trends

This chapter provides an overview of recent developments of implantable and wearable biosensors. Both label-free and label-based biosensors are being studied and developed for detection and measurement of biological parameters. Label-based biosensors

Figure 7.14 A prototype of a wearable medical monitoring device.
U. Anliker, J.A. Ward, P. Lukowicz, G. Troster, F. Dolveck, M. Baer, et al., AMON: a wearable multiparameter medical monitoring and alert system, IEEE Transactions on Information Technology in Biomedicine 8 (2004) 415–427.

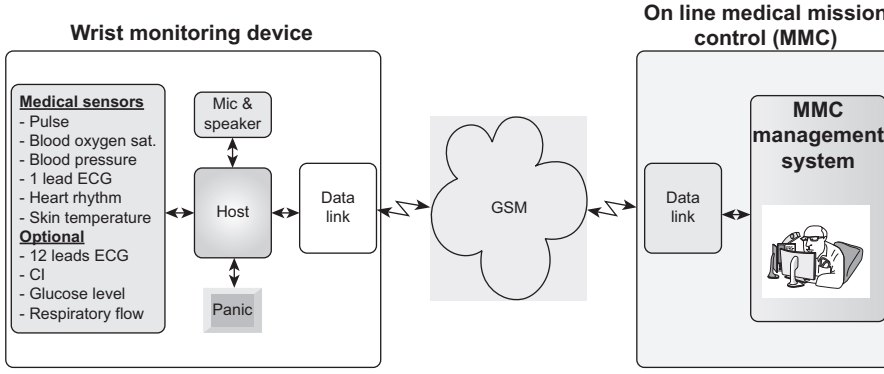

Figure 7.15 System level overview of the wrist-worn medical device with GSM/ Universal Mobile Telecommunication System (UMTS) link.
U. Anliker, J.A. Ward, P. Lukowicz, G. Troster, F. Dolveck, M. Baer, et al., AMON: a wearable multiparameter medical monitoring and alert system, IEEE Transactions on Information Technology in Biomedicine 8 (2004) 415–427.

require fluorescent labels for biomolecules and optical measurement systems, which place some constraints for the use in POC diagnostic devices. Recent POC devices focus on label-free biosensing techniques. The electrochemical and electrical biosensors with interface circuits are very suitable for wireless POC devices. The fundamental elements of these biosensors and the interface or readout circuits are discussed in detail in this chapter. A capacitive pH sensor and interface circuit design to measure gastric acid is presented in detail. Because biosensors are implanted or deployed inside the body, the challenges for smaller size, flexibility, and biocompatibility issues should be addressed. Integrating biosensors and interface circuits with wireless transceivers on semiconductor chips can decrease the size considerably. Because flexible sensors need to be less than 100 μm thick, the challenges to manufacture small and thin biosensors and circuits on chip need to be addressed in the future. Biocompatibility issues are critical for biosensor devices, as the sensitive biomaterial may affect the body adversely as well as degrade over time. Moreover, electrode passivation and interference with other species also limit sensor sensitivity and selectivity. The biocompatible materials for sensors and integrated circuits require expedited advances in the future. It is also crucial to adapt a suitable packaging for the environment into which the biosensors are placed.

Wireless telemetry systems are used to connect biosensor devices to a central node acting as a data acquisition unit. The challenges for such wireless systems are the power radiated through the antenna, the power consumption for data transfer, and the type of network protocol and topology. All the nodes are connected to a central medical database system using the traditional Internet or mobile networks. Three wireless POC systems for implantable and wearable devices are explored to demonstrate the development of wireless integrated sensors functionalized with biologically sensitive elements, integrated interface circuits, and wireless transceiver systems for specific applications. These systems are battery powered, and hence adapting wireless energy harvesting techniques to replace the bulky batteries will lead to new opportunities in the design of biosensors.

The recent developments of the wireless implantable and wearable biosensors for POC applications enable dedicated patient health management. The future trends for POC biosensors outline the need for smaller and flexible integrated systems, new biocompatible materials applicable for different applications, new packaging techniques, energy-efficient wireless systems, improved antenna designs, and efficient wireless power transfer and energy-harvesting techniques.

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Point of care (POC) medical biosensors for cancer detection

8

L. Bueno, W.R. de Araujo, T.R.L.C. Paixão
University of São Paulo, São Paulo, SP, Brazil

8.1 Introduction

Point of care (POC) medical devices could be defined as medical/clinical analysis at or near the location of patient care (Kost et al., 2006; Nichols, 2002). One of the first POC tests reported in the literature was documented in 1550 BCE, wherein an Egyptian researcher used ants to evaluate glycosuria (excretion of glucose into the urine) in patients suspected of having diabetes mellitus (DuBois and Clarke, 2014). Hence, the goal of POC devices is to provide fast, convenient and easy-to-use diagnostic testing that shortens the therapeutic turnaround time when compared with testing at a core laboratory. These devices can provide rapid diagnosis in a hospital or in resource-poor countries that lack a core laboratory infrastructure, thus permitting immediate or remote clinical management decisions to improve patient medication, cure or direct any changes in medical procedure. These POC biosensor devices can be classified by their biorecognition and transducer systems, as depicted in Fig. 8.1.

One of the major challenges in the medical application of POC biosensors is to make possible the use of these devices to diagnose and monitor severe diseases like cancer. Hence, at the start of the chapter, it is important to define cancer and its types to show how each cancer can be detected using POC biosensors with some approaches in the development of medical biosensors for cancer detection.

8.2 Definition of cancer

Cancer is a group of diseases characterized by uncontrolled growth and spread of abnormal cells, and results in death if its spread is not controlled. Damage to a gene or specific molecule could disrupt communication amongst cells, ultimately leading to the termination of all biological functions that sustain a living organism. Cancer is caused by external factors, such as tobacco, infectious organisms and an unhealthy diet, and by internal factors, such as inherited genetic mutations, hormones and immune conditions. These factors may act together or in sequence to cause cancer, and manifestation of the disease may occur at variable periods between the exposure to external carcinogenic factors and detection of cancer markers (American Cancer Society, 2015a; Simon, 2010) or after changes in the genetic code and the formation of cancer cells (Fig. 8.2). Proto-oncogenes will be discussed in Section 8.3.

Medical Biosensors for Point of Care (POC) Applications. http://dx.doi.org/10.1016/B978-0-08-100072-4.00008-3

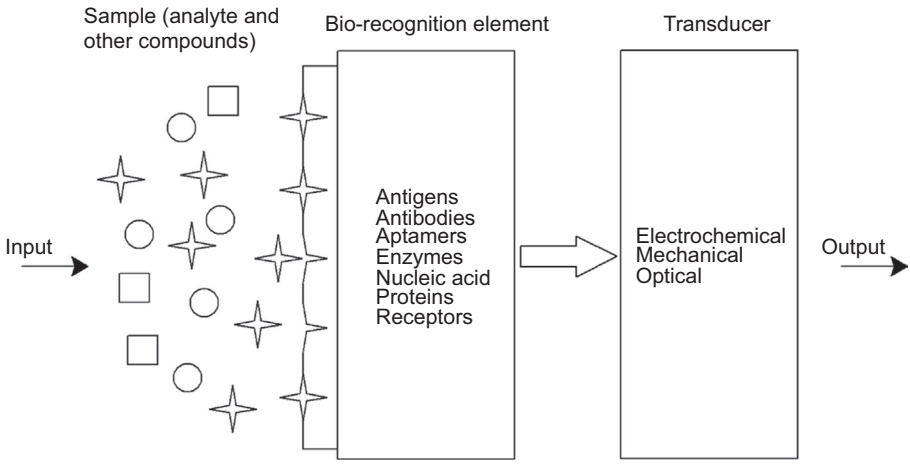

Figure 8.1 Schematic diagram of POC biosensor device.

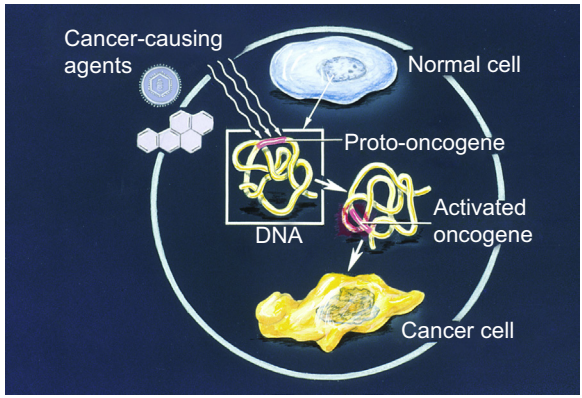

Figure 8.2 Conversion of a normal cell to a cancer cell, when an oncogene is activated. This image (with the ID 2351) was released by the National Cancer Institute, an agency that is a part of the National Institutes of Health. The image is in the public domain and can be freely reused.

8.3 Cancer biomarkers

Biomarkers are defined by the National Cancer Institute at the National Institutes of Health (http://www.cancer.gov/) as 'biological molecules found in body fluids or tissues that can sign a normal or abnormal process, or of a condition or disease'. They can also be used to check how well the body responds to a treatment for a disease or condition. There is tremendous variety in biomarkers, which can include proteins (eg, an enzyme or receptor), nucleic acids [eg, a microRNA or other noncoding ribonucleic acid (RNA)], antibodies and peptides, amongst other categories. A biomarker can also be a collection of alterations, such as gene expression, proteomic and metabolomic signatures. Genetic biomarkers may be inherited and detected as sequence variations

in germ line deoxyribonucleic acid (DNA) isolated from whole blood, sputum or buccal cells, or they may be somatic and can be identified as mutations in DNA derived from tumour tissue (Henry and Hayes, 2012).

Besides being found in body fluids and tissues, some markers are associated with the smell of breath and skin. The correlation between breath smell and disease has been reported in literature (Matsumura et al., 2010; Horvath et al., 2009; Silveira-Moriyama et al., 2008), and these signature molecules are volatile organic compounds found at concentrations lower than the ppb range. These markers are formed as metabolic products generating a 'fingerprint' of the diseased state (Simon, 2010).

The choice for a correct candidate as a biomarker to be monitored or detected will reflect various pathophysiological conditions of the biological system and enable better disease diagnosis and/or treatment outcomes, because it can indicate the disease stage of the patient the best treatment option (Chen et al., 2015). A unique marker can signalize different diseases or different stages of the same disease. Table 8.1 summarizes some of the important biomarkers that can be used to detect different cancer types, and they can be employed for different clinical purposes: prognosis, diagnosis, staging, monitoring, screening and selection of therapy (Ludwig and Weinstein, 2005).

8.4 Types of cancer

In this section, the most common types of cancer according to the National Cancer Institute and American Cancer Society are listed (National Cancer Institute, 2015b) (Table 8.2). They were chosen because of their high incidence and mortality, and due to the potential application of POC sensors to monitor these types of cancers. Based on our literature search, we found that POC devices have not been developed for some of the cancers listed in Table 8.2.

8.4.1 Breast cancer and point of care devices

Breast cancer is a type of cancer that affects mostly women (only 1% of men are affected) and is the most diagnosed cancer worldwide, with more than 230,000 estimated new cases in 2015 in the United States alone (National Cancer Institute, 2015b). However, the chances of cure for breast cancer are above 80%, in contrast to lung cancer, in which approximately 30% of the cases can be cured (American Cancer Society, 2015a). Although the number of breast cancer cases has decreased in the last few years, breast cancer is still a cause for concern, as it is the second largest cancer with a high mortality rate in women; lung cancer tops the list, with the highest mortality. The most common type of breast cancer is ductal carcinoma, which begins in the lining of the milk ducts (thin tubes that carry milk from the lobules of the breast to the nipple). Another type of breast cancer is lobular carcinoma, which begins in the lobules (milk glands) of the breast. Invasive breast cancer is breast cancer that has spread from where it began in the breast ducts or lobules to surrounding normal tissue.

Because cancer progression results in modification of nucleic acids, as illustrated by Fig. 8.2, these large molecules are commonly used as main targets to detect cancer.

Table 8.1 Biomarkers used to detect different cancer types and their clinical use

Biomarker	Cancer type	Clinical use
Chromosomes 3, 7, 9 and 17	Bladder	Screening and monitoring
NMP22	Bladder	Screening and monitoring
Fibrin/FDP	Bladder	Monitoring
BTA	Bladder	Monitoring
High molecular weight CEA and mucin	Bladder	Monitoring
CA 15-3	Breast	Monitoring
CA 27-29	Breast	Monitoring
Cytokeratins	Breast	Prognosis
Oestrogen receptor and progesterone receptor	Breast	Selection for hormonal therapy
HER2/NEU	Breast	Prognosis, selection of therapy and monitoring
21 gene recurrence score	Breast	Prognosis
BRCA1 germline mutation	Breast and ovarian	Estimated risk of developing cancer
Pap smear	Cervical	Screening
CEA	Colon	Monitoring
Epidermal growth fat or receptor	Colon	Selection of therapy
KRAS mutation and anti-EGFR antibody	Colon	Selection of therapy
KIT	GIST	Diagnosis and selection of therapy
CA 125	Ovarian	Monitoring
CA 19-9	Pancreatic	Monitoring
PSA (total)	Prostate	Screening and monitoring
PSA (complex)	Prostate	Screening and monitoring
PSA (free PSA %)	Prostate	Benign prostatic hyperplasia versus cancer diagnosis
α-Fetoprotein	Testicular	Staging
Human chorionic gonadotropin-β	Testicular	Staging
Thyroglobulin	Thyroid	Monitoring

BRCA, breast cancer biomarker; *BTA*, bladder tumour-associated antigen; *CA*, cancer antigen; *CEA*, carcinoembryonic antigen; *EGFR*, epidermal growth factor receptor; *FDP*, fibrin degradation protein; *KIT*, proto-oncogene c-Kit; *KRAS*, colorectal cancer mutation biomarker; *NEU*, human epidermal growth factor receptor 2; *NMP22*, nuclear matrix protein 22; *PSA*, prostate-specific antigen.
Data extracted from Ludwig, J.A., Weinstein, J.N., 2005. Biomarkers in cancer staging, prognosis and treatment selection. Nature Reviews Cancer 5, 845–856 and Henry, N.L., Hayes, D.F., 2012. Cancer biomarkers. Molecular Oncology 6, 140–146.

Briefly, these biosensors or nucleic acid sensors exploit the interaction of the analysed target with immobilized complementary nucleic acid (biorecognition layer) at the surface of the sensor/transducer, as shown in Fig. 8.3. As the complementary nucleic acid has a specific sequence, the recognition is very specific, conferring this sensor a higher specificity. Hence, after recognition by the biorecognition element, the signal

Table 8.2 Number of deaths for each common cancer type (26 January 2015)

Cancer type	Estimated deaths
Thyroid	1950
Melanoma	9940
Endometrial	10,170
Kidney (renal cell and renal pelvis) cancer	14,080
Bladder	16,000
Non-Hodgkin lymphoma	19,790
Leukaemia (all types)	24,450
Prostate	27,540
Breast (female–male)	40,440
Pancreatic	40,560
Colon and rectal (combined)	49,700
Lung (including bronchus)	158,040

Retrieved from National Cancer Institute at the National Institutes of Health, 2015b. http://www.cancer.gov/cancertopics/types/commoncancers.

can be transduced by different types of optical, mechanical (Quartz Crystal Microbalance and acoustic sensors) and electrochemical techniques (Tothill, 2009; Soper et al., 2006). Fig. 8.3 shows an example of an electrochemical readout, based on guanine oxidation mediated by a ruthenium complex in solution. If the number of guanine molecules increases in the recognition layer, the signal increases, and if the nucleic acid recognized is expressed in cancer, this sensor can be used a good candidate for rapid screening of cancer.

Being an aggressive disease, breast cancer should be detected and treated early in a simpler and faster way, if possible, to achieve better results. With this view, some research groups have worked on nucleic acid sensors with the possibility of miniaturization to make the diagnostic process easy for doctors and patients. A combination of electrochemical and optical approaches was used as a readout to create an ultrasensitive method for detection of messenger RNA (mRNA) for the proto-oncogene (a normal gene that, with slight alteration by mutation, becomes a gene that has cancer-causing potential; Fig. 8.2) c-Myc in Michigan Cancer Foundation-7 (MCF-7) cells (breast cancer cell line). In the device, the authors modified the anodic pole with antisense DNA to be used as recognition element and detected nucleic acid from tumour cells on an indium tin oxide bipolar electrode in a poly(dimethylsiloxane) microchannel (Wu et al., 2012). The electrochemiluminescence biosensor was able to detect 2203 copies of the nucleic acid in MCF-7 cells compared with 13 copies in an immortal hepatic cell line control, indicating that the miniaturized device can be used as a good POC device for breast cancer.

Other genes can also be used as targets for breast cancer detection. BRCA1 and BRCA2 (breast cancer 1 and 2) are genes that produce tumour suppressor proteins that help repair damaged DNA and, therefore, play a role in guaranteeing the stability of the genetic material in the cell. Mutations in BRCA1 and BRCA2 genes are associated

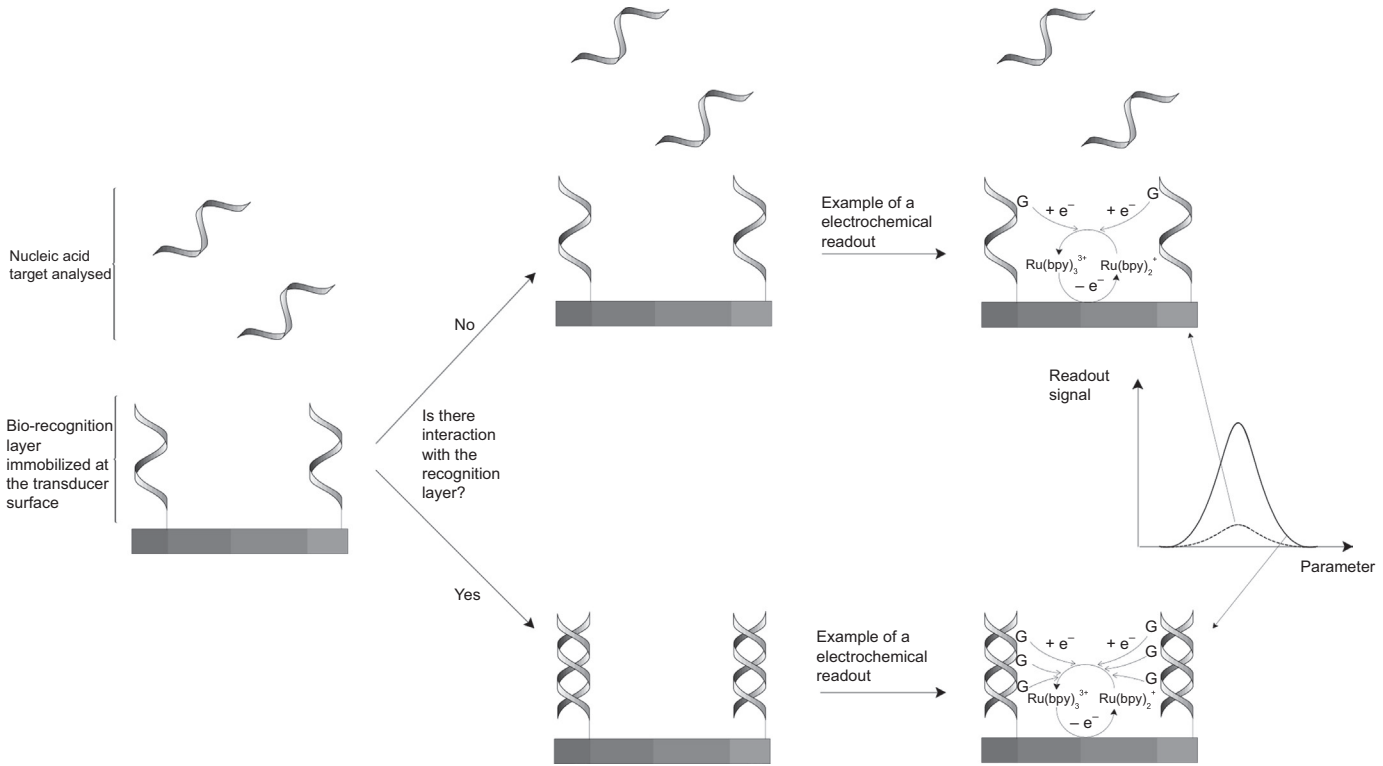

Figure 8.3 Schematic representation of nucleic acid sensor design to detect cancer. An example of an electrochemical readout using a ruthenium complex to mediate the guanine oxidation is shown.

with breast cancer syndromes, and some researchers have already identified a large number of mutations in the BRCA genes, which are associated with a risk of cancer. Based on this finding, the detection of BRCA gene mutant can lead to an interesting POC biosensor. An electrochemical nucleic acid sensor that is able to detect BRCA1 mutant using zinc oxide nanowires to immobilize oligonucleotide probe/biorecognition element has been developed (Mansor et al., 2014). A detection limit of 3.32 μM was obtained after employing differential pulse voltammetry techniques to recognize the target molecule. Other approaches in the literature exploit similar ideas of using nucleic acid sensors for BRCA1 detection (Rasheed and Sandhyarani, 2014; Li et al., 2012). This type of approach is important not only to diagnose cancer but also to monitor its development.

Human epidermal growth factor receptor 2 (HER2) is a marker associated with aggressive breast cancer, and its monitoring is important to choose the best treatment option. HER2 is a receptor tyrosine kinase protein that controls growth factors and hormones. Alterations in HER2 are related to abnormal processes in the cell, indicating their use as biomarkers for some types of cancer, such as breast and ovarian cancer (Zwick et al., 2001). A proposed biosensor by Gohring et al. (2010) to detect HER2 in human serum combines optical ring resonator architecture with microfluidics. Antibodies were attached to the sensor surface and the device was able to measure concentrations of HER2 ranging from 13 to 100 ng/mL, reported by the authors as medically relevant concentrations for real cases.

Another optical sensor has been used by Jokerst et al. (2009) to simultaneously quantify three important cancer markers, namely carcinoembryonic antigen (CEA), cancer antigen 125 (CA125) and HER2. In this approach, the authors integrate semiconductor nanoparticle quantum dots (QDs) into a microfluidic biosensor, which is able to quantify the markers in both saliva and serum. QDs have some advantages compared to organic fluorophores in POC devices due to their unique spectroscopic properties, because their narrow emission peaks enable multiplexed analysis and increase in signal-to-noise ratio (Soper et al., 2006). However, some drawbacks reported by Soper et al. (2006) stress the need to improve the stability of the QDs in biological medium, by capping to prevent aggregation and by development and optimization of the conditions to realize stable and active QD bioconjugates as challenges for successful use of QDs in cancer diagnostics. According to some researchers (Harris et al., 2007), the simultaneous detection of multiple biomarkers can help ensure the best diagnosis and suitable treatment for each patient. It is important to highlight that CEA and CA125 are nonspecific antigens for some types of cancer, but they are used as markers because their levels are elevated in the presence of tumours.

8.4.2 Colon and rectal cancer and point of care devices

Cancers of the colon and the rectum are often associated and are mostly referred to as colorectal cancer. Colorectal cancer is the most lethal cancer in the United States after lung cancer and can be avoided with screening. The procedures for detecting abnormal growths in the colon or rectum are quite simple; such masses can be removed before turning cancerous or at early stages, curing the disease in most of the cases (Prevention, 2015).

In cases in which the number of deaths is very high, the development of an analytical method wherein the targets indicating the cancers can be detected in a small amount is an urgent requirement. Biosensors can operate on a very low density of cells, which is a very attractive feature in the diagnostic area, especially to detect and study rare and uncommon cells hidden in a large heterogeneous cell population (Zhang et al., 2014b). It is also interesting that these sensors can be portable, fast and easy to operate so that patients and health professionals can understand and apply the results of the measurements obtained to help in treatments. An electrochemical device based on the biosensing approach with a great potential to be used as a 'point of care' sensor was tested in mice; this device could discriminate between normal and cancerous epithelial tissues (Vernick et al., 2011). The device was developed to measure the enzymatic activity of intestinal alkaline phosphatase (ALP), an enzyme known to be downregulated in cancerous cells. In colorectal cancer cells, its activity was shown to be as low as <0.0001 unit/mg of protein, whereas a healthy differentiated intestinal epithelial cell could exhibit an activity of >0.7 unit/mg protein (Gum et al., 1987). The activity of this enzyme was measured in slices of a biopsy using a carbon-counter electrode, gold working electrode and Ag/AgCl reference electrode screen-printed on ceramic within a few minutes on a cheap device.

A relatively common electrochemical type of sensor is a DNA-based biosensor reported earlier. In a specific case (Wang et al., 2008), the K-ras gene (which is highly associated with colorectal cancer) was detected by a sensor based on a horseradish peroxidase (HRP)-labelled probe modified with a sulfhydryl group (–SH) group and chemically adsorbed on the gold electrode through self-assembly. The target DNA containing the complementary sequence to the probe DNA was captured by a hybridization process, as depicted in Figure 8.3. The HRP-labelled oligonucleotide (detection DNA), which is complementary with another part of target DNA, was hybridized in the form of a sandwich. Then, H_2O_2 electroreduction current catalysed by HRP was measured amperometrically in the presence of hydroquinone as the mediator. In another case (Feng et al., 2006), a modified glassy carbon (GC) electrode with a nanoporous CeO_2/Chitosan composite film was used to immobilize a DNA probe, hybridized and its signal was recorded using methylene blue as an indicator and by the differential pulse voltammetry technique. The detection of the target sequence associated with colorectal cancer gene has a relatively wide linear range, low detection limit, high sensitivity and satisfactory reproducibility.

Cancer research includes several optical sensors, including works with microwave, fluorescence (Tao et al., 2012) and magnetic-based assays. The basic principle of these sensors is to analyse the electromagnetic properties of the targets. Zhang et al. (2014b) showed, in an interesting study, that it is possible to find electromagnetic signatures between benign and malignant cells and to estimate the phase of the tumour, because cancer diagnosis is based on staging of the tumours. As the stage increases (from zero to four), the malignant cells are more aggressive and obtain the propensity to proliferate and produce metastases in remote tissues. In the study, five colorectal cell lines in different stages were selected; data were obtained using 15 passive sensors and four tunable frequency sensors. This study demonstrated the potential of intracellular dielectric permittivity analysis at microwave frequencies to evaluate the degree of

aggressiveness of malignant cells. This technology is indeed promising for targeting uncommon or rare cells and providing an early prognosis with the objective to assess and prevent tumour spread and metastasis development.

A magnetic bead-based assay showed that it is possible to detect the enzyme telomerase in presence of colon and bladder cancer (Rothacker et al., 2007). Telomerase, also called telomere terminal transferase, is a ribonucleoprotein enzyme that catalyses the addition of repeated DNA sequences TTAGGG or telomeres at the end of chromosomal DNA. In cancer cells, cell division can occur indefinitely and telomere lengths are maintained by expressing telomerase. In contrast, in normal cells, telomerase expression is repressed and telomeres shorten progressively with each cell division (Greider, 1996). Telomerase has been identified in over 80% of tissue samples derived from bladder carcinomas, regardless of the tumour stage or degree of differentiation (Muller, 2002), and is being considered as a biomarker in cancer. In a previously published study (Rothacker et al., 2007), colon and bladder cancer cells were captured using antibody-coated magnetic beads. Telomerase activity was detected using a biosensor connected to an oligonucleotide containing the telomerase recognition sequence, also covalently coupled to magnetic beads. Magnetic beads are an effective tool for extracting and concentrating biomarkers. Using this dispersed solid-phase approach, magnetic beads are mixed with large volumes of patient sample to capture biomarkers. These magnetic beads are then recollected magnetically, and the captured biomarkers are released from the bead surface into a more amenable reaction buffer (Bordelon et al., 2013). This approach helped distinguish between cancer patients and normal healthy volunteers, indicating a high sensitivity and specificity for this method.

8.4.3 Bladder cancer and point of care devices

Squamous cell carcinomas and adenocarcinomas develop in the inner lining of the bladder as a result of chronic irritation and inflammation (National Cancer Institute, 2015a). According to National Health Services in the United Kingdom, bladder cancer mostly affects the elderly and cannot always be prevented; however, some lifestyle modifications such as smoking cessation and dietary changes can reduce its risks. An early diagnosis is essential to ensure minimal suffering in cancer patients. Some that focus on this purpose have been reported in the literature. Some Chinese researchers have suggested a method to detect specific DNA sequences of bladder cancer (Zhang et al., 2014a). In this study, an electrochemical biosensor was developed by the modification of GC surfaces with CdTe QDs—semiconductors that help to improve the electrode area—for subsequent immobilization of a DNA probe. In the next step, target DNA sequences were hybridized, and in the final stage, methylene blue was used to obtain electrochemical signals using differential pulse voltammetry. Some factors such as ease of operation, good selectivity and sensitivity, rapid response and low cost render this sensor useful to achieve good results in the medical field.

Another approach, a label-free technique, was used to detect telomerase activity in human urine (Kim et al., 2013). The device is a silicon-based micro-ring resonator biosensor using telomerase extracted from two bladder cancer cell lines, J-82 and HT-1376, in a buffer solution as well as spiked urine. This chip-based sensor is fast,

cost-effective and can function as a POC device in contrast to the Polymerase Chain Reaction (PCR)-based telomeric repeat amplification protocol (TRAP) method, which is the standard test for telomerase activity (Hou et al., 2001). The TRAP assay is time-consuming, requires expensive equipment and reagents and does not provide precise quantitative information, besides other problems related to other conventional PCR methods (Sharon et al., 2010).

Field-effect transistors (FETs) biosensors have attracted attention, because they present the advantages of miniaturization and superior sensitivity besides offering rapid, inexpensive, label-free detection and high selectivity achieved by immobilization of biomolecules to bind to targets. Chen et al. have reported the first FET biochip to diagnose bladder cancer from urine specimens (Chen et al., 2015). In this study, graphene sheets were used to improve the conductivity and nanowires were employed to increase the superficial area to obtain a better immobilization of anti-APOA2, the antibody for apolipoprotein A-II (APOA2) recognition, which was recently identified as a new biomarker that showed elevated rates in pooled bladder cancer (Chen et al., 2010, 2012).

8.4.4 Kidney cancer and point of care devices

Renal cell carcinoma, also known as *renal cell cancer*, is the most common type of kidney cancer. About 9 out of 10 kidney cancers are renal cell carcinomas (American Cancer Society, 2015c). This cancer forms in the lining of very small tubes in the kidney that filter the blood and remove waste products. Transitional cell cancer of the renal pelvis is kidney cancer that forms in the centre of the kidney in which urine is collected. Wilms' tumour is a type of kidney cancer that usually develops in children under the age of 5 (based on information of the National Cancer Institute). An estimated 61,560 new cases of kidney (renal) cancer are expected to be diagnosed in 2015 in the United States. This estimate largely reflects renal cell carcinomas, but also includes cancers of the renal pelvis (5%), which behave more like bladder cancer, and Wilms' tumour (1%) (American Cancer Society, 2015a).

Unfortunately, there are no blood or urine tests that directly detect the presence of kidney tumours. A combination of imaging studies [ultrasound and computer tomography (CT) scan] is usually required to completely evaluate a suspected tumour (Urology Care Foundation, 2014). Very few studies on biosensors for kidney cancer have been reported. However, an American patent offers an approach for early diagnosis of renal cancer with a label-free technique. The process is based on surface-enhanced Raman spectroscopy (SERS) and/or localized surface plasmon resonance (LSPR) as transducer platforms; these methods read the fingerprint of the proteins and allow the measurement of the exact amount of these proteins in the urine. Two proteins, aquaporin-1 (AQP1) and adipophilin (ADFP) were used as markers, using imprinted artificial receptors for noninvasive and rapid screening of kidney cancer with surface-enhanced Raman scattering (SERS) and/or localized surface plasmon resonance (LSPR) in a biosensor based on metal nanostructures (Singamaneni et al., 2012). The authors suggest some ways to create the invention and obtain the proposed results. One of these methods is a paper-based plasmonic biosensor for sensitive and specific detection of

target proteins in urine for rapid screening of kidney cancer, which has been proposed in an article by the same research group (Tian et al., 2012). As a result, the created biochip offers numerous advantages such as low cost, easy storage and POC diagnosis. Besides this, paper-based LSPR substrates transformed into printable microfluidic devices to enable the detection of multiple bioanalytes in complex physiological fluids are considered one of the challenges for the future application of POC biosensors for cancer treatment.

8.4.5 Leukaemia and point of care devices

Leukaemia is one of the most known, studied and aggressive types of cancer. It starts in blood-forming tissues such as the bone marrow, and causes large numbers of abnormal blood cells to be produced and entered into the bloodstream (National Cancer Institute, 2014). Leukaemia can be divided into four main groups according to the cell type and rate of growth: acute lymphocytic leukaemia (ALL), chronic lymphocytic leukaemia (CLL), acute myeloid leukaemia (AML) and chronic myeloid leukaemia (CML). Majority (91%) of the leukaemia patients are 20 years and older (American Cancer Society, 2015a; Abdulhalim et al., 2008). A sensitive and accurate diagnosis is essential for effective treatment of this disease. Methods currently used for the detection of leukaemia cells include flow cytometry, polymerase chain reaction and fluorescence measurement (Shan et al., 2014). Accordingly, a sensitive and quantitative biosensor that can be applied in diagnostics and treatment with POC properties has been proposed (Liu et al., 2009). The device combines optical properties of gold nanoparticles and the advantages of aptamers (single-stranded oligonucleotides that bind to a specific target molecule) in comparison with conventional molecular probes that include stability, ease of production and high specificity amongst others (Jayasena, 1999). Ramos cells (lymphoma cells) interacted with aptamers conjugated with gold nanoparticles, allowing the rapid qualitative and quantitative detection of cancer cells in bloodstream at low cost; in addition, it is possible to use different aptamers to detect several types of cancer cells in the near future.

Optical transducers are common in the cancer biosensor research area. In the literature, we can find studies on fluorescence, field-effect transistors and nanoparticles amongst others (Soper et al., 2006). Surface plasmon resonance (SPR) is an optical technique that enables the characterization of biomolecular interactions on the surface in real-time without labelling, because it is sensitive to the changes in the refractive index of biomaterials at the interface between a thin gold film and an ambient medium (Abdulhalim et al., 2008). An SPR biosensor that combines the cited technique and spectral imaging has been used (Fang et al., 2011) to measure a type of specific myeloid antigen, CD33. This antigen acted as a cell surface marker for the clinical diagnosis of and to determine the preferred treatment for myeloid leukaemia. The obtained results were compared with the standard flow cytometry test, and they corroborate statistically. Additionally, this method is easy to reproduce, less time-consuming, enables direct analysis and is cheaper than the conventional methods.

DNA-based biosensors, as reported previously, are common types of biosensors for cancer detection (in any stage) and are mainly used in combination with an

electrochemical readout. An example of this combination is the work reported by Lin and collaborators (Lin et al., 2007), wherein an 18-base DNA sequence relating to Chronic Myelogenous Leukaemia (CML, Type b3a2) was used along with methylene blue (MB) as the hybridization indicator. The DNA probe was attached to a GC surface and the interaction between MB and the CML gene generated a voltammetric signal similar to the signal shown in Figure 3 in the absence or presence of the CML gene. The proposed method is very simple and sensitive and could be applied for real testing for clinical diagnostics.

Piezoelectric sensors are rarely used as transducer readouts; however, some studies have been reported, in which the authors suggest using *p*-aminophenylboronic acid conjugated gold nanoparticles (AuNPs) (Shan et al., 2014). AuNPs were used as catalysts for silver ion reduction in the presence of the reducing agent hydroquinone. A specific aptamer for human acute lymphoblastic leukaemia was immobilized on the gold quartz crystal microbalance electrode and this interaction enabled the defined cancer cells to be captured. The leukaemia cells were indirectly monitored in real time through the resonant frequency change of the quartz crystal microbalance caused by the deposition of silver metal. A good relationship between frequency response and number of cancer cells with a detection limit of 1160 cells/mL shows the promise of this method for future applications.

8.4.6 Melanoma and point of care devices

According to the National Cancer Institute (NCI) and American Cancer Society (National Cancer Institute, 2015b), melanoma is the most dangerous form of skin cancer, a malignancy of the melanocyte, the cell that produces pigments in the skin. Melanoma is most common in individuals with fair skin, but can occur in people with all skin colours. Most melanomas present as a dark, mole-like spot that spreads and, unlike a mole, has an irregular border. The tendency of developing melanoma may be inherited, and the risk increases with overexposure to the sun and sunburn.

Melanoma can be diagnosed through the monitoring of tyrosinase, a cytoplasmic melanocyte differentiation protein, which is a key enzyme in melanin synthesis and has been listed as important melanoma biomarker. Mossberg et al. (2014) developed an electrochemical biosensor platform with an amperometric detection mode to detect the enzymatic activity of tyrosinase in fresh biopsy samples without pretreatment of the samples. The combination of this method with modern portable devices can provide interesting POC sensors in the future.

An interesting approach was developed by D'Amico et al. (2008), in which they used an electronic nose sensor with a good sensitivity towards volatile organic compounds emitted by skin lesions from melanoma patients, and the method seems to be effective for identification of malignant lesions. The gas sensor used gas chromatography mass spectrometry detection and shows satisfactory accuracy. This approach does not use a biosensor but combines chemometric tools with a sensor that can be a favourable approach to detect a pattern in POC biomedical devices.

8.4.7 Lymphoma and point of care devices

The NCI defines lymphoma as a cancer that begins in cells of the immune system. There are two basic categories of lymphomas. One is Hodgkin's lymphoma, which is marked by the presence of a type of cell called the Reed–Sternberg cell. The other is non-Hodgkin's lymphoma, which includes a large, diverse group of cancers of immune system cells. Non-Hodgkin's lymphomas can be further divided into cancers that have an indolent (slow-growing) course and those that have an aggressive (fast-growing) course.

Mansur et al. (2014) have shown the potential applications for in vitro diagnosis of non-Hodgkin's lymphoma (NHL) tumours using novel multifunctional immunoconjugates composed of QDs as the fluorescent inorganic core and antibody-modified polysaccharide as the organic shell. The QDs/immunoconjugates have shown binding affinity to antigen CD20 (aCD20) expressed by malignant B-lymphocytes.

8.4.8 Lung cancer and point of care devices

Lung cancers usually form in the cells lining the air passages of the lung. The two main types of lung cancer are small-cell lung cancer and nonsmall-cell lung cancer, based on how the cells appear under a microscope (National Cancer Institute, 2015b).

The main biomarkers for lung cancer are thymidine kinase 1 (TK1) and CEA, an acidic glycoprotein of which CEA is a non-specific marker that could be elevated in breast, ovarian, lung and liver cancers. It is used to monitor cancer recurrence after surgery and to follow patients during therapy. Serum CEA levels are typically below 5 ng/mL in healthy individuals (Ronkainen and Okon, 2014). CEA studies have revealed an association between highly elevated marker levels, metastases and poor prognosis (Ronkainen and Okon, 2014). Li et al. (2010) developed a rapid and sensitive detection of nitrated ceruloplasmin, a significant biomarker for cardiovascular disease, lung cancer and stress response to smoking. The authors created a portable fluorescence biosensor based on QDs and a lateral-flow test strip, and the results hold great promise for POC and in-field analysis of protein biomarkers. Drawing on the same biomarker, Gao et al. (2011) constructed an amperometric immunosensor for detection of CEA, utilizing uniform carbon nanotubes (CNTs)-based film with gold nanoclusters and anti-CEA immobilized antibodies. This immunosensor had a detection limit of 0.06 ng/mL and good stability, showing potential for screening of CEA levels. On the other hand, Alegre et al. (2014) developed a traditional Enzyme-Linked Immunosorbent Assay (ELISA) to detect TK1 in serum.

8.4.9 Pancreatic cancer and point of care devices

In pancreatic cancer, also called exocrine cancer, malignant (cancer) cells are found in the pancreatic tissues. The exocrine cells and endocrine cells of the pancreas form different types of tumours. It is very important to distinguish between exocrine and endocrine cancers of the pancreas, because they have distinct risk factors and causes, different signs and symptoms and tests for diagnosis (American Cancer Society, 2015b).

Measurement of serum carbohydrate antigen (CHA) 19-9 has shown satisfactory sensitivity and predictive potential in pancreatic cancer patients (Kim et al., 2004). Zhang et al. (2014c) demonstrated a sandwich-type electrochemical immunosensor for the detection of CHA 19-9 antigen based on the immobilization of primary antibody (Ab1) on a three-dimensional ordered macroporous magnetic (3DOMM) electrode. The 3DOMM electrode was fabricated by introducing core–shell Au–SiO_2@Fe_3O_4 nanospheres onto the surface of a three-dimensional ordered macroporous Au electrode.

Chang et al. (2013) developed a high-throughput biosensor based on metal-enhanced fluorescence technique for detection of a pancreatic cancer marker, UL16-binding protein 2 (ULBP2), in diluted human serum. The authors describe this biosensor as a cost-effective high-throughput sandwich immunoassay; compared with the limit of detection (LOD) of the conventional ELISA method, the LOD of the proposed biosensor for ULBP2 is significantly improved 100-fold under the same conditions.

8.4.10 Prostate cancer and point of care devices

Prostate cancer affects the prostate gland, which is a part of the male reproductive system and is located below the bladder and in front of the rectum. One of the most commonly used biomarkers for prostate cancer is prostate-specific antigen (PSA), which is found at elevated levels in cancer patients (Jolly et al., 2015). PSA is a serine protease that is synthesized specifically in the epithelial cells of the prostate gland and its expression therein is regulated by the androgen receptor. The normal reference range for PSA is 0–4 ng/mL and its cancer sensitivity as well as its tissue specificity makes PSA the most useful tumour marker available for screening and managing prostate cancer (Ronkainen and Okon, 2014).

Damborsky et al. (2015) used SPR for measuring real-time quantitative binding affinities and kinetics of the interactions of specific antibodies with different epitopes of free and complexed prostate-specific antigen (PSA) to be used in microfluidic immunoassay-based platforms for POC devices. The authors describe a selective, sensitive and reliable biosensor for prostate cancer diagnosis as a lab-on-chip device.

Wan et al. (2011) describe a CNT-based, multiplexing, electrochemical immunosensor utilizing a sandwich-immunoassay type on a disposable screen-printed carbon electrode for sensitive and simultaneous determination of PSA and interleukin 8 (IL-8), another cancer biomarker.

Azmi et al. (2014) created a handheld, POC system, in which a silicon nanowire biosensor (SiNW) chip is wire-bonded to a ‘bio-smartcard’ and subsequently slotted into an electronic readout device. This sensor detects an oxidative stress biomarker, 8-hydroxydeoxyguanosine (8-OHdG), which has been related to prostate cancer risk.

8.4.11 Thyroid cancer and point of care devices

The thyroid gland is an organ at the base of the throat that produces hormones that help control heart rate, blood pressure, body temperature and weight. The four main types of thyroid cancer are papillary, follicular, medullary and anaplastic thyroid cancers, based on how the cancer cells appear under a microscope (National Cancer Institute, 2015b).

Thyroglobulin (Tg) is used as a tumour marker for thyroid cancer, and is used post-surgically to monitor disease recurrence (Krahn and Dembinski, 2009). Burne et al. (2005) describe POC assays for autoantibodies to thyroid peroxidase (TPO) and to thyroglobulin. Both assays are based on the ability of autoantibodies in test samples (whole blood, plasma or sera) to inhibit the binding of monoclonal antibodies to TPO or to Tg. These assays require no special equipment and yield results in 10 min.

Choi and Chae (2009) created a microfluidic sensing platform for the detection of Tg using competitive protein adsorption. The authors engineered two surfaces covered by two known proteins, immunoglobulin G (IgG) and fibrinogen, with different affinities. This microfluidic device offers selective protein sensing by being displaced by a target protein, Tg, on only one of the surfaces. The adsorption and exchange are evaluated by fluorescent labelling of these proteins.

Thus, POC biosensors could be used to detect cancer with the help of different biomarkers. Further research in this area is warranted for this method to be applicable and accessible to the public. Additionally, the field of laboratory medicine also needs some modifications (DuBois and Clarke, 2014). Keeping the future in perspective, Soper et al. (2006) made a list with 11 points that should be considered for the future development of POC biosensors. In two of these points, they mention the necessity of (1) developing POC biosensors based on a molecular signature or a panel of signatures, like a fingerprint for cancer. In this regard, we believe that it is important to include the necessity of (2) combined POC biosensors with chemometrical approaches such as pattern recognition tools to evaluate this complex signature. These approaches could include, for example, Principal Components Analysis (PCA), Artificial Neural Networks (ANN), K-nearest neighbours (KNN) and Soft Independent Modelling of Class Analogy (SIMCA). The other nine points are important to (3) increase the selectivity of biosensors due to the complexity of sample media, (4) work in tissue samples, (5) process small amounts of sample, (6) decrease detection limits and costs. In addition, (7) develop sensors set apart for screening, (8) decrease the number of false positives and false negatives, (9) make devices for real-time monitoring, (10) integrate POC with sample handling and processing and (11) develop companion devices for research. For the two last points, we believe that the incorporation of multivariable calibration and detection is important to make devices for simultaneous detection of different types of cancers.

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Point of care (POC) blood coagulation monitoring technologies

9

L. Harris[1], *R.S. Lakshmanan*[2], *V. Efremov*[2], *A.J. Killard*[3]
[1]Dublin Institute of Technology, Dublin, Ireland; [2]Dublin City University, Dublin, Ireland;
[3]University of the West of England, Bristol, United Kingdom

9.1 Introduction

9.1.1 Brief historical perspective

Although the monitoring of blood disorders or bleeding times has been in existence for many years with clot-based assays, the modern era of coagulation monitoring began with the introduction of automated coagulation analysers.[1] Many of the first coagulation analysers operated on the basic principle of the time taken for blood to coagulate, indicating a normal, rapid or prolonged bleeding time. As knowledge accumulated and research advanced in the area of coagulation and clotting disorders, early simple clotting assays were modified. Researchers in the 1950s realised that upon contact with certain 'foreign' materials including glass, kaolin and silica, blood would clot more rapidly in vitro.[2] Although the in vivo situation is more complex as blood is exposed to injured vessels and natural contact factors, both in vitro and in vivo clotting processes are reliant on the presence of so-called thromboplastins, which are now understood to be a combination of either tissue factor and/or platelet phospholipids. Using this information, several manual clot-based tests have been developed and have been used as indicators of clotting disorders and have been adapted for use in automated laboratory analysers.

9.1.2 Traditional laboratory-based testing

Laboratory-based testing has now become reliant on automation, the output of which includes high-throughput analysis, rapid turnaround times, improvements in diagnostic accuracy and assay precision.[3] Such technological advances have a knock-on effect in the clinical setting including improved patient care and treatment. This has been particularly evident in the area of coagulation and haemostasis. Blood-clotting disorders were traditionally monitored using the bleeding time at the bedside, involving a measurement of the length of time that blood flows following rupture to the skin. Such simple tests evolved into the clotting-time tests we are familiar with today, because of the research on the coagulation cascade and the interplay of its activators.[4] Research and assay development has expanded the panel of clotting-time tests, such as the prothrombin time (PT), the activated partial thromboplastin time (aPTT), the activated clotting time (ACT) and the thrombin time (TT) or thrombin clotting time (TCT) over the years, rendering it

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more convenient to the central laboratory to have automated systems in place.[1,5] Central laboratories are no longer reliant on the visual detection of clot formation as coagulometers or haemostasis instruments use a range of automated detection methodologies including optical, electrochemical or mechanical. In addition, automation offers the added benefit of improved standardisation of testing across laboratories provided the same instrumentation and reagents are used, which is of particular importance in haemostasis measurements owing to the physiological complexity of the coagulation cascade.

9.1.3 Introduction to point of care coagulation testing

Point of care testing (POCT) or near-patient testing involves the use of miniaturised medical devices and assays to test a patient and is performed outside the physical facilities of the central clinical laboratory. Results are typically obtained in relatively short times, ultimately improving patient care and empowering patients as it allows them to take more responsibility for their own health.[6] POC testing first became available in the form of glucose and pregnancy tests, both of which are excellent examples of the successful implementation of home testing.[7] The rapid uptake and acceptance of these methods has driven further research into the development of miniaturised diagnostics and biosensors. The development of such technologies usually involves the integration of a biological assay onto a miniaturised chip that also houses a detector – essentially the combination of a biological component with a physicochemical detector component.[8]

The advantages of POCT include features such as simple sample collection, faster turnaround times resulting in more timely treatment which is imperative in anticoagulant administration in the self-testing, in-patient or emergency-care settings.[9] Traditional clotting tests, including the PT, aPTT and ACT were first adapted from their bench-top formats into POC devices.[10] Based on their dependence on clot formation, these assays were easy to transfer to miniaturised devices, but also suffered from drawbacks including poor specificity, reproducibility and reliability. As a result, subsequent research has been conducted in the development of more factor-specific assays such as D-dimer, thrombin and fibrinogen which then became targets for POC assay development.[11] Although knowledge of specific coagulation factors is crucial to understanding the process of coagulation, clinicians and researchers alike are now searching for tests that offer an insight into a patient's overall clotting processes. The future of POCT in coagulation and haemostasis is focused on the development of a global coagulation test which has the potential to measure the coagulation process under more realistic physiological conditions and be more representative of the in vivo situation. In addition, global haemostatic tests that can be used reproducibly in different laboratories will allow for improved standardisation of testing.

9.2 Development of point of care coagulation monitoring devices

9.2.1 Clot-based point of care assays

The evolution of POC technology began with the need for rapid turnaround of results to improve patient care. A variety of technologies were initially developed that were

essentially adaptations of some of the traditional assays of coagulation. These conventional assays measure clotting time via the intrinsic and extrinsic pathways of the coagulation cascade, and include PT, aPTT, ACT and the TT or TCT tests. Each assay measures a different part of the coagulation cascade[5] (Fig. 9.1). For example, the PT uses tissue factor (TF) as an activator of clotting via the extrinsic pathway. TF is usually activated, exposed and released upon damage to the vascular endothelium which initiates the sequential activation of coagulation factors, leading to the formation of

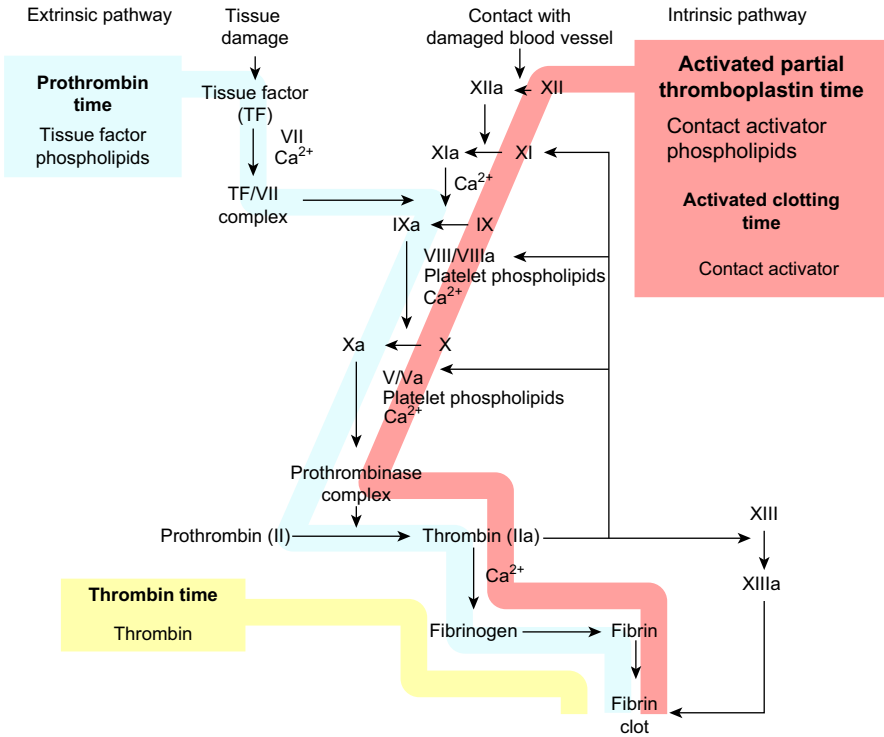

Figure 9.1 The coagulation cascade and clotting-time tests. The coagulation cascade has two primary, interconnected pathways: the extrinsic and intrinsic pathways. In the intrinsic pathway, damage to vessel walls reveals negatively charged surface activators which cause the sequential activation of coagulation factors (indicated by Roman numerals) which eventually leads to the formation of thrombin and the conversion of soluble fibrinogen to insoluble fibrin clot. The extrinsic pathway is activated via damage to the epithelium and the release of tissue factor (TF) which also leads to clot formation. The Prothrombin Time (PT) test mimics activation via the extrinsic pathway through the addition of TF and phospholipids. The Activated Clotting Time (ACT) test uses contact activator to measure high heparin dosage in whole blood. The Activated Partial Thromboplastin Time (aPTT) test uses contact activator and phospholipids in plasma to more rapidly activate the contact pathway. The Thrombin Time (TT) test adds thrombin to plasma to measure fibrinogen.
Adapted from Harris LF, Castro-López V, Killard AJ. Coagulation monitoring devices: past, present, and future at the point of care. *TrAC Trends Anal Chem* 2013;**50**:85–95. Copyright Elsevier.

thrombin and the conversion of fibrinogen to fibrin to form a blood clot. The PT assay has been the only coagulation assay to have been effectively standardised via the International Normalisation Ratio (INR). The aPTT assay is a measure of the intrinsic or contact pathway and involves the in vitro activation of Factor XII (FXII) in plasma using a negatively charged 'contact activation' material such as glass, celite or kaolin in the presence of phospholipids (partial thromboplastin). This test is sensitive to deficiencies in several clotting factors, most notably those involved in haemophilia (FVIII, FIX and FXI), as well as being sensitive to the effect of anticoagulants such as heparin. It should be noted that the extrinsic pathway is the in vivo pathway to activation of coagulation. The ACT is also based on activation of the intrinsic pathway, but uses whole blood in combination with a contact activator to initiate clotting. It is typically used to measure high doses of heparin within the surgical environment that are out of the range of aPTT measurements. Finally, the TT assay is a simple assay in which thrombin is added in excess to a plasma sample, and the time taken to clot is proportional to the concentration of fibrinogen in the sample. This can be further quantified by comparison with standard fibrinogen concentrations using the Clauss assay.

A variety of technologies have been developed to automate these assays which employ mechanical, optical and electrochemical detection methodologies. The prothrombin time/International Normalisation Ratio (PT/INR) assay has been widely exploited in terms of application to miniaturised POC devices and is by far one of the more successful assays commercially available in a miniaturised device format. The PT/INR is used to measure warfarin treatment, which remains one of the most widely used oral anticoagulants since its introduction into clinical medicine by Karl Paul Link in the 1950s.[12] By combining the assay with microfluidics, Roche then introduced the CoaguChek device in the 1990s for PT self-testing to monitor warfarin therapy. In the original device, a blood sample is applied to a disposable test strip containing a thromboplastin reagent and iron filings. An electromagnetic field moves the filings, which is detected optically. Upon clot formation, the movement ceases which is returned as the clotting time. However, recent versions of the device now employ a fully electrochemical transduction mechanism which employs an electrogenic thrombin substrate. Other devices include the Alere INRatio device which uses electrochemical impedance in combination with the PT/INR assay to generate clotting-time measurements.

9.2.2 Fibrinolysis

Fibrinolysis refers to the dissolution of a clot. Plasminogen is an inactive circulating precursor of the enzyme plasmin, which cleaves fibrin. Plasminogen is activated by a number of activators, notably tissue plasminogen activator (tPA). Measurement of fibrinolysis is now being targeted by many new POC devices as this process is extremely important in haematology and cardiovascular medicine. Although methods such as thromboelastography (TEG) measure clot dissolution (discussed further in section in viscoelastic methods), fibrinolysis-specific tests [plasmin, tPA and plasminogen activator inhibitor (PAI)] are no longer present on the standard test panels for thrombophilia testing as the prevalence of fibrinolytic disorders is low.[13] Lysis of fibrin results in fibrin degradation products (FDPs) which include D-dimer. Current

testing focuses on measurement of FDPs and D-dimer specific assays which reflect the amount of fibrin degradation and can identify disorders of the fibrinolytic system or diagnose venous thrombosis, as D-dimer is specific for degradation of cross-linked fibrin and is only present following clot formation.[14] Several POC tests are available for monitoring D-dimer levels in emergency-care settings to rule out venous thromboembolism (VTE). Sidelmann and colleagues evaluated the Radiometer AQT90 FLEX POC system for D-dimer determination in whole blood.[15] This particular POC device is an antibody-based assay, with all reagents in dry form within a special cup. Upon completion of sample incubation with the assay reagents, including a europium-labelled antibody, the europium signal detected is proportional to the D-dimer concentration. Europium exhibits long-lived fluorescence and is useful as a label in time-resolved fluorescence measurements which increase sensitivity by allowing background biofluorescence to decay before measurement of the label.

9.2.3 Thrombin

Thrombin generation assays (TGA) first evolved from standard clotting tests such as the PT, aPTT and TT, as they are essentially measuring the formation of thrombin through the formation of a fibrin clot. With the advancement of colorimetric assays and thrombin-specific peptide substrates, thrombin could be measured directly, eliminating interference from biological variables that often affect clot-based assays. In addition, clot-based assays are only truly reflective of the first burst of thrombin, whereas a measurement of the amount of thrombin is a better marker of clotting function.

Many of the early POC assays that used thrombin as an indicator of clotting time were developed using electromechanical or electrooptical detection methods. The most well known of these was the CoaguChek S (Roche Diagnostics) which used magnetic particles moving in a magnetic field with an optical detection system to detect cessation of movement when clotting had occurred. However, devices that are more recent have employed less complex and less costly electrochemical methods, which also eliminate issues relating to sample colour and turbidity. Commercially available devices such as the CoaguChek XS (Roche Diagnostics) and the i-STAT (Abbott Diagnostics) are simple biosensors that incorporate both electrodes and electrogenic substrates that are cleavable by thrombin.[11] Although these two devices focus on the return of a clotting time, there are POC tests that can directly measure thrombin activity. A single-use miniaturised electrochemical sensor that houses both clotting activators and an electrogenic substrate specific for thrombin was shown to effectively measure thrombin in whole-blood samples of 10 μL volume.[16]

9.2.4 Fibrinogen

The Clauss assay is the most widely employed method for the measurement of functional fibrinogen, measuring fibrinogen-dependent clot formation.[17] With this assay, fibrinogen concentration is inversely proportional to the time recorded for clot formation. The Clauss assay is commonly performed on an automated coagulometer, although

a simple, single-use microfluidic device was reported by Dudek et al.[18] who claim to measure fibrinogen concentration in the normal range (1–7 g/L) with a turnaround time of 5 min. The concentration of fibrinogen is related to the distance travelled by the sample on a lateral flow device that contains biological activators of clotting.[18]

Fibrinogen measurements are also recorded in the central laboratory using the prothrombin time-fibrinogen (PT-Fg), which is an assay based on clotting time. Fibrinogen concentrations are measured because of the change in optical density or light scatter on an automated coagulometer.[19] Although few POC tests have been developed in this space for human diagnostics, POC fibrinogen assays are popular in veterinary diagnostics. One example is the Solo Fibrinogen Test (Euro Lyser Diagnostica) which uses an immunoturbidimetric assay and light scatter, all incorporated into a miniaturised bench-top instrument and returning a result within 8 min.

9.2.5 Antifactor Xa assays

The current standard assay for monitoring antifactor Xa (FXa) levels uses a simple enzymatic assay and relies on colorimetric detection. This 'gold standard' assay is the result of the development of peptide substrates in the 1960s specific for a range of different coagulation proteins, thrombin being one of the first targets for this type of assay development. FXa is located at a critical junction in the coagulation cascade and in addition to the increased administration of low molecular weight heparin (LMWH) therapy, the need for monitoring was realised. The anti-Xa assay works on the principle of FXa cleavage of an FXa-specific peptide substrate with an attached chromophore. Upon addition of excess FXa to a heparinised patient sample, a heparin–antithrombin (AT) –FXa complex forms and as FXa cleaves the substrate, the released chromophore is detected colorimetrically and is indirectly proportional to the heparin concentration in the sample (Fig. 9.2).

Anti-Xa assays show promise for monitoring of the newly developed FXa inhibitors. Miniaturisation of the assay and incorporation into a biosensor-type platform has been reported in the literature.[20] The anti-Xa assay was initially developed using fluorescence as the method of detection, instead of the standard absorbance assay[21] and was shown to work with a range of anticoagulant drugs. The disposable biosensor correlated well with standard chromogenic and fluorogenic assays for a range of antithrombotics with rapid turnaround times of 60 s. Further development for a fully integrated device is continuing.

9.2.6 Techniques to measure blood viscoelasticity

Thromboelastography (TEG) was first described by Dr. Hellmut Hartert in 1948[22] and was trademarked by Haemoscope Corporation in 1996 as TEG. It is used to describe the trace produced from the measurement of the viscoelastic changes associated with blood coagulation process. The TEG can be considered as a simplified version of the conventional cone and plate rheometer. Similar devices such as ROTEM (Pentapharm GmBH) and Sonoclot Analyzer (Sienco Inc.) are now commercially available that use similar principles with differing technology to

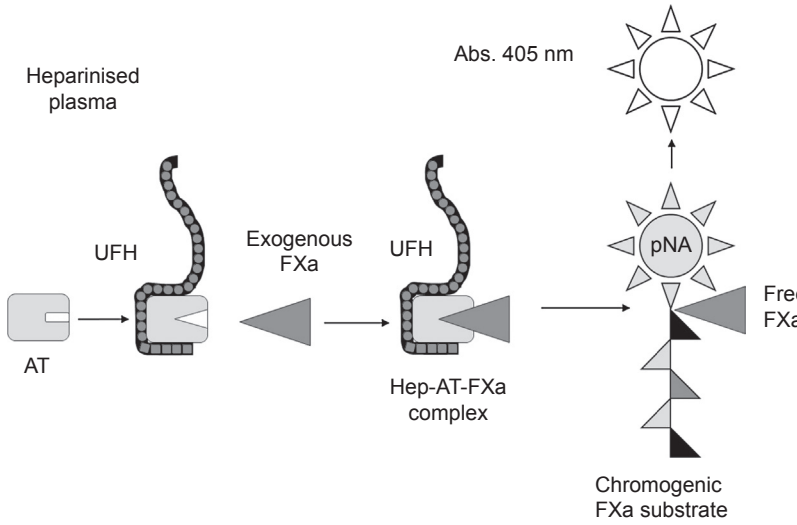

Figure 9.2 Anti-Xa chromogenic assay principle used in the measurement of heparin. Adapted from Harris LF, Killard A. Heparin monitoring: From blood tube to microfluidic device. In: Piyathilake DE, Liang R, editors. Heparin, properties, uses and side effects. New York: Nova Science Publishers (2012).

produce the characteristic traces to assess global haemostatic function by monitoring viscoelastic changes.

The TEG system consists of a cup that holds the blood sample and oscillates through an angle of 45′ in 10 s. A pin is suspended in the blood by a torsion wire and is monitored for motion. As blood starts to clot, the fibrin formation and its interaction with platelets links the motion of oscillating cup to the pin and thus the torsion wire. The motion of this torsion wire is transformed into a displacement (displayed on a trace in millimetres) dependent on the strength of the formed fibrin-platelet links. ROTEM has a similar cup and pin arrangement but differs from TEG in that the deflections are measured optically. In addition, in ROTEM the cup containing the blood is stationary and the pin is oscillated and the resistance to the rotation of the pin is quantified as the strength of the forming blood clot. A schematic of the TEG and ROTEM traces generated along with the parameters measured are shown in Fig. 9.3 and the main parameters of TEG/ROTEM curves analysis are shown in Table 9.1.

TEG and ROTEM have found numerous applications in assessing patient coagulation status, particularly in surgery and trauma care. The TEG/ROTEM is a very useful tool for global coagulation and fibrinolytic monitoring and has been used in cardiac and liver units for many years to assess fibrinolysis and the efficacy of antifibrinolytic therapy.[23] The investigation of novel antifibrinolytic therapies can also be undertaken using this technology.[24] The use of the TEG to monitor the effect of unfractionated heparin, low molecular weight heparin and heparinoid has also been assessed.[25] The haemostatic monitoring of other anticoagulants such as hirudin has also been accomplished using the TEG.[26]

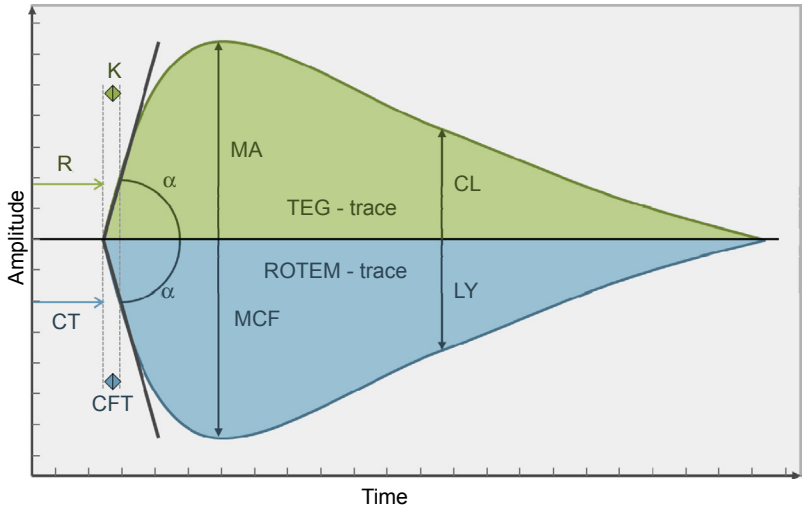

Figure 9.3 Schematic illustrating TEG and ROTEM traces.

Table 9.1 List of main TEG/ROTEM parameters

TEG parameter	ROTEM parameter	Description
R Value (reaction time)	CT (clotting time)	Time taken for the trace to exceed a threshold value (typically 2-mm displacement) due to clot formation.
K Value (clot formation time)	CFT (clot formation time)	The time (s) between an increase in amplitude from 2 to 20 mm
α angle	α angle	The tangent to the clotting curve through the 2-mm point.
MA (maximum amplitude)	MCF (maximum clot firmness)	The MA (mm) reached in the tracing, which correlates with platelet count, platelet function and the concentration of fibrinogen.
LY60	LI (Lysis Index)	Amplitude change percentage measured at 60 min of achieving maximum amplitude.
TMRTG (time to maximum rate of thrombus generation)	t-maxVel (time to maximum velocity clot curve.)	Time to reach the maximum of first derivative of displacement trace.
MRTG (maximum rate of thrombus generation)	maxVel (maximum velocity of clot curve.)	First derivative of displacement trace.

9.2.6.1 Cardiac surgery

Bleeding is a major cause of morbidity during long and complex cardiac surgery. Cardiac surgery causes an imbalance in the haemostatic system of a patient resulting in an increased risk to bleeding, particularly for patients with an underlying haemostatic disorder, and thus requiring transfusion of blood products. This contributes to significant perioperative cost, particularly towards coagulation management, with anticoagulants during surgery and in maintaining hemostasis after surgery. Thus, there is a need for a rapid and reliable method to guide clinicians with optimal transfusion management algorithms. Conventional tests such as aPTT, PT, fibrinogen, platelet count and platelet activity assays are time-consuming (about 1 h) to guide transfusions for severely bleeding patients. The TEG and ROTEM measurements being a global haemostatic measure are capable of providing the required information in less than 20 min.[27] TEG, ROTEM and Sonoclot analyser have been used as rapid and cost-effective POC viscoelastic devices to guide transfusion algorithms.[28–30] Although von Kier et al.[31] and Shore-Lesserson et al.[32] showed a clear advantage based on a TEG-guided algorithm for blood transfusion to be better than the traditional POC devices, Spalding et al.[28] showed similar results with the use of an ROTEM-guided transfusion algorithm in which they show that in study of about 700 patients undergoing surgery resulted in a 25% reduction in RBC units, 50% decrease in platelet concentrates in terms of costs. There have been extensive studies suggesting the utility of this type of viscoelastic testing in guiding transfusion therapies during cardiac surgery.

9.2.6.2 Trauma

In severe trauma injury, an understanding of the haemostatic balance is of critical importance. This is because during the acute phase it is important to control and manage excessive bleeding and haemorrhage and after treatment it is important to prevent hypercoagulability or thromboembolism, thus a better understanding of the haemostatic balance using a thromboelastograph is significantly better than the conventional coagulation screening tests.[33] In a recent detailed review, Da Luz et al.[34] have investigated the literature on efficacy of TEG/ROTEM in critical trauma care and found that the main shortcoming is the unavailability of a gold-standard method with which to compare the tests. However, the reports suggest the utility of TEG in predicting hypocoagulability, hypercoagulability, platelet dysfunction and fibrinolysis.[35,36] Kane et al.[37] suggested that a longer reaction time (R-value in TEG) was an indicator of mortality with 52% of pelvic injury cases resulting in death. Sankarankutty et al.[38] in their review suggested that TEG and ROTEM are superior in providing results of hyperfibrinolysis better than any other conventional tests. Levrat et al.[35] used ROTEM and found that trauma patients exhibiting hyperfibrinolysis were severely injured, with coagulation factor irregularities and eventually resulting in death. Jeger et al.[39,40] showed the utility of RapidTEG, a tissue factor formulation used as the activator, was better at providing rapid information on coagulopathy for trauma patients.

9.2.6.3 Thrombophilia and haemophilia screening

In the laboratory, the 'global' aspects of the TEG are used to identify or monitor specific defects. It has been demonstrated that the TEG can clearly distinguish a group of patients as hypercoagulable from a cohort of patients who have experienced a venous thromboembolic (VTE) event. However, this hypercoagulable group is not necessarily the same as the group identified by routine thrombophilia screening.[41] It was demonstrated that 34% of patients had a positive thrombophilia screen, whereas 45% had a positive TEG trace. This study did show that some patients fell into the hypercoagulable group by both screening methods (33% of those with an abnormality) and questions whether these individuals are at greater risk of further thrombotic events.

Furthermore, it was suggested that those individuals with a positive history of VTE, hypercoagulable TEG and normal routine thrombotic screening may be good candidates for linkage analysis to identify novel constitutional or acquired thrombophilic traits.[41] It has been reported that current thrombophilia screening tests do not predict for future risk of thrombosis.[42] Currently, the significance of an abnormal TEG in patients referred for thrombophilia screening with regard to future thrombotic risk is unknown. A prospective surgical study investigated the relationship between preoperative TEG parameters and the risk of postoperative VTE.[43] They found that the maximum amplitude (MA) of the TEG was predictive of deep vein thrombosis (DVT) development with a sensitivity of 72% and a specificity of 69%. Further work needs to be carried out to determine whether an abnormal TEG/ROTEM alone or in combination with another finding can identify a group of patients at increased thrombotic risk. A newer TF-based activation method on TEG, called the rapid TEG, has been used to identify hypercoagulation[36] and in study of coagulopathies in trauma.[39]

There are few reports of the use of TEG/ROTEM for the screening of inherited bleeding disorders. When the use of the TEG/ROTEM has been reported in inherited bleeding disorders, it has been in association with the monitoring of therapeutic intervention using factor concentrates, deamino-delta-D-arginine vasopressin (DDAVP), fresh frozen plasma (FFP), cryoprecipitate, activated prothrombin complex concentrate or recombinant activated factor VII.[44–46] Quite recently, the ROTEM has been shown to detect minor changes in factor XIII with a deficiency being associated with reduced maximum clot firmness.[47] The TEG/ROTEM testing has been used to monitor activated-prothrombin complex concentrates and recombinant activated-factor VII in the treatment of haemophilia A patients with specific factor inhibitors and rare coagulation disorders.[44–46]

There has been a wider acceptance of TEG for coagulation monitoring due to its ability to provide coagulation profiles in an acceptable time. However, one of the disadvantages of TEG and similar methods is the presence of moving mechanical parts that induce a significant strain interfering with the clotting sample. This, in some cases, results in an underestimation of clot strength. The TEG also lacks standardised methods and thus has an inherently large coefficient of variability.[48] These tests also do not consider the effect of endothelial cells on the formation of a clot, though there has been a one-off study looking at the effect that endothelial cells have on this system,[49]

which can provide a better understanding of the in vivo processes. A large amount of literature suggests that TEG/ROTEM tests are currently being used for diagnostic purposes and more widely in surgery and trauma; however, whether the TEG and ROTEM can be compared directly would require standardisation of the reagents used for activation.[38]

9.3 Novel point of care coagulation tests and recent innovations

9.3.1 Ultrasound elastography

Sonorheometry is a method developed at the University of Virginia, United States, that measures blood coagulation with a combination of acoustic radiation force and ultrasonic motion tracking.[50–52] The acoustic waves propagate through coagulating blood and a 'relative compliance' as a function of time is measured. Essentially, the relative compliance reflects the ability of a blood/clot to be a conductor of acoustic waves. A typical curve for relative compliance provided by this device, very similar to a TEG trace, is used to measure the following parameters: two clotting times indicating start and end of fibrin formation (TC1 and TC2, respectively), time for start and end of fibrinolysis (TL1 and TL2, respectively), clot formation rate (CFR) and minimum compliance (S) (shown schematically in Fig. 9.4). Furthermore, it is hypothesised that the basics of diagnostic curve obtained are similar to the TEG method.

Viola et al.[50] have shown that the measurement system evaluates contributions of fibrin using glycyl-L-prolyl-L-arginyl-L-proline (GPRP), a potent inhibitor of fibrin formation, contributions of platelets by analysing the effect due to blocking the glycoprotein IIb/IIIa (GPIIb/IIIa) receptor and the effect of fibrinolysis to the physical properties of the clot. Mauldin Jr. et al.[52] also established the efficacy of the method in studying effects of different heparin concentrations on the trace characteristics. Sonorheometry shows great promise mainly due to the fact that it provides viscoelastic information, without any moving mechanical parts (unlike in TEG, ROTEM and Sonoclot) and thus prevents large strains from interfering with the forming clot. However, a wider study on patients with differing clinical aetiologies has not been evaluated and the system has to go through several optimisations for compatible assays and reagents for measuring coagulation.

9.3.2 Electrochemical detection and impedance-based methods

Research in the area of electrochemical sensors that detect changes in blood and blood chemistry has been ongoing since the early 20th century.[53,54] The advancement of technologies such as screen-printed electrodes has allowed for the fabrication of miniaturised, disposable electrochemical sensors that are well suited for integration into POC technologies.[55] High levels of sensitivity and selectivity are associated with these new electrochemical sensors which allow for the rapid, simple and sensitive detection of biological targets that would otherwise go unnoticed.

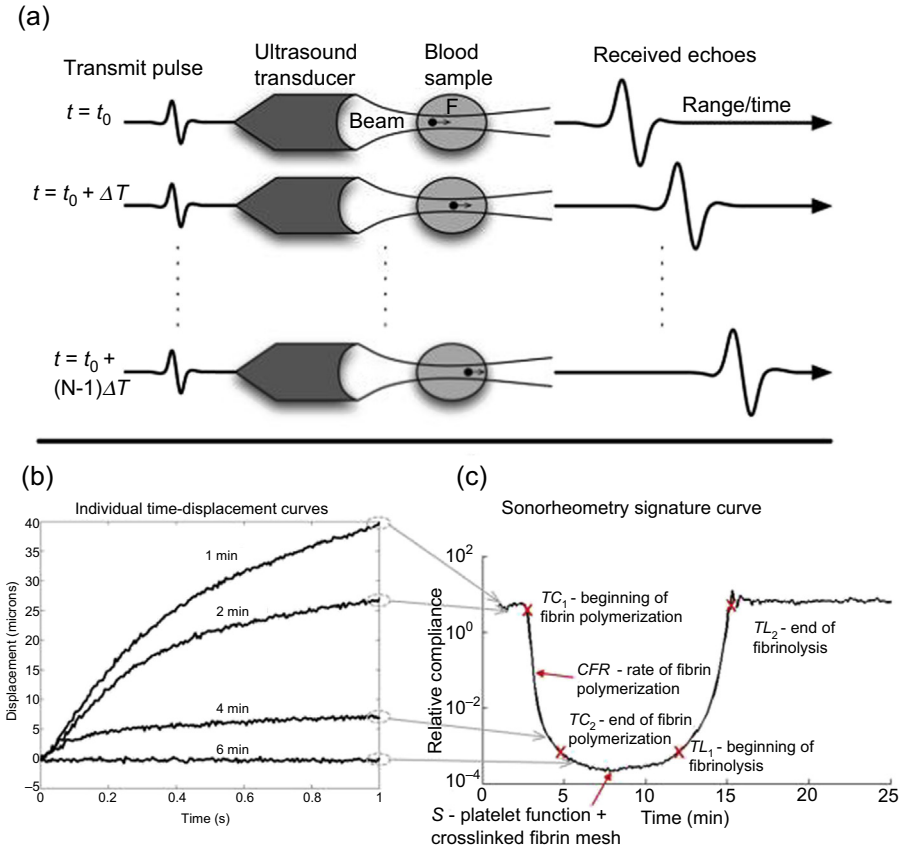

Figure 9.4 Typical sonorheometry principle and graph of relative compliance as a function of time.
Taken from Viola F, et al. A novel ultrasound-based method to evaluate hemostatic function of whole blood. *Clin Chim Acta* 2010;**411**:106–13.

Electrochemistry works using the principles of oxidation–reduction reactions which generate electric currents or, more simply, the conversion of chemical information into an electrical signal. Electrochemical cells or sensors usually contain a working electrode, to which a potential is applied, and a reference electrode. The oxidation–reduction reaction that ensues is then recorded as an electric current which is a measurement of the analyte from the reaction.[16] Electrochemical methods can be further subdivided into amperometric (measures current), potentiometric (measures potential), conductometric (measures the conductive properties of the medium), impedimetric (measures resistance and reactance) or field effect (measures current through charge accumulation at a gate electrode).[56]

Typical electrochemical biosensors (Fig. 9.5) are made up of a bioreceptor which binds to the biological target, the interface architecture within which the biological event occurs and generates a signal, which is picked up by the transducer. The

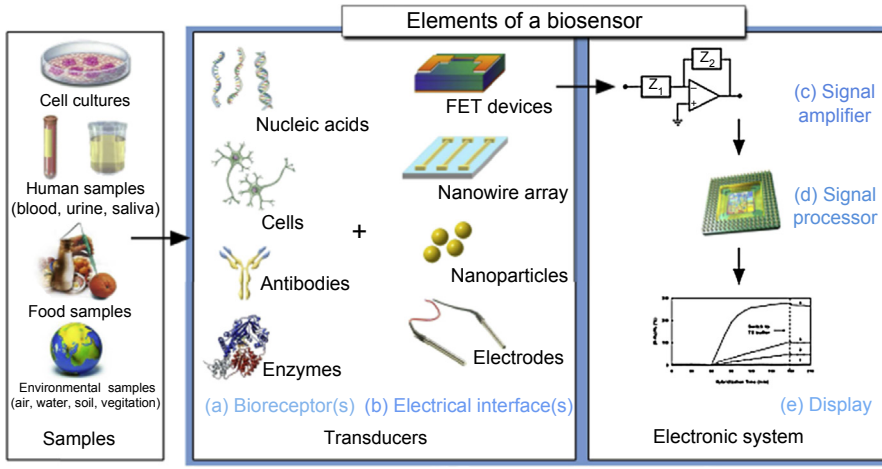

Figure 9.5 Components of a typical electrochemical biosensor.
Taken from Grieshaber D, MacKenzie R, Vörös J, Reimhult E. Electrochemical biosensors – sensor principles and architectures. *Sensors* 2008;**8**:1400–58.

transducer signal is then converted to an electronic signal which is amplified and processed using computer software.[56]

As technology evolves, diagnostic devices are becoming more advanced in addition to the detection and monitoring of more complex analytes and biological processes. One such recent development is a high-throughput microfluidic device that uses real-time electrical impedance to monitor blood-coagulation processes.[57] One of the most complex and dynamic physiological processes, coagulation poses many difficulties in terms of monitoring and analysis of the various components of the process. Electrochemistry has been advocated as the best method for whole-blood coagulation-time measurement, the reproducibility of the generated signals, in addition to device miniaturisation and disposability are all favourable in the development of POC devices.[57] The microfabricated chip developed in this study comprised glass and polydimethylsiloxane (PDMS) with seven wells, each containing a pair of aluminium electrodes. Blood samples were loaded into the wells and coagulation was monitored under varying temperatures and haematocrit concentrations. Results indicated that impedance measurement of coagulation time is as fast and reliable as traditional methods and is a promising technique for incorporation in the clinical setting.

The use of electrical impedance to monitor blood coagulation has been around since 1948[58] and has found a wider acceptance in measuring haematocrit levels and erythrocyte sedimentation rates.[59] As technology evolves, microfabrication techniques are enabling miniaturisation of diagnostic devices making them highly suitable for a POC setting. This has led to few high-throughput microfluidic devices,[57,60,61] which utilise microfabricated microfluidic chips with electrodes to monitor impedance changes in a clotting-blood sample. The changes in impedance across electrodes provide a trace of the entire coagulation process. Lei et al.[57] have studied a simple planar aluminium two-electrode setup housed in a glass and a PDMS chip. They provided

an equivalent circuit representation of the blood in contact with the electrodes by studying effect of haematocrit on impedance changes occurring at a certain frequency during recalcification of citrated blood. However, the studies are very preliminary and are of interest mainly due to their size requiring only about 1 μL of a blood sample. Another POC impedance-based device developed by Ramaswamy et al.[61] comprised a two-channel patterned-electrode (Au/Cr) structure on glass, with a capillary flow PDMS channel to deliver blood to the electrodes. Upon addition of the blood sample with the activators, the coagulation response was recorded and a difference in clotting and nonclotting samples was observed. The authors also tested the impact of heparin on clotting and spiked blood with increasing concentrations of heparin proportional to increasing clotting times. The reproducibility of this device has potential applicability in the monitoring of cardiovascular and coagulation disorders.

The combined use of aptamers and electrochemistry is quite a popular area of research in the diagnostic device space. Aptamers are short single-stranded in vitro ribonucleic acid (RNA) or deoxyribonucleic acid (DNA) molecules and have been used to replace antibodies owing to their chemical versatility and enhanced selective binding to their targets.[62,63] Amongst the newer technologies reported in the literature, aptamer-based recognition is frequently employed to target thrombin with over 900 publications on thrombin aptamers to date.[62] One such reported device can determine thrombin levels on nanochannels using voltammetric detection.[64] Using a highly specific aptamer/thrombin binding interaction in conjunction with a nanoporous material for the sensor, thrombin was detected in whole-blood samples at picomolar levels which are in the clinically relevant range.

Thrombin, a major protein in coagulation was targeted by Xu et al. for the development of a label-free impedimetric aptasensor for monitoring of thrombin.[65] A histidine-labelled thrombin aptamer was immobilised onto electropolymerised poly [pyrrole-nitrilotriacetic acid (NTA)] Cu_2 electrode films. The response of the sensor to thrombin, without a labelling step, was measured using impedance and a linear response was recorded. Not only does this device allow for label-free monitoring of thrombin, it also has a renewable sensing layer which are both promising characteristics in terms of ease of application to clinical measurement.

Another thrombin biosensor was also developed using synthesised hyperbranched polyester microspheres with carboxylic acid functional groups, ultimately developing a label-free electrochemical aptamer sensor. A thrombin-binding aptamer was used as a receptor for measuring thrombin levels, and hyperbranched polymers were investigated in this instance to prevent biofouling of the electrode surface after application of whole-blood samples resulting in a wide detection range and a low limit of detection.[66] The combination of gold nanoparticles and aptamer-based electrochemistry into a diagnostic device was reported to allow for the detection of thrombin.[67]

9.3.3 Optical detection

Optical detection techniques are also incorporated into microfluidic devices, as many of the traditional coagulometers use the principle of changes in optical density to compare clotting and nonclotting samples. The feasibility of miniaturising optical detectors

has also allowed for the development of optical-based POC devices. Although many POC devices using the prothrombin (PT) method of clotting activation, a device has been developed that uses an optical sensor, an electrical processing and control circuit to monitor changes in coagulation once activated by a PT reagent.[68] The sensor comprised a glass slide to which the blood sample and activator were added. To monitor the change in transmitted light intensity, a light-emitting diode (LED) was used as a transmitter and a photodiode detector. Performance of the optical device was compared to standard assays and high correlations were recorded for 153 whole-blood patient samples. In addition, fibrin formation was also measured adding another dimension to this test, in addition to its low cost and rapid turnaround of results, making it an ideal replacement for standard coagulometers.

Nanoparticles are increasingly used in the diagnostic and drug delivery space owing to their unique optical and physical properties and their multifunctionality (Fig. 9.6). Nanotechnology has led to many discoveries in the biodiagnostics, drug delivery and cancer therapy.[69] Gold nanoparticles in particular have received substantial recognition from the research community because of their compatibility with biological applications, chemical stability and ease of fabrication.[70]

An example of nanotechnology in the development of coagulation diagnostic devices includes a label-free assay using gold nanoparticles to detect coagulation proteins, thrombin and fibrinogen.[71] Thrombin cleavage of fibrinogen to fibrin causes aggregation of Au nanoparticles which allows for the sensitive detection of thrombin. Using thrombin aggregated Au nanoparticles, fibrinogen can be analysed through fibrinogen-induced aggregation of thrombin Au nanoparticles.

A more novel microfluidic platform to perform multiplexed hypercoagulability panel testing for anthithrombin, protein C, protein S and factor VIII antigens using a droplet of blood, was developed by one research group.[72] An on-chip Enzyme-Linked Immunosorbent Assay (ELISA) was developed using magnetic beads coated with antibodies and a fluorescence signal was generated. On-chip assays correlated well with bench-top assays.

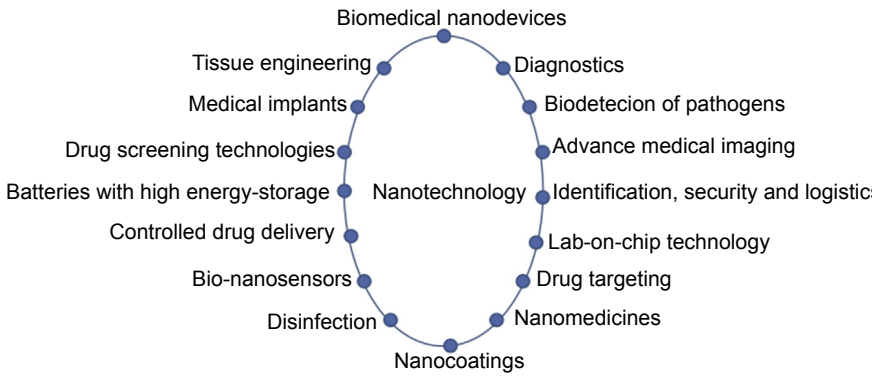

Figure 9.6 Schematic illustration of nanotechnology revolutionising biomedical sciences. Taken from Shrivastava S, Dash D. Applying nanotechnology to human health: revolution in biomedical sciences. *J Nanotechnol* 2009:1–14.

9.3.3.1 Optical thromboelastography

There are a few POC coagulation-monitoring devices in the market using optical methods for detection [Hemochron Whole Blood Coagulation System (ITC Nexus, New Jersey, United States), Sysmex (Sysmex UK Limited, United Kingdom) and GEM analyser (Instrumentation Laboratory, Massachusetts, United States)]. The typical optical methods used for coagulation monitoring are based on turbidimetry and nephelometry.[73] In turbidimetry, the change in the transmitted light directed through a cuvette during the coagulation process is measured and, in nephelometry, the light scattered by a coagulating-blood sample is measured using detectors at different angles. A few novel approaches have been proposed in a few recently published works in scientific literature.[74–77] These methods are based on optical coherence tomography (OCT) or laser speckle rheometry (LSR). Fu et al.[77] used simplified OCT which is based on illuminating a cuvette containing blood with a near infrared coherent light source. The method was based of the known effect of blood light-scattering coefficient change during coagulation. They monitored and quantified light penetration depth into the sample to estimate the effect of pH on the clot formation time. The LSR method, developed by Nadkarni and co-workers,[74,75] illuminates a blood sample with a laser and the characteristic speckle pattern fluctuations caused by the Brownian motion of blood cellular components. The viscoelastic changes occurring in blood during coagulation affect the Brownian motion and hence the fluctuations in the speckle patterns provide a TEG-like trace for blood coagulation. Margalith et al.[78] used LSR for tradition PT assay and showed the capability of the system to perform blood oximetry.

9.4 Resonator-based methods

9.4.1 Quartz crystal microbalance (QCM)

QCM is a piezoelectrically actuated shear-mode oscillator that is extremely sensitive to changes in physical properties at the surface. It was originally considered as a device to measure the mass of films attached to the quartz crystal surface and is thus called a microbalance. However, with better understanding of the device and its ability to work well in liquids, it goes beyond and is able to monitor viscoelastic changes of the attached layers. Some of the earlier work in monitoring blood coagulation using QCM was by Muramatsu et al.[79,80] studying the effect of fibrinogen concentration on the time taken for a clot to form. Since then, there have been several reports[79–90] showing frequency shift as the only parameter of QCM as a measure of change in plasma viscosity during coagulation. Blood coagulation on QCM has been studied using different activation methods such as prothrombin time (PT),[87,91] activated partial thromboplastin time (aPTT),[81–83,89,90,92] thrombin clotting time (TCT)[79] and recalcification.[85,93] However, the unique feature that QCM provides is the measurement of a dissipation, which in combination with frequency changes can provide more meaningful viscoelastic information on the clot. Real-time monitoring of the process allows also the study of kinetics of the clot formation. Bandey et al.[94] considered the frequency and dissipation changes and used an equivalent circuit model of QCM to distinguish between

different stages of coagulation in a whole-blood sample and settling behaviour of red blood cells based on deviations from Newtonian behaviour. In another study, Muller et al.[91] used diluted plasma and PT-based activation and studied changes in frequency and bandwidth shifts and showed near-Newtonian fluid behaviour during coagulation. Lakshmanan et al.[95–97] used frequency and dissipation characteristics and showed using an aPTT activation that the QCM can be a very useful tool in studying viscoelastic property changes occurring during coagulation. QCM is particularly interesting to be used in a point of care (POC) setting, with a miniaturised multichannel system which can conduct several coagulation assays in a single run will have great potential. Although in its early stages of research, a wider validation using a range of assays and patient cohort will prove a significant advantage over the existing methods for blood-coagulation monitoring.

9.4.2 Magnetoelastic transducers for monitoring coagulation

Other modern technology based on magnetoelastic resonators is represented.[98,99] Magnetoelastic transduction is used as a means to monitor the changes that occur during both the coagulation and the fibrinolytic processes. The magnetoelastic measurements are performed on a Helmholtz coil configuration, using a sinusoidal alternating current (AC) magnetic field for interrogation of the magnetoelastic films. When exposed to a time-varying AC magnetic field, magnetoelastic sensors oscillate at a fundamental frequency that is dependent on the chemical composition and the physical dimensions of the strip. A direct current (DC) field is also used to offset the magnetic anisotropy of the strip by aligning the magnetic domains to maximise its vibrational amplitude. The oscillation of the sensor creates a magnetic flux, which can then be detected remotely by a pickup coil. Magnetoelastic sensors behave similarly to surface acoustic-wave devices (eg, QCM) in that they respond to changes in physical parameters, such as mass loading, viscosity and pressure with corresponding changes in their resonant frequency of oscillations. The magnetoelastic sensors have been used to study blood coagulation.[98–103] Puckett et al. demonstrated the use of these sensors to monitor coagulation first by measuring recalcification[99] of citrated plasma from horse and rats and later studied human plasma to measure clotting activated using prothrombin reagent and fibrinolysis using the addition of plasmin. They showed the ability of these devices to monitor the entire coagulation process but attributed the response to changes in viscosity. Zeng et al.[100] and Roy et al.[100,102,103] took a more systematic approach and looked at contribution of individual components in blood to their contribution to the overall response. However, the full advantage of such resonator devices lies in the fact that they can obtain viscoelastic information. All the work on magnetoelastic sensors demonstrates the ability of these sensors to measure changes but does not provide insights into the viscoelastic properties of the forming clot. The advantage of these inexpensive magnetoelastic devices is that they can be queried remotely and do not require direct physical contact with the sensing element. In addition, reducing dimensions of magnetoelastic sensors[104–107] has been shown to improve sensitivity of these devices which make it amenable to miniaturisation and find applications in a POC setting.

9.4.3 Other novel research methods

Paper-based microfluidics has been around for a long time and has applications in many dipstick and lateral-flow diagnostic devices for glucose and protein detection. Paper offers many advantages as part of a POC device as it is functional, portable and inexpensive compared to other substrates and materials.[108] Recently a paper-based lateral-flow device was developed for blood-coagulation screening.[109] Using the principles of size separation of blood components, viscosity changes with blood coagulation and the porosity of the device membrane, the authors were able to determine the clotting time by measuring the distance travelled by red blood cells, the colour of which was also used as a visible marker.

Although lateral-flow assays are bountiful in device development, the range of analytes that can be detected is ever increasing. Two such recent devices are aimed at quantifying platelet and fibrin accumulation on-chip. A microfluidic assay of haemophilic blood clotting, focusing on the detection of platelet and fibrin deposition was developed.[110] A flow-based microfluidic device modified with collagen was shown to mimic platelet activation and fibrin generation as triggered by the intrinsic pathway allowing for the detection of dysfunctional thrombin generation. Members of the same research group also carried out further research and validated a microfluidic device for measuring this platelet and fibrin accumulation following recalcification of citrated whole blood on chip.[111] As outlined by the authors, millions of clotting tests require recalcification prior to testing, and this integrated development allows for on-chip recalcification and drug dosing for assays of platelet function under flow.

In addition to the vast literature showing the utility resonator-based methods such as QCM and magnetoelastic resonators, some other approaches are of particular interest which use novel approaches and can have an impact in the paradigm of POC coagulation monitoring devices. Advances in microfabrication techniques have been ever-reducing the size of devices without a compromise in sensitivity; in fact, in some cases these techniques appear to exhibit superior sensitivity. The detection of anticoagulant therapy can be carried out in a number of ways and one new development used surface acoustic wave (SAW) technology in a microfluidic device to detect clinically relevant doses of anticoagulants including unfractionated heparin (UFH), argatroban, dabigatran and rivaroxaban. SAW technology, essentially a label-free technology, allows for the sensitive detection of biologically important molecules by transduction of an electrical signal into a mechanical signal.[112] In this particular study, SAW was used to mix and recalcify citrated whole blood, and, upon quantification of clot formation, results were shown to be comparable to standard clotting tests for the drugs tested.[113] In another study,[114] an SAW resonator was used to monitor coagulation processes in combination with impedance measurements to estimate haematocrit levels. Such a multiparametric measurement modality holds great potential in future POC coagulation-monitoring devices. Microfabricated contour-mode film bulk acoustic resonators have also been used for monitoring viscoelastic changes occurring during blood coagulation.

9.5 Future perspectives and challenges

9.5.1 Drawbacks of measuring novel anticoagulants

Anticoagulant therapy, such as warfarin, unfractionated heparin and low molecular weight heparin, has long been established as are the well-documented drawbacks of these drugs. As a result, the pharmaceutical industry has been in pursuit of novel drugs that offer greater advantages over the traditional antithrombotic. The ideal anticoagulant would only require oral administration, have a predictable anticoagulant response, excellent specificity and the absence of a need for routine patient monitoring. The new anticoagulants can be classified into direct thrombin inhibitors (DTIs) such as dabigatran, argatroban, bivalirudin, direct FXa inhibitors such as rivaroxaban and apixaban, and indirect FXa inhibitors such as fondaparinux and danaparoid.

Routine coagulation tests may not be suitable for monitoring the new cohort of antithrombotics, in terms of prolongation or reduction of the clotting time resulting in misleading clinical readings. Siemens Healthcare recently produced a document on these new emerging drugs and their effects on routine coagulation assays (Table 9.2). According to one author, assays such as the PT, aPTT and TCT are unsuitable for monitoring thrombin and FXa inhibitors die to hypersensitivity or insensitivity.[115] With the promise of newer anticoagulants, it was envisaged that the requirement for monitoring would be minimal. However, there remains the issue that some patient cohorts will always require monitoring of their anticoagulant status, indicating the need for the development of some form of monitoring assay or even a companion diagnostic.

Table 9.2 Influence of new anticoagulants on routine coagulation assays

	Direct thrombin inhibitors	Direct FXa inhibitors	Indirect FXa inhibitors
PT in sec and INR	↑	↑	No
aPTT	↑	↑	No
Thrombin time	↑↑	No	No
Fibrinogen (Clauss)	No/↓	No	No
Multifibren U	↓↓	No	No
Derived fibrinogen	No/↓	No/↓	No
D-dimers	No	No	No

Taken from Mani, et al. Influence of new anticoagulants on coagulation tests. *White Paper*. Siemens Healthcare Diagnostics Ltd.; 2011. Single arrow: Moderate increase (up) or decrease (down) in clotting time or assay value. Double arrow: Significant increase (up) or decrease in clotting time or assay value.

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Nanostructured materials and nanoparticles for point of care (POC) medical biosensors

10

L.C. Brazaca[1], L. Ribovski[1], B.C. Janegitz[2], V. Zucolotto[1]
[1]University of São Paulo, São Carlos, São Paulo, Brazil; [2]Federal University of São Carlos, Araras, São Paulo, Brazil

10.1 Introduction

Defined as "a natural, incidental or manufactured material containing particles, in an unbound state or as an aggregate or as an agglomerate and which, for 50% or more of the particles in the number size distribution, one or more external dimensions is in the size range 1–100 nm" by the European Commission on 18 of October 2011, nanomaterials have properties that provide large applicability in sensing platforms.[1] Due to their reduced size, nanomaterials behavior is driven by quantum effects, which leads to variations in physical–chemical properties, eg, electrical conductivity and melting point, when compared to bulk materials.[2–4] High electron mobility and specific surface area, as well as the unique electrical and thermal conductivities exhibited by some nanomaterials, compose a few of the outstanding properties that make these materials suitable for sensors and biosensors development.

Diagnosis and monitoring of diseases are complex subjects in medicine due to the necessity of detecting and quantifying several molecules, usually in very small amounts. There are several laboratory tests to screen molecules of interest, however, point of care (POC) devices are required because most people do not have regular access to these technologies and the logistics of forwarding a sample to the laboratory for it to be tested might require days or weeks, in addition to having a qualified staff. In case of an outbreak, the lack of an easy detection system might lead to erroneous diagnosis because a group of conditions may present similar symptoms, eg, dengue fever symptoms might be mistaken for flu or other viruses.[5] Various strategies have been reported to clinical analysis application, although establishing a reliable and accurate biosensor depends on many requirements.

Regarding sensitivity and selectivity, nanomaterials offer a great improvement to transducers and architectural variability. For example, prostate specific antigen (PSA) is one of the best-known cancer biomarkers and a promising biomolecule for POC devices. To improve response and achieve a more reliable PSA immunosensor, reports using gold nanoparticles (AuNPs)[6,7] (Fig. 10.1),[8] quantum dots (QDs),[9,10] carbon nanotubes (CNTs)[11,12] (Fig. 10.2),[13] silica nanoparticles (SiNPs),[14] and graphene[15] (Fig. 10.3)[16,17] are frequent in the literature. For example, Li and co-workers described an immunosensor to detect PSA using graphene as the sensor

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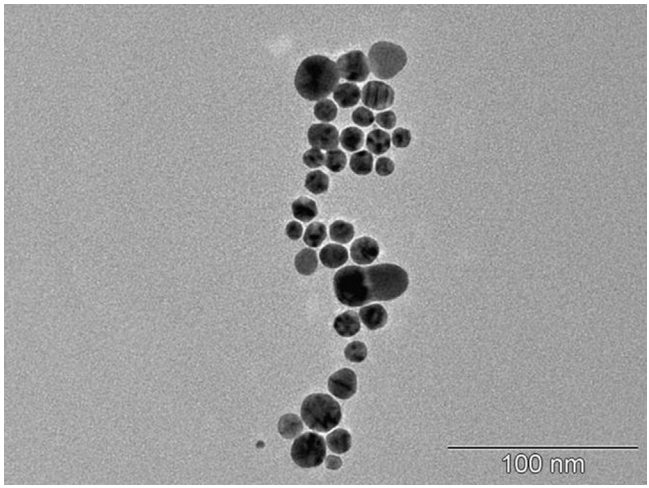

Figure 10.1 Transmission electron microscopy (TEM) of PAMAM-coated AuNPs.
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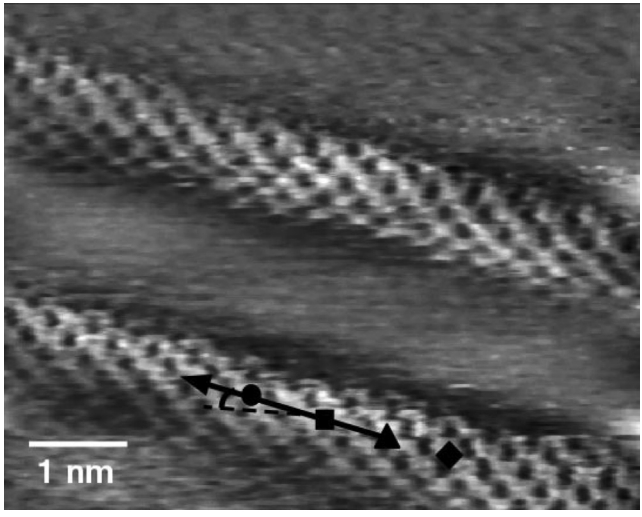

Figure 10.2 Scanning tunneling microscopy (STM) image showing the atomic structure of isolated single-walled carbon nanotubes (SWCNTs).
Reprinted with permission from Odom TW, Huang JL, Kim P, Lieber CM. Atomic structure and electronic properties of single-walled carbon nanotubes. *Nature* 1998;**391**:62–4, © 1998, Nature Publishing Group.

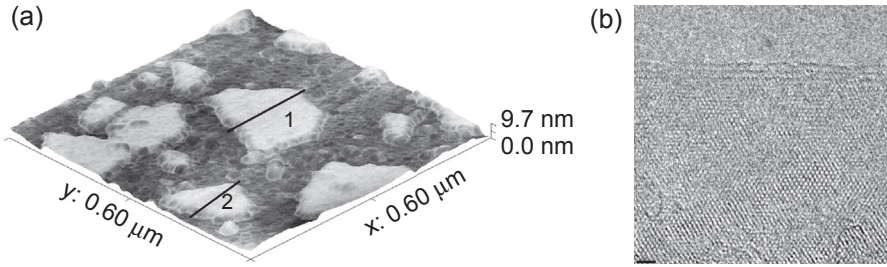

Figure 10.3 (a) Atomic force microscopy (AFM) image of graphene oxide flakes. The image was constructed by combining the three-dimensional (3D) topography with phase contrast information. *(Reprinted with permission from Nogueira PFM, Nakabayashi D, Zucolotto V. The effects of graphene oxide on green algae Raphidocelis subcapitata. Aquatic Toxicology 2015;**166**:29–35, © 2015 Elsevier, BV.)* (b) TEM image showing the atomic structure of two to four layers graphene membranes. *(Reprinted with permission from Meyer JC, et al. The structure of suspended graphene sheets. Nature 2007;**446**:60–3, © 2007, Nature Publishing Group.)*

platform, which increased the effective surface area of the biorecognition layer, and, as a consequence, more antibodies were captured, thus increasing sensitivity.[18]

Another type of biosensors, the enzymatic ones, were the first biological-based biosensors developed, produced, and commercialized. Leland C. Clark, one of the pioneers in this field, had first demonstrated the concept of biosensors in 1962 by using glucose oxidase immobilized on a working electrode and monitoring the oxygen consumption of the catalytic reaction.[19] Glucose biosensors technology is well established and widely applied to measure glucose levels in blood and its success illustrates the potential of enzymes as bioreceptor agents, highlighting its application on the development of POC biosensors. Several reports based on nanomaterial-modified glucose biosensors clearly demonstrate the advances in the field by employing nanoparticles,[20] graphene,[21] nanotubes and a variety of nanocomposites[22] and nanostructured films.[23] However, it is important to note that the use of nanomaterials is not essential to any biosensor, but it is helpful if the analytical characteristics achieved are not satisfactory.

Considering deoxyribonucleic acid (DNA) sensors, easy-to-use devices would revolutionize disease diagnosis, from viral and bacterial infections to cancer. Furthermore, DNA sensors (or genosensors) would allow customizing cancer treatments by profiling cancer cells according to germline or acquired mutations. However, sample pretreatment is not simple. It includes amplification steps and sample processing. Besides, most scientific papers describing DNA sensors employ synthetic oligonucleotides as proof of concept, which leads to new challenges regarding the manipulation of complex samples.[24] Due to their high electron mobility, surface-to-volume ratio, and great biocompatibility, nanomaterials have been widely applied in the design of DNA sensors as labels, or integrated within the transducer to amplify signal transduction and to obtain more sensitive and stable sensors.

Small-sized nanomaterials, as metallic nanoparticles, are suitable to tag oligonucleotides. For example, a target DNA, ie, the DNA strand that will be complementary

to other DNA strand (probe), labeled with a nanoparticle will provide better mismatch discrimination.[25] Nanomaterial-modified electrodes show an increased in the electroactive area which leads to an easier attachment of probe oligonucleotides.[26]

This chapter focuses on electrochemical biosensors that incorporated nanomaterials in their design, including the synthesis and characterization of these nanostructured materials and their use with biological recognition elements.

10.2 Synthesis, characterization, and application of nanomaterials

Nanomaterials present high potential for improving electrochemical POC device performance. Due to its unique properties, such as increased superficial area, extremely high electrical conductivity, small size—adequate to interact with biological molecules[27]—and stability, these nanostructures have been widely explored for POC medical applications. Metallic nanoparticles, CNTs, and graphene have been extensively studied due to their individual properties and will be the focus of this section, in which we will discuss main methods for synthesis, characterization, and application of these nanomaterials in POC medical devices.

10.2.1 Synthesis

Electrical, optical, and catalytic properties of nanomaterials depend strongly on their characteristics, such as size, composition, shape, and purity. The choice of synthesis method and its execution conditions can influence significantly these parameters, which has been an object of extensive study. In the following, we present the most actual and relevant CNTs, metallic nanoparticles, and graphene synthesis techniques.

10.2.1.1 Chemical reduction

One of the most common approaches on synthesizing metallic nanoparticles includes the chemical reduction of ions by inorganic or organic agents—for example, sodium citrate, sodium borohydride, or ascorbate.[28–30] Usually, an aqueous solution containing ions of the desired metal (such as Ag^+ or Au^{3+}) is used as starting point of the reaction. The reducing agent is then added and the ions are reduced to their metallic form, which is followed by agglomeration and formation of clusters. These clusters grow and lead to the formation of nanoparticles—which may be stabilized by the presence of a stabilizer agent, such as polymers, citrate, or any surface agent.[29] Corbierre and Lennox have prepared 1–2 nm AuNPs stabilized by thiol by reducing Au(I)-thiolate complexes with lithium triethyl borohydride.[30] Graphene and graphene nanostructures may also be produced reliably in large quantities by chemical reduction techniques.[31–33] Stankovich and co-workers have synthesized graphene nanosheets using chemical reduction of exfoliated graphite oxide.[31]

10.2.1.2 Seeded-growth method

The seeded-growth method is envisioned for the growth of metallic nanoparticles of controlled shape. Although methods such as chemical reduction are only able to produce spherical nanoparticles, the seeded-growth procedure allows the formation of rods, cubes, and tubes, among others.[34] In this case, seeds are produced using a metallic ion and a strong reducing agent, being further added to a solution containing a metal salt, a weak reducing agent, and a structure-directing agent. The latter prevents the formation of new agglomerates and provides the growth in an ordered and desired manner.[35] For example, Xu and colleagues studied the conditions for producing gold nanorods with different characteristics using silver nucleation.[34]

Commonly, transition metals nanoparticles—such as Fe, Co, and Ni—are used as seed for growth of CNTs in different techniques. When in contact with a hydrocarbon source or gaseous carbon, for example, a deposit of carbon is observed on the particle surface.[36] The base of the nanotube is then structured around the nanoparticle catalyst—which determines the diameter of the nanotube as its own. CNTs characteristics may be controlled by changes in reaction temperature, type of gas, metal concentration, and gas pressure, for example.[36]

10.2.1.3 Chemical vapor deposition (CVD)

One of the most promising techniques for producing graphene in large quantities and low temperatures is chemical vapor deposition (CVD).[36] To this end, a transition-metal substrate—such as Ni, Au, or Cu[37]—is saturated with carbon at high temperatures using a hydrocarbon gas—methane, or benzene, for example. As the temperature decreases, the solubility of carbon into the transition metal is lowered and the compound leaves the metal, forming graphene on the surface of the substrate.[38] CVD graphene presents interesting electronic properties for several applications. Thin, Ni-grown graphene may be used as flexible and transparent electrodes for the development of organic photovoltaic cells. Also, large-grain graphene may be produced using Cu foil, presenting electronic properties for field effect transistors application.[39]

CVD may also be used as a simple, energy-efficient method to produce single- or multi-walled carbon nanotubes (SWCNTs and MWCNTs, respectively) with growth control, high yield, and purity.[36] In this case, catalyst nanoparticles of transition metals (as Ni, Co, and Fe) are used as seeds.[40]

10.2.1.4 Electric arc discharge

CNTs and graphene synthesis may also be performed by high-temperature physical methods using graphite, such as electric arc discharge and laser ablation.[36,38] This method produces the best-structured CNTs, but also yields impurities such as graphite, residual catalyst, and amorphous carbon. In addition, it is necessary to disperse, purify, and functionalize the nanotubes before use.[36] Despite such disadvantages, the method is very simple and has potential for mass production of these nanomaterials,[41] in addition to the possibility of producing pure graphene.[42] Electric arc discharge has also been efficiently used in the synthesis of core-shell nanoparticles.[43]

For the production of carbon-based nanomaterials, electric arc discharge method usually relies on the application of a voltage between two graphite electrodes, in a buffer atmosphere composed of helium or hydrogen, for example. When the electrodes are brought close together, electric arc discharge occurs, causing the formation of plasma. Carbon atoms sublimate from the anode and deposit on the cathode and, depending on the experimental conditions, such as gas pressure, geometry, and positioning of the electrodes, atmosphere, temperature, arc current, the use of catalysts, and others, different structures may be formed.[42,44] If the electrodes are facing each other, MWCNTs may be synthetized, and if there is catalytic metal present on the anode, SWCNTs may arise.[41] Synthesis of MWCNTs in liquid environment was also described by Antisari and co-workers, in 2003.[41] The first pure graphene synthesis using arc-discharge method was described by Subrahmanyam and co-workers, in 2009, using an H_2 atmosphere.[45]

10.2.1.5 Laser ablation

Laser ablation has been applied for synthesizing high-purity and high-quality SWCNTs[46] and metallic nanoparticles—such as platinum, gold, silver, and/or copper.[47] The principles of this technique are similar to the electric arc discharge. In this case, the energy necessary for the synthesis of nanoparticles is provided by a laser. For CNTs synthesis, the laser hits a graphite substrate containing catalyst materials (Ni or Co, usually).[48] For metallic nanoparticle synthesis, the laser may be applied to the bulk material in solution, for example. One of the main advantages of this technique is the absence of reactants in the final solution.[29,47] The characteristics of the produced nanomaterials depend mainly on the wavelength of the laser, the duration of the pulses, bulk environment, and others.[29] Synthesis of metallic nanoparticles in pure solvents usually generates spherical particles from 3 to 30 nm, although the presence of irregularities in shape is frequent.[47] Mafuné and co-workers described the synthesis of stable 1–7 nm platinum nanoparticles in an aqueous solution of sodium dodecyl sulfate (SDS), with an absorbance spectrum similar to the ones chemically prepared.[49] Zhang and co-workers prepared SWCNTs using nitrogen atmosphere and Ni and Co as catalysts with a yield of over 50%, similar to the one obtained using argon.[50]

10.2.2 Characterization

The existence of sophisticated characterization techniques has allowed nanotechnology to advance in the last few years. The ability of studying nanomaterial size, shape, surface properties, composition, purity, stability and dispersion has been a fundamental feature in nanotechnology development. Here, we discuss the most fundamental techniques used in this area.

10.2.2.1 UV–Visible absorbance spectroscopy

The ability of UV–Visible (UV–Vis) spectroscopy to investigate nanoparticle size, concentration, aggregation state, and bioconjugation[51] in a simple, rapid, and low-cost manner has made it one of the most-used techniques in nanomaterial characterization. UV–Vis spectroscopy is based on the passage of UV and/or visible light through a

sample, producing an absorbance spectrum. In particular, some metallic nanoparticles—such as Au and Ag—absorb light at a determined wavelength with exceptional efficiency due to the so-called surface plasmon resonance (SPR). The light absorption related to this phenomenon depends strongly on the nanomaterial composition, size, shape, and environment, allowing its precise characterization.[52] It has been described by Tomaszewska and co-workers that polydispersed samples may lead to erroneous results.[53]

Lim and co-workers have used UV–Vis absorbance to follow the transformation of a DNA-covered AuNP into gold "nanobridged nanogap" particles, which are able to generate reproducible and stable surface-enhanced Raman scattering signals.[54] In another paper, Chou and co-workers used DNA to assemble nanomaterials upon producing colloidal superstructures for controlled delivery and elimination. In this case, UV–Vis spectroscopy has shown to be able to differentiate between the different building blocks and assembled superstructures.[55]

10.2.2.2 Dynamic light scattering (DLS)

Dynamic light scattering (DLS), one of the most common light-scattering modalities, is an efficient, elegant, and simple technique for measuring nanomaterial hydrodynamic size distribution and aggregation state based on their Brownian motion,[51] especially in the range of 2 to 500 nm.[53] It is important that the particles present a narrow size distribution, as polydispersed samples may distort the results.[53]

Every particle or molecule in suspension is constantly in contact with solvent molecules that are moving due to thermal energy. In turn, this causes the molecules or particles to move in a particular manner, called Brownian motion, which depends on their size. When the sample is illuminated with a laser, the intensity of scattered light fluctuates depending on particle Brownian motion velocities—and, therefore, their size. Through the analysis of intensity fluctuations, Brownian motion velocity can be acquired and the radius of the particle can then be determined by the Stokes–Einstein relationship.[52] He and co-workers have developed a technique for hierarchical DNA self-assembly into polyhedral architectures, and, using DLS, they compared the hydrodynamic radius of the assembled complexes with the predicted ones.[56]

10.2.2.3 Zeta potential

When charged colloidal particles are dispersed in an ionic solution, an electrical double layer is formed around each particle. While the inner layer, also called the Stern layer, is composed of opposed charge ions tightly packed around the particle, the outer—or diffuse—layer is formed by mobile, solvated ions with the same charge as the dispersed material.[57] To measure the particle's Zeta potential, an external electrical potential is applied across the solution, forcing the movement of particles toward the electrode. The determination of the electrophoretic mobility is then used to obtain the particle Zeta potential.[58] Usually, absolute values above 30 mV indicate a stable aqueous suspension, whereas smaller values may lead to particle aggregation due to van der Waals interparticle attractions.[59] It is important to notice that Zeta potential is not absolute and depends strongly on the environment to which the particles are submitted, such as pH and ionic strength.[51] Krishnan and co-workers have developed a method for electrostatic trapping nanomaterials in a

fluid. Upon using Zeta potential measurements, the authors were able to calculate important values such as charge densities of gold nanoparticles and slit surfaces used in the development of the device.[60]

10.2.2.4 Fourier transform infrared spectroscopy (FTIR)

The molecular absorption of infrared (IR) light causes transitions between rotational and vibrational modes in energy ground levels. For a many-atom molecule, the resulting vibrational frequency is a combination of several pure vibrational modes, which may present shifts due to electrical and steric effects, composition, electronegativity, and size of neighboring atoms.[61] The set of specific vibrational modes may identify a material with great precision, being one of the most outstanding features of FTIR. Besides having inexpensive and fast measurements,[51] FTIR technique has been widely employed for nanomaterials characterization due to its ability to evaluate the interaction between nanomaterials and biomolecules[62] or even displaying the conformational states of the bound proteins.[63–65] Changes in the secondary structure of bovine serum albumin (BSA) upon the interaction with AuNPs were studied using ATR-FTIR (FTIR combined with attenuated total reflection spectroscopy) by Shi and co-workers.[64] Li and co-workers have studied the alterations induced by carbon-based nanoparticles in lung cells using ATR-FTIR.[66] Also, Zeng and co-workers used ATR-FTIR for confirming the covalent immobilization of graphene oxide QDs onto amino-modified polyvinylidene fluoride membranes, increasing significantly its bactericidal and antibiofouling properties.[67]

10.2.2.5 Transmission electron microscopy (TEM)

Transmission electron microscopy (TEM) is a technique in which electrons are accelerated toward a thin sample using high voltage, creating images with a spatial resolution of less than 0.1 nm.[68,69] This technique can yield important information about the morphology, orientation, and structures of narrow regions of the nanomaterials. Furthermore, high-resolution TEM images (HREM) are able to present information about atomic structure and growth of nanomaterials.[68,70] Hansen and co-workers studied the dynamic shape changes in the atomic scale of copper nanocrystals on different supports using TEM.[69] The directional growth of inorganic nanowires using graphene as a template was observed in atomic resolution using TEM by Lee and co-workers.[71] Furthermore, arrays of SWCNTs densely packed for optimized electronics performance have been developed and characterized in detail using TEM by Cao and co-workers.[72]

10.2.2.6 Atomic force microscopy (AFM)

Atomic force microscopy (AFM) is a scanning probe technique commonly used for profiling of surfaces. As it presents good horizontal and vertical resolution (of 0.2 and 0.01 nm, respectively), it has been used for nanomaterials characterization.[68] Nanoparticle size and shape are commonly investigated.[73–75] AFM in tapping mode is usually applied for graphene thickness determination. However, because it has been considered a challenging task to determine the number of layers and the correlation between

the measurement and the actual thickness of graphenes, a secondary technique such as Raman spectroscopy is often employed.[76] AFM has also been used for determining graphene stiffness and elastic properties,[77] as well as for determining its flatness.[78] Cuenot and co-workers also used AFM for studying the effect of nanoscale on elastic properties of 30–250 nm silver and lead nanowires and polypyrrole nanotubes.[79] Wang and co-workers used a voltage pulse applied by a metallized AFM tip to produce molecular-sized pores on graphene for molecular valves development.[80]

10.2.3 Application of nanomaterials in point of care medical biosensors

The use of nanomaterials in the development of medical biosensors for enhancing the POC biosensor devices performance is one of the main subjects currently studied in the area. Immobilization of nanostructures in thin films or directly on the working electrode may improve the electrical interactions between sensor components—such as direct electron transfer (DET) of a redox process catalyzed by an enzyme. Besides, labeling specific probes using nanomaterials has enabled lower detection limits and more sensitive sensors by electrical signal enhancement. Multiple labels using different nanomaterials have also enabled simple, fast, and cost-effective simultaneous biomarker detection, which is of importance for the diagnosis of several diseases. Furthermore, electrochemical assays may be multiplexed by the use of electrode arrays, not depending on bulky components such as optical techniques—being, therefore, suitable for POC devices.[81] The advances in nanomaterial biosensing opens up the possibility of detecting several biomarkers—such as cancers—that cannot be quantified by conventional techniques, because it requires extremely low detection limits, high sensibility and precision.[82]

AuNPs have been envisioned for POC application due to their excellent conductivity, good catalytic activity, and high surface-to-volume ratio.[81] Several AuNPs' POC-based biosensors may be found in the literature.[81,83–86] Chikkaveeraiah and co-workers have developed an antibody-based assay for the simultaneous detection of two cancer biomarkers in serum: prostate specific antigen (PSA) and interleukin-6 (IL-6).[87] For this purpose, supermagnetic particles heavily loaded with horseradish peroxidase (HRP) enzyme were conjugated to specific antibodies, whereas the working electrode was decorated with 5 nm AuNPs conjugated to another antibody—acting as a sandwich assay (Fig. 10.4). The use of AuNPs provided a higher surface area for depositing the biological material, forming a biocompatible surface. Detection of sub-pg/mL was achieved in an assay total time as low as 1 h 15 min, and the results presented an excellent correlation with Enzyme Linked Immuno Sorbent Assay (ELISA) technique. Furthermore, the cost of each microfluidic chip for the simultaneous detection of four proteins was approximately $10.

AuNPs have also been used for DNA-specific sequence detection. Lee and co-workers developed silicon/glass-based devices for simultaneous Polymerase Chain Reaction (PCR) target amplification and sequence-specific electrochemical detection.[88] A PCR closed chamber was connected to patterned indium tin oxide (ITO) or gold electrodes modified with specific DNA sequences, which served as an efficient electrochemical

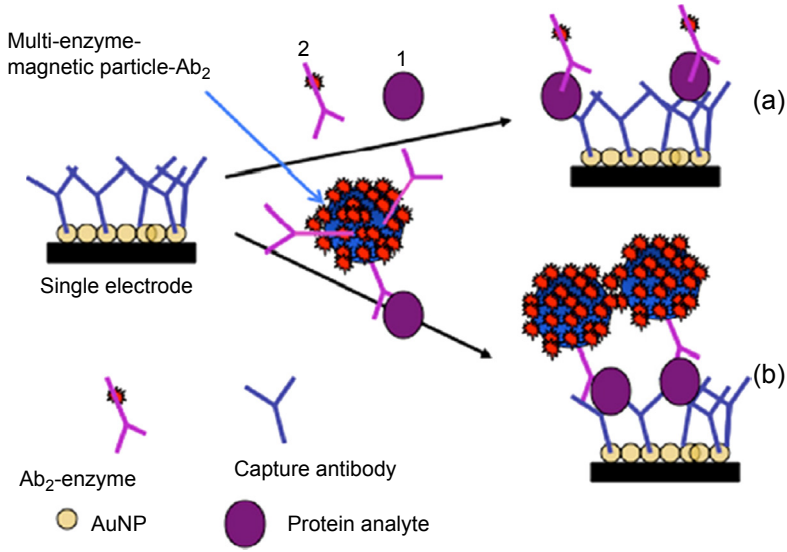

Figure 10.4 Schematic representation of different strategies of detection using labeled antibodies and a platform with AuNPs and capture antibodies. (a) Represents the conventional method, in which each antibody is labeled with a single enzyme. (b) Represents the structure proposed by Sun and co-workers. In this case, each of the antibodies is labeled with multiple enzymes, enhancing the electrochemical signal.
Reprinted with permission from Chikkaveeraiah BV, Mani V, Patel V, Gutkind JS, Rusling JF. Microfluidic electrochemical immunoarray for ultrasensitive detection of two cancer biomarker proteins in serum. *Biosensors & Bioelectronics* 2011;**26**:4477–83, © 2011, Elsevier, BV.

detection platform. At first, PCR target amplification is carried out with the assistance of platinum heaters and temperature sensors, patterned on the surface of the device. Sequence-specific recognition is then performed as the target hybridizes to the modified electrode. Streptavidin-labeled AuNPs were used to achieve sensitive target detection, improving the hybridization rate of the DNA strands. Furthermore, AuNPs were used as substrate for catalytic silver deposition, enhancing the electrochemical signal. Using this approach, several hundred copies of DNA could be detected.

Biosensors based on AgNPs and different nanoparticles have also been reported in the literature. Baccarin and co-workers have immobilized hemoglobin and AgNPs encapsulated in poly(amidoamine) dendrimer (PAMAM) on glassy carbon electrodes for hydrogen peroxide determination.[89] It is important to mention that the proposed method can be applied to immobilize and study the DET of other redox enzymes. Furthermore, a sensor based on Fe_3O_4 coated with cetyltrimethyl ammonium bromide (CTAB) and poly(sodium 4-styrenesulfonate) (PSS) layer-by-layer films were developed as a biomimetic system of peroxidase by Guivar and co-workers.[90] The latter structures are of great interest for overcoming several biomaterials disadvantages, such as activity loss after immobilization and lack of stability. Ballesteros and co-workers reported the use of Fe_3O_4 coated with AuNPs for dopamine detection.[91] The particles were immobilized on ITO electrodes in conjunction with poly(vinyl

sulfonic acid) (PVS), and the final modified electrode was capable of reducing the oxidation potential for dopamine to 0.30 V.

A diversity of nanocrystals composed by inorganic materials have been employed as tags for multiplexed clinical testing. Liu and co-workers[92] and Wang and co-workers[93] have demonstrated the use of these materials for the multitarget detection of proteins and DNA, respectively. A sandwich assay based on four different nanocrystal labels (cadmium sulfide, zinc sulfide, copper sulfide, and lead sulfide) has shown to be able to identify targets independently using stripping voltammetry. Each peak, differing in position and magnitude, is related to a single target. The multiple detection of biomarkers is a key feature in several disease diagnosis, such as cancer[82] and Alzheimer's disease.[94]

The use of carbon-based nanomaterials such as CNTs and graphene for the development of POC biosensors has attracted attention due to their physical, electrical, electrochemical, and chemical properties.[36,95] In particular, one-dimensional nanostructures are very attractive for designing high-density arrays for ultrasensitive protein detection due to their high surface-to-volume ratio and electron-transport properties. Such nanostructures represent a promise for the development of multiple biomarkers assays in ultrasmall sample volumes, being of special interest for medical areas.[82]

Chikkaveeraiah and co-workers developed a system for simultaneous and real-time measurement of four prostate cancer biomarkers in human serum based on SWCNT forests.[96] The high-density CNTs extending out of the electrodes provide a conductive, high surface area for the immobilization of a large number of capture antibodies. After the reaction of the sensor with 10 μL of serum sample, a secondary antibody was added, labeled with HRP for enhanced electrochemical detection. The latter design has enabled the sensitive measurement of biomarkers with great correlation with ELISA test. A novel sensing architecture using electrospun polyamide 6/poly(allylamine hydrochloride) (PA6/PAH) nanofibers functionalized with MWCNTs was proposed by Mercante and co-workers for dopamine determination.[97] The developed system may be extended to other electrochemical sensors and did not suffer any interference from uric and ascorbic acid, substances commonly present in biological samples.

Chitosan-modified graphene electrodes were developed by Alwarappan and co-workers for DNA mutation analysis.[95] Graphene was covalently modified with positively charged chitosan, allowing the immobilization of a single-stranded-DNA capture sequence, negatively charged. In the presence of the target DNA, cyclic voltammetric analysis displayed a significantly higher redox peak current, in comparison with a mismatch DNA. The developed platform presented high potential for use in POC devices for the identification of specific DNA mutations associated to disease conditions.

Graphene can also be applied on electrodes in its reduced form. A reduced-graphene oxide (RGO) film deposited on a glassy carbon electrode was reported by Janegitz and co-workers to provide a selective electrode for estradiol analyses.[98]

The identification of whole virus and bacteria is important for fast and precise infection determination, as well as for identifying potentially risky areas and contaminated equipment in health centers, avoiding hospital-acquired infections. POC devices have been envisioned as great opportunities for whole organism detections mainly due to their simple preparation, avoiding the typical steps of virus isolation, extraction,

purification, and amplification of biomolecules.[36] Mandal and co-workers developed a CNTs-based device for detecting a model virus—M13-bacteriophage—with high sensitivity and cost effectiveness, being able to detect quantities as low as 550 viruses.[99]

10.3 Biological recognition elements

10.3.1 Enzymes

Enzymes are biocatalysts for many biochemical reactions of every living organism, from viruses and bacteria to mammals. These macromolecules are indispensable in most cellular metabolic processes, allowing reactions to be performed much faster. They show great specificity to a substrate or an ensemble of substrates, making them interesting biological recognition elements for biosensors. Despite enzymes specificity, it is still necessary to improve some analytical characteristics of enzymatic biosensors, mostly sensitivity and selectivity. Incorporation of nanomaterials in the design of these devices may represent a good strategy to overcome these problems.

A working electrode modified with AuNPs, CNTs, and/or graphene, for example, enables the DET between electrode surface and enzyme active site. The latter is an advantage, since some enzymes, eg, metalloproteins,[3,89,100,101] such as Tyrosinase (Tyr) (E.C. 1.14.18.1), are unable to oxidize or reduce at any potential because their redox center is encapsulated in the enzyme's tridimensional structure. Nanomaterials, on the other hand, are capable of reducing the electron tunneling distance and consequently enabling the DET.[2,102] Janegitz and co-workers describe a Tyr-based biosensor immobilized on boron-doped diamond electrode modified with AuNPs, in which DET was observed between Tyr and the modified electrode.[101]

Likewise, stability is a crucial factor for efficiency of biosensors, which can be enhanced using nanomaterials that offer a suitable microenvironmental to immobilize enzymes, retaining its activity.[2,103]

10.3.2 DNA

Nucleic acids are responsible for storing and transferring genetic information, occurring in the form of DNA or ribonucleic acid (RNA). However, DNA structure is more stable and more suitable to store genetic information. Although more stable, DNA can still mutate. Commonly associated with diseases and perceived as harmful, not all mutations are disadvantageous since mutation is one of the mechanisms that allowed organism variability and evolution. Furthermore, DNA molecules are capable of annealing according to base-pairing rules with elevated stability and specificity. These features make DNA molecules particularly attractive as biological recognition elements and, consequently, to be used as building blocks for sensors.

The advances in sequencing methods over the past years have boosted research to identify genetic differences between organisms and mutations related to diseases. Taking advantage of these differences, several DNA sensors have been suggested to detect cancer-related mutations and transgenic organisms and to distinguish pathogenic

organisms from nonpathogenic. Advantages of DNA-based sensors are their relatively low cost and POC features, because sequencing methods are still expensive and require highly trained staff for experimental procedure and analysis. Many research groups in the field of DNA sensors have considered the use of nanomaterials as a strategy to improve sensors efficiency and performance. Wang and co-workers[104] describe a DNA sensor using AuNPs and high-fidelity *Escherichia coli* DNA ligase to detect a point mutation. The oligonucleotides were modified with NPs to improve the sensitivity of sensors in the differentiation of DNA strands with only one nitrogenous base.

An interesting use of a DNA sensor is related to its use in detection of genetically modified organisms (GMOs). Sun and co-workers developed a DNA sensor to detect transgenic maize using reduced graphene to modify a carbon ionic-liquid electrode. Reduced graphene increased the amount of probe oligonucleotide immobilized on the surface of the electrode[105] (Fig. 10.5). Another example is the DNA sensor for pathogens diagnosis. Miodek and co-workers[106] report a gold electrode modified by MWCNTs coated with polypyrrole and poly(amidoamine) generation-4 dendrimers (PAMAM G4). The authors observed an increase of the active surface area and consequent electrochemical signal amplification.

10.3.3 Antibodies

The immune system comprises two main mechanisms: innate and acquired immunity.[107] The acquired immunity is the ability of an organism to improve its defenses after being exposed to specific molecules.[108] In this case, specific antibodies are produced and secreted by lymphocytes. Immunoglobulins (antibodies) are "Y"-shaped proteins of approximately 150 kDa composed of four polypeptide chains: two heavy and two light chains, linked by disulfide bonds.[108] They present both constant and variable sequences, the latter ones being responsible for specific antibody–antigen interactions.[108]

Both monoclonal and polyclonal antibodies are powerful tools in the manufacturing of biosensors due to their ability to form stable complexes with specific molecules with great specificity, being constantly used as recognition elements.[108,109] Currently, the advent of recombinant antibodies has allowed the rapid production of characteristic-controlled, low-cost antibodies with improved features for use in biosensors.[110]

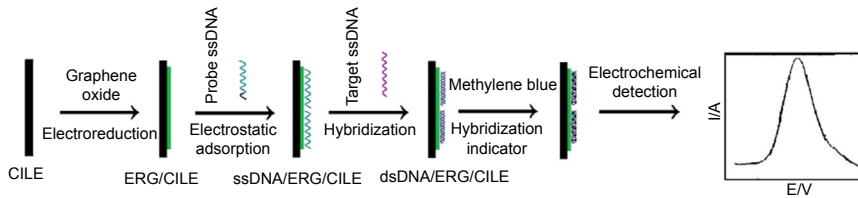

Figure 10.5 Construction, structure, and mechanism of the biosensor developed by Sun and co-workers.
Reprinted with permission from Sun W, et al. Electrochemical sensor for transgenic maize MON810 sequence with electrostatic adsorption DNA on electrochemical reduced graphene modified electrode. *Sensors and Actuators, B: Chemical* 2014;**202**:160–6, © 2014, Elsevier, BV.

These facts, combined with the possibility of producing antibodies for a great diversity of molecules, has made the development of immunosensors one of the most common and more reliable approaches in the area. Diverse electrochemical techniques—such as potentiometric, amperometric, and impedance—may be applied in different biosensor designs.[108] The use of nanomaterials in the development of immunosensors has enabled enhanced sensing properties due to the improvement of electron-transfer properties, enabling a higher surface area for receptors immobilization, providing biocompatible surfaces and multiple labeling for simultaneous detection of biomarkers.[87,92,111]

Interesting immunosensors for POC diagnostics have been proposed. Du and co-workers developed an antibody-based lateral-flow test strip combined to a nanomaterials-based electrochemical sensor for the selective, sensitive, and quick quantification of pesticides exposure.[112] Lateral flow-based POC devices are an efficient strategy to the development of simple biosensors which are based on the flow of a sample through strips containing regions with specific functionalities.[113] The mentioned assay is based on the difference between the active form of acetyl cholinesterase (AChE) and its total concentration. Antibodies specific to AChE were immobilized in specific zones of a paper strip. When a sample flows through the device, AChE and its phosphorylated form are captured by the antibodies. Then, specific areas of the strip are exposed to acetyl thiocholine (ATCh), making the captured AChE catalyze ATCh, forming thiocholine. Thiocholine is then quantified using CNT-modified screen-printed electrodes, promoting its redox reactions in lower potentials.

Neves and co-workers developed a nanostructured immunosensor for celiac disease diagnosis.[114] Dual Screen Printed Carbon Electrodes (SPCEs) were modified with MWCNTs and AuNPs, promoting increased electron-transfer rates (Fig. 10.6). Each one of the working electrodes was then coated separately with gliadin and tTG, specific antigens for the antigliadin and anti-tTG antibodies that characterize celiac disease. Alkaline phosphatase (AP) labeled antibodies were used for specific binding to antigliadin and anti-tTG antibodies, allowing their precise quantification upon determination of the enzymatically deposition of metallic silver using cyclic voltammetry. The performance of the biosensor was similar to the ELISA kits, being an excellent platform for potential POC development.

10.4 Electroanalytical techniques

Electroanalytical methods have been extensively applied in sensing and biosensing. Potentiometry, amperometry, cyclic voltammetry, linear voltammetry, differential pulse voltammetry, square-wave voltammetry, and electrochemical impedance spectroscopy (EIS) represent the most-used electrochemical techniques used for biosensor fabrication and detection.

Potentiometry has been used for electroanalytical purposes for many years.[115–117] In biosensing in particular, this technique is based on ion-selective electrodes and ion-sensitive field effect transistors. Basically, the setup of a potentiometric measurement presents an indicator and a reference electrode (connected to the two terminals

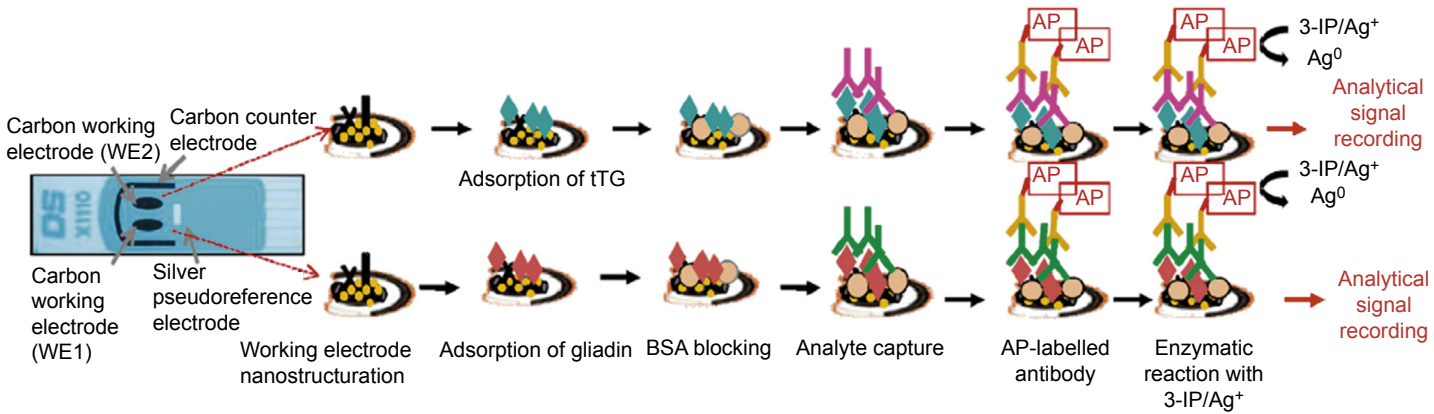

Figure 10.6 Schematic representation of the biosensor proposed by Neves and co-workers for multiplexed detection of celiac disease biomarkers. Reprinted with permission from Neves M, Gonzalez-Garcia MB, Delerue-Matos C, Costa-Garcia A. Multiplexed electrochemical immunosensor for detection of celiac disease serological markers. *Sensors and Actuators B-Chemical* 2013;**187**:33–9, © 2013, Elsevier, BV.

of a voltmeter) which are immersed in a sample solution. A potential difference (mV) appears, proportional to the logarithm of the activity of the analyte of interest. Recently, few works have used potentiometric measurements for biosensing, and it is an interesting field that can be explored to detect biological samples. Some modern and simple strategies have been applied for potentiometric determination that could be employed for POC detection. Abd-Rabboh and co-workers reported an electrochemical method to detect the determination of trypsin at low levels by using a polymeric membrane with polyion-selective electrodes and polyion-cleaving enzyme.[118] Potentiometric immunosensors have also been reported for medicine. Chumbimuni-Torres and co-workers have designed a potentiometric immunosensor for protein detection by using silver-enlarged AuNP labels in a sandwich immunoassay.[119] Figueiredo and co-workers developed a disposable Au potentiometric immunosensor for nonstructural protein 1 (NS1), a dengue biomarker, which is secreted in the first days of infection.[120] Using anti-NS1 antibodies, the authors obtained a detection limit 0.09 μg/mL. A biomimetic potentiometric biosensor using graphene was proposed by Truta and co-workers[121] to detect carnitine, a potential biomarker of ovarian cancer, present in urine. In another interesting work proposed for POC testing, Miller and co-workers[122] determined K^+ by using porous graphene ion-selective electrodes in normal physiological concentrations in the presence of interfering ions.

Amperometry consists in the application of a fixed potential in a determined time and recording the anodic or cathodic current. Since Clark and Lyons proposed the glucose biosensor in 1962,[19] amperometric biosensing has been used and can be applied for POC diagnosis.[123]

The application of electrodes in live-cell analysis is an important challenge. An amperometric biosensor for detection of hydrogen peroxide secreted in live-cell macrophages was developed by Sun and co-workers[124] using the hybrid CNT/graphene decorated with Pt nanoparticles in a paper electrode. Some disposable amperometric biosensors could be easily applied for POC testing. Sánchez and co-workers[125] proposed an immunosensor using screen-printed electrodes modified with CNT/polysulfone (PSf) (Fig. 10.7). The authors used the immunoglobulin G (IgG) as a model of an antibody which could be replaced by other antibodies of interest.

Cyclic voltammetry and linear-sweep voltammetry are dynamic electrochemical measurements. In both cases, the potential is ramped linearly versus time. Cyclic voltammetry also presents a scan of a return to a chosen potential—usually, the initial one. The response are anodic and/or cathodic peak currents that are proportional to the concentration of the electroactive species. These techniques are used for electrochemical characterization of working electrodes, and in some cases they are applied for determination of analytes of interest. Zhang and co-workers[126] have applied cyclic voltammetry to detect a pathogen using an antibody labeled with horseradish peroxidase which reacts with H_2O_2. A voltammetric immunosensor for *Enterobacter sakazakii* was developed by employing MWCNTs/ionic liquid/thionine-modified electrode which presented a limit of detection of 7.7×10 CFU/mL. Monteiro and co-workers[127] related a biosensor that can be applied in POC diagnostics based upon a carbon-paste screen-printed electrode coated with a cytochrome c nitrite reductase (ccNiR)/carbon ink for the determination of nitrite in different media, including in clinical samples.

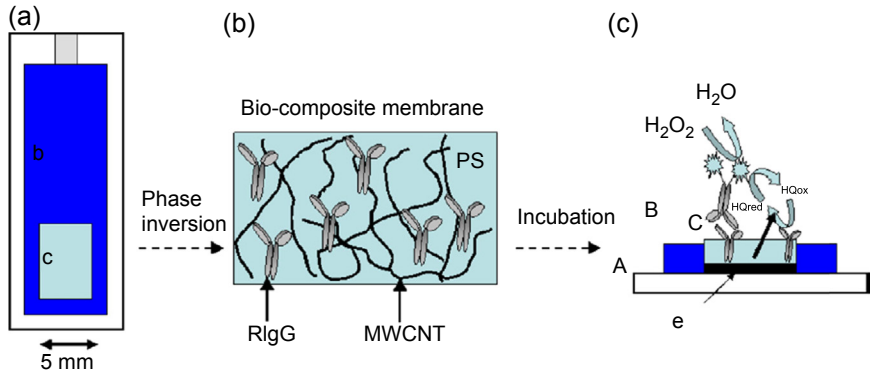

Figure 10.7 Schematic representation of the immunosensor proposed by Sánchez and co-workers. (a) Top view of the MWCNT/PSf screen printed device, (b) structure of MWCNT/PSf/IgG composite. (c) Cross section of the biosensor after incubation with anti-IgG-HRP antibody. (A) polycarbonate substrate, (B) insulator layer, (C) MWCNT/PSf. Reprinted with permission from Sánchez S, Pumera M, Fàbregas E. Carbon nanotube/polysulfone screen-printed electrochemical immunosensor. *Biosensors and Bioelectronics* 2007;**23**:332–40, © 2007 Elsevier, BV.

Differential pulse voltammetry and square-wave voltammetry are the main pulsed techniques used in biosensing. The main advantage exhibited by these techniques is the low capacitive current, which can improve the sensitivity of the voltammetric procedures. Differential pulse voltammetry is usually applied in irreversible systems and in systems that present slow-reaction kinetics. Square-wave voltammetry is usually applied in reversible systems and in rapid reaction kinetics systems.

Moreira and co-workers[128] developed a disposable biosensor for myoglobin, a cardiac biomarker using a screen-printed Au electrode. By performing square-wave voltammetry, a limit of detection was obtained of 0.8 µg/mL. A highly sensitive DNA biosensor based on MWCNTs was developed by Cai and co-workers.[129] For monitoring the DNA hybridization reaction, differential pulse voltammetry was applied using daunomycin as an electroactive intercalator. The use of CNTs improved the amount of DNA hybridized, as well as the detection sensitivity due to its increased surface area and charge-transfer characteristics. Ferapontova and co-workers developed a label-free RNA aptamer-based biosensor for detection of the bronchodilator theophylline in serum.[130] The redox properties of a ferrocene redox probe were monitored using differential pulse voltammetry, displaying a dynamic range of 0.2–10 µmol/L.

EIS is one of the most-used methods among impedance-based techniques and is able to provide diverse information about the surface of modified electrodes, such as capacitance and charge-transfer resistance. EIS is especially useful for the monitoring of binding molecules that do not produce electroactive species.[131–133] Experimentally, an electric signal of small amplitude is applied in several frequencies (typically in the range of 10^4 to 10^{-3} Hz), whereas the real and imaginary parts of impedance are recorded. Well-characterized redox molecules such as $K_4[Fe(CN)_6]$ can be used as electron donors to investigate the system. EIS-based biosensors present great potential

for the development of POC devices, as they are easily miniaturized and undergo label-free detection.[134] However, it is still very challenging to apply EIS techniques to POC devices, mainly due to instrumentation limitations.[134] Recently, Zhang and co-workers developed a simple, cost-effective handheld device for performing EIS measurements with the control of a smartphone. Its applicability as a POC biosensor was illustrated by detecting BSA and thrombin in concentrations as low as 1.78 μg/mL and 2.97 ng/mL, respectively.[135]

10.5 Conclusion

Modern devices for medical purposes are very important to assist in the diagnosis and treatment of several diseases which may improve healthcare. POC testing is a relatively new trend that can be increased in the next years. In particular, electroanalytical methods can be applied because of the possibility of equipment miniaturizing ease of use. On the other hand, disposable electrodes open a field for this application, including electrodes made of paper, plastic, and ceramic, which can be discarded or incinerated. In addition, the development of nanomaterials in the recent years, such as CNTs, graphene, and metallic nanoparticles have improved the selectivity and sensitivity of the electrochemical biosensors for POC diagnosis. Fluidic system strategies have joined the analysis of various biomarkers, collaborating in the development of biosensors that present better and reliable responses for detection of different diseases. Less-invasive devices for live cells and/or living systems have also been a frontier in the field.

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Microfluidic platforms for point of care (POC) medical diagnostics

11

M. Azhar, D. Dendukuri
Achira Labs Pvt. Ltd., Bangalore, India

11.1 Introduction and background

Microfluidic technology platforms provide an essential set of building blocks for building point of care (POC) medical tests. Through miniaturizing, integrating, and automating fluid flow on disposable cartridges, they can enable a set of complex operations, usually performed in laboratory settings, to be performed in a POC or field setting. There are several excellent reviews of the use of microfluidics platforms for POC tests (Chin et al., 2012; Lee et al., 2010; Yager et al., 2006). In this chapter, we will focus on some of the most recent academic developments in the field and also highlight platforms that have already been successfully commercialized in different test categories. This is important because microfluidic technologies have to compete with existing POC technologies and offer clear advantages over and above those tests that exist today.

11.1.1 Point of care tests

Although there is no universally accepted definition for POC testing, it generally refers to testing that is performed at distributed settings away from central labs. This could be in a physician's office, a public health center, or even at home. The fundamental requirement for such tests is well described by the acronym ASSURED which has been coined by the World Health Organization (WHO) to reflect the properties required of POC tests, namely Accuracy, Sensitivity, Specificity, User Friendliness, Rapid and Robust, Equipment-Free (or lite), and Delivered at settings away from traditional lab infrastructure (Peeling et al., 2006). POC tests have many advantages because they can reduce time required to diagnose critical illnesses, solve for sample degradation due to lack of cold supply chain, plug leaks in healthcare practice in which patients do not return with test results, and reduce indirect costs by avoiding travel. Further, POC testing is likely to be the primary form of testing that many patients in the developing world will have access to in the future (St John and Price, 2014). Similar to the way cell phones have helped billions of people leapfrog technological advances, POC testing allied with the improved connectivity provided by smartphones could radically alter the way healthcare is practiced and delivered in both the developed and developing world (Ozcan, 2014; Topol, 2015).

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11.1.2 Microfluidic platforms

There are several different microfluidic platforms that have been demonstrated for POC testing. These platforms can be classified by the motive forces required for performing operations like pumping, valving, and mixing on chip (Mark et al., 2010). The platforms include centrifugal microfluidics (Madou et al., 2001), digital microfluidics (Sista et al., 2008), droplet microfluidics (Basova and Foret, 2015), continuous flow/pneumatic microfluidics (Kartalov et al., 2006), and programmable capillary microfluidics (Yetisen et al., 2013). From an application point of view, the specific test category and clinical area addressed are more relevant than the technological tool box used to construct the test. Therefore, this chapter is organized according to the different kinds of tests that have been enabled by microfluidics rather than focusing on the technology elements. We will also restrict ourselves to microfluidic technologies that offer a level of fluid control over and above that demonstrated by simple capillary flow such as that seen in lateral-flow Rapid Diagnostic Tests (RDTs).

11.2 Categories of in vitro diagnostic tests

The focus of this chapter is on IVD or in vitro diagnostic tests which are commonly performed on body fluids such as blood, serum, saliva, or urine. In existing central labs, large automated equipment is used to perform the battery of 1000+ tests, which can be prescribed by a clinician (https://labtestsonline.org/map/aindex/). These tests fall into several different categories such as molecular diagnostics, immunoassays, microbiology, hematology, clinical chemistry, histopathology, and more. The existing paradigm for lab technology automation in the central lab is based on robotic liquid handling, which enables high throughput and multiparameter analysis to be performed efficiently. Robotic liquid handling requires a certain minimum physical footprint and cost which makes it difficult to translate such technologies to portable POC analyzers. Further, handling sub-μL volumes of fluid using such technology has many practical challenges such as evaporation of fluids and loss of precision in metering. Microfluidics offers a fundamentally different paradigm for fluid handling by using devices and motive forces which enable the handling of fluids at the nL scale and even smaller. This also means that tests performed using standardized methodologies in the central lab have to sometimes be done in fundamentally different ways in POC microfluidic devices. Several academic reports published over the last two decades have focused on the development of both platforms for different categories as well as individual tests. In the rest of this chapter, we have organized the different microfluidic POC tests according to the IVD category to which they belong.

11.2.1 Polymerase chain reaction/nucleic acid amplification

Nucleic acid tests have been steadily increasing in their importance and in the market share of all IVD tests over the last decade. They cover a range of different clinical applications from infectious diseases, in which they are establishing themselves as the gold standard for many tests, to new genetic tests for applications ranging from

cancer to cardiac disease. Genetic material in biological samples is usually segmented inside cells and is in much smaller quantities per unit volume than the typical protein or metabolite. Nucleic acid testing therefore usually requires thorough sample preparation to isolate and separate the material of interest before amplification to detect the analyte. Although polymerase chain reaction (PCR) testing has now been used for over 30 years, it has been restricted to central labs thanks to the complex sample preparation steps required. Although several microfluidic technologies and miniature thermocyclers have been proposed for nucleic acid amplification over the years, sample preparation on chip has been a challenge (Dineva et al., 2007). The method used for sample preparation depends critically on where the nucleic acid material lies. For pathogen detection in whole blood or sputum, removal of erythrocytes and other interfering materials is key to obtaining a sensitive result. For some pathogens like malaria which are present inside erythrocytes, selective separation of such cells may be required. Several innovations have been reported over the past few years to address this challenge. Many reviews have focused on improved methods for sample preparation on microfluidic chips (Cui et al., 2015; Mach et al., 2013; Park et al., 2011). Sample preparation for PCR needs to include effective methods to first separate, then lyse, and finally extract nucleic acid material. Several methods including membrane filtration, sedimentation, flow-based separation, and separation using acoustic, magnetic, and dielectrophoretic forces have been reported. Some of these methods exploit favorable physics seen at the microscale. For lysis, methods can be broadly classified into those that use chemical, thermal, electric, and mechanical techniques. For extraction, silica-based solid-phase extraction and magnetic bead-based extraction, both used at the macroscale, have been demonstrated inside microfluidic devices. The Immiscible Filtration Assisted by Surface Tension (IFAST) technique uses paramagnetic particles to first bind and then to isolate whole cells, proteins, and nucleic acid material from complex sample matrices such as whole blood (Berry et al., 2011). The technique relies on bead-bound analyte traversing across interfaces causing almost instantaneous separation. Liquid–liquid and liquid–air interfaces have been exploited for this purpose. A similar technique that uses surface tension-based valves inside capillaries has also been proposed and demonstrated for concentration of DNA from urine (Bordelon et al., 2013) and for RNA separation (Bordelon et al., 2011). In the past few years, sequencing technologies have made great strides, and we are now close to achieving the "$1000-genome." Sequencing technologies allied with microfluidic sample preparation and amplification technologies could provide an answer to the problem of identifying unknown species at the POC. In particular, the problem of antibiotic resistance is growing rapidly, and there is a clear need for better diagnostic tests based on nucleic acid amplification that can help avoid or at least slow the spread of resistant bugs (Laxminarayan et al., 2013).

11.2.2 Immunoassays

Immunoassays exploit the very specific reaction between antibody and antigen to measure a range of different protein markers implicated in infectious disease, cardiovascular disease, endocrine dysfunction, vitamin deficiencies, and several others. There have been hundreds of publications on immunoassays on microfluidic platforms

and some excellent reviews on the same subject (Han et al., 2013; Ng et al., 2010). Microfluidic platforms for POC immunoassays have used different substrates including beads and microchannel surfaces to immobilize antibodies. Immunoassay panels are often required to be multiplexed and microfluidics offers a distinct advantage over existing techniques in this regard. Although existing commercial platforms use well-based approaches in which cross-talk between species limits multiplexing, microfluidics offers different methodologies including spatially encoded arrays (Ghodbane et al., 2015) and barcoded beads (Appleyard et al., 2011) to provide for multiplexing. One of the main advantages of microfluidics for immunoassays is the potential reduction in sample volume and assay time enabled by the smaller-length scales enabled by microfluidic assays (Miller et al., 2011). The final readout for the assay could be optical, electrochemical, or mechanical. Within these methodologies, optical methods have found the most favor. In contrast to bulk immunoassays in which a large path length is available, microfluidic methods typically use path lengths on the order of ~100 μm. Fluorescence methods have therefore emerged as the favorable detection methodology as they provide the required sensitivity with shorter path lengths. More recently, immunosensors on capillary platforms like paper (Mu et al., 2014), thread (Zhou et al., 2012), and fabric (Bhandari et al., 2011) have been reported which combine the simplicity of lateral-flow platforms with a rudimentary level of flow control provided through patterned capillary substrates.

11.2.3 Metabolites and clinical chemistry

Metabolites are chemical species created during the metabolic activities of the human body. Among the metabolites most relevant to POC testing include glucose, creatinine, sodium, potassium, blood gases, and calcium. There are several commercial platforms that can perform these tests at the POC. Among these, Abbott's i-STAT platform is a notable example given the breadth of test menu and multiplexing capabilities provided. This handheld, blood-analyzing device primarily uses electrochemical analysis of blood samples using cartridges containing specific enzyme-coated electrodes or ion-specific electrodes, a calibrant, and a waste reservoir. There are several similar platforms for the i-STAT like immunoradiometric assay, Epoc, and Opti-1 (Erickson and Wilding, 1993). Although offering a wide range of analyte detection, i-STAT is relatively expensive and requires the reagents to be stored at cold temperatures, which is a challenge in resource-poor settings. For this chapter, we focus on microfluidics-based advances of recent years which add significant value to these previously commercialized platforms. The value added could be in terms of cost reduction, improved stability of reagents, faster or more accurate testing, new methodologies provided through microfluidics, etc. Some viable and cost-effective POC alternatives to i-STAT for specific analyte measurements are discussed later.

11.2.3.1 Glucose

The two most widely used techniques for glucose detection are photometry and amperometry. In both cases, an enzyme, usually glucose oxidase, oxidizes the glucose.

Chronoamperometry is the most popular technique to measure glucose from whole blood samples (Wang, 2008). In photometry, a dye acts as the detectable medium in the reaction, and, in the amperometric method, a mediator-enabled electrochemical reaction at the working electrode generates a concentration-dependent current. Recent microfluidic advances in glucose sensors have included exploring both paper (Dungchai et al., 2009; Nie et al., 2010) and fabric (Choudhary et al., 2015) as constituent materials for the sensors. Screen-printed carbon and Ag/AgCl electrodes were integrated with a chromatography paper-based fluidic channel or fabric that had been patterned into hydrophilic and hydrophobic regions. Reduction in costs down to two cents per sensor has been proposed as the advantage of these over existing commercial sensors. Further, paper-based electrochemical sensors were used in conjunction with commercially available glucometers (Nie et al., 2010) to detect glucose, cholesterol, and lactate which can help significantly bring down costs by breaking the prevalent closed system model in which sensors made by one company only work with proprietary glucometers. There is a tremendous interest in connected and wearable diagnostics that can be interfaced with a mobile phone and provide real-time information to a patient or a doctor. In this context, advances in glucose detection have focused on continuous glucose monitoring through contact lens-based detection (Badugu et al., 2004; Yao et al., 2011), tattoos integrated into the skin (Bandodkar et al., 2015), and subcutaneous insertion of sensors underneath the skin (Bindra et al., 1991; Heller and Pishko, 2003; Strategies for calibrating a subcutaneous glucose sensor – Abstract – Europe PMC, n.d.).

11.2.3.2 Lipid profile (cholesterol, triglycerides, HDL, and LDL)

Monitoring blood lipid profile involves measuring cholesterol levels [total, low-density lipoproteins (LDL), high-density lipoproteins (HDL), and triglycerides]. LDL and total cholesterol levels can be vital indicators of heart attack and stroke risks. POC techniques for measuring lipid profile include reflectance photometry (Plüddemann et al., 2012) of venous or finger-prick blood. Commercial lipid profiling devices are provided by Alere, Roche, and Polymer Technology Systems (Plüddemann et al., 2012). Research has also focused on lipoprotein separation and analysis by combining microfluidic/capillary electrophoresis with fluorescence detection (Huifei Zheng, 2010; Weiller et al., 2002). A microfluidics-based isoelectric focusing device was used to perform electrophoresis and characterize lipid samples from elderly and young patients (Jang et al., 2011). The mobility, spread, and isoelectric points of the HDL3 fraction of the sample could be used to predict the extent of aging from different subjects.

11.2.3.3 Kidney profile (creatinine, urea, and uric acid)

Creatinine measurement in whole blood or serum/plasma can facilitate diagnosis and treatment of chronic kidney diseases. Nova Statsensor POC and ABL800 flex analyzer are among the popular POC devices for creatinine and estimated Glomerular Filtration Rate (eGFR) measurements. However, negative interferences can offer challenges to these platforms (Shephard, 2011; Straseski et al., 2011). Microfluidic approaches for creatinine detection are also being researched have shown promising correlations with conventional methods (Lin et al., 2013; Songjaroen et al., 2009).

11.2.3.4 Liver function (AST/SGOT, ALT/SGPT)

Aspartate transaminase (AST) or the equivalent serum glutamic oxaloacetic transaminase (SGOT) and alanine transaminase (ALT) or the equivalent serum glutamic pyruvic transaminase (SGPT) are useful indicators of liver conditions such as alcoholic liver disease (Cohen and Kaplan, 1979). A multiplexed, paper-based microfluidic assay designed for quick, semiquantitative measurement of AST and ALT in a finger-stick specimen has been tested clinically (Pollock et al., 2012). Such paper-based techniques have also been supplemented by optical detection (Swanson et al., 2015). Microfluidic channels combined with electrochemical sensors have also used to measure ALT and AST (Song et al., 2009).

11.2.3.5 Blood gases

Microfluidic systems can combine microdialysis and optical monitoring have been used for detecting blood gases (pH, pCO_2, and pO_2) (Cooney and Towe, 2004). Rapidlab 1200 from Siemens is one commercially available POC device for blood gas monitoring (Mielsch et al., 2010).

11.2.3.6 Electrolytes

Electrolytes like sodium, potassium, and chloride are important parameters in monitoring the health of critically ill patients (Kapoor et al., 2014). Sodium monitoring in sweat by means of a conductometric microfluidic sensor can indicate levels of dehydration (Liu et al., 2014). Multichannel capillary electrophoresis has been used to concurrently detect multiple cations and anions (Mai et al., 2016). Paper microfluidics using a salt bridge could potentially measure chloride ion concentration in blood serum (Jang et al., 2015).

11.2.3.7 Uric acid, lactate, bilirubin, gamma-glutamyl transferase

Electrochemical-based measurements using screen-printed electrodes on paper have been used for multiplexed detection of glucose, lactate, and uric acid (Dungchai et al., 2009). Lactate ProAnalyzer from Arkray Inc. is a commercial POC device for measuring lactate (Gaieski et al., 2013). Electrochemical tattoos are an interesting noninvasive technique of measuring lactate in sweat (Jia et al., 2013). Bilirubin level monitoring can be useful for monitoring neonatal jaundice (Carceller-Blanchard et al., 2009). Bilirubin levels and gamma-glutamyl transferase (GGT) detection have been integrated on centrifugal microfluidic or "lab on disc" platforms (Nwankire et al., 2013). Colorimetric techniques can also be used for determining GGT levels in serum (Del Corso et al., 2006).

11.2.4 Hematology/cell enumeration

11.2.4.1 Complete blood count

Hematology deals with the analysis of the cellular content of blood and is an invaluable tool for physicians for diagnosis. Population estimates of the different categories

of blood cells can provide key indicators to patient health. Complete Blood Counts (CBCs) are vital parameters by which a physician makes a diagnosis or monitors treatment of patients. CBCs include Red Blood Cell (RBC) counts, White Blood Cell (WBC) counts, platelet counts, hematocrit, and hemoglobin levels. In addition, the five-part differential which includes the five different categories of WBCs is often performed today. Traditional methods of hematology include manual counting under the microscope using a hemocytometer. In the past 30 years, automation of the cell enumeration process has been facilitated by electrical methods that make use of the low electrical conductivity of cells which are measured by Coulter counters. COULTER Ac·T from Beckman Coulter for example can provide a complete blood count with a 60 samples per hour throughput. The cells are channeled between two closely spaced electrodes and the difference in current is used to measure the population and volume of the specific cells. Flow cytometry is another cell enumeration technique that uses optical scattering in a flow of hydrodynamically focused cells. Although current flow cytometers are mostly bulky and expensive instruments, inexpensive equivalents using cheaper laser diodes have been studied (Habbersett et al., 2007).

With increased access to cheap computing power and sophisticated image processing algorithms, image processing-based methods are now being revived again. Hematology can now be performed using a combination of a microfluidic device and a camera. HemoCue AB (von Schencket et al., 1986) has been among the most successful manufacturers of instruments for hemoglobinometry that uses the technique of dual wavelength optical absorbance (von Schenck et al., 1986). Portable versions have also been used recently (Nkrumah et al., 2011). EasyCell *assistant* employs image analysis algorithms to automatically group on the display by cell type for confirmatory review. The company Pixcell has a product Hemoscreen that combines viscoelastic focusing and image analysis to provide a complete blood count (Heikali and Carlo, 2010). A cluster of differentiation 4 (CD4) POC test marketed by Alere (Waltham, MA), the Pima CD4 test uses dual-fluorescence image analysis to count $CD3^+$ and $CD4^+$ using labeled anti-hCD3 and anti-hCD4. Chempaq XBC (Rao et al., 2008) and Sysmex pocH-100i (Whisler and Dahlgren, 2005) are a couple of commercially available automated hematology analyzers that offer CBCs.

11.2.4.2 Microfluidic methods for hematology

POC diagnostics for hematology has generated worldwide interest because of lower operating costs, simplicity, and quick result availability. These features are particularly important as diseases like Human Immunodeficiency Virus (HIV) and malaria are prevalent in developing countries. Microfluidic cell counting primarily relies on channeling/focusing blood cells so that they can be analyzed. Techniques such as impedance-based measurements (Morgan et al., 2006), hydrodynamic focusing (Chang et al., 2007), dielectrophoretic focusing (Holmes et al., 2006), and optofluidic fluorescence (Holmes et al., 2006; Morgan et al., 2006) have been used to reduce hematology to lab-on-chip platform. Magnetic and acoustic waves have also been used to channel blood cells in chips for analysis (Tsutsui and Ho, 2009).

HIV tests

In HIV infections, $CD4^+$ T Lymphocytes count in blood can be used to infer the response to drug treatment. With image-processing algorithms, fast automating of cell quantification on HIV microfluidic devices can be performed. This approach has several advantages over manual counting, giving higher throughput and reducing manual labor. Microfluidic Enzyme Linked Immuno Sorbent Assay (ELISA) has been used for CD4 counting with cell phone-based image processing is a promising approach to POC for HIV diagnosis (Wang et al., 2014). Daktari Diagnostics, Inc. (Boston, MA) is developing a CD4 test that uses a novel microfluidic-affinity chromatography/shear-gradient technique to differentially capture $CD4^+$ cells from whole blood (Boyle et al., 2012).

Blood cell count/hematology

Circulating tumor cells are being studied for monitoring cancer progression and metastasis. The challenge in detecting these cells in blood is mainly due to their sparse concentration. A Microfluidic chip-based micro-Hall detector (μHD) can measure single, immunomagnetically tagged cells in blood (Issadore, 2015). Microfluidic Impedance cytometers have been proposed as a full blood count POC device. Differential Leukocyte Counts (DLC) were also measured on capillary and venous blood (Hollis et al., 2012; Holmes et al., 2009).

Malaria

Several groups have been looking into using image processing to automate malaria parasite counting in blood. Translation of malaria diagnostics to POC domain has been much desired in developing countries. BinaxNow from Alere is a popular immunochromatographic assay that provides results in an easy to understand and quick manner (Murray et al., 2008). There has been a unique approach to image-processing analysis, by crowd sourcing the visual identification of infected and healthy cells. People untrained in medical diagnosis differentiate between cells by means of a mobile gaming application (app). The reported accuracy of diagnosis of malaria-infected red blood cells was within 1.25% of the diagnostics made by a trained medical professional (Mavandadi et al., 2012).

Optofluidic techniques have also been proposed for malaria diagnosis in which differential optical absorption is observed between malaria affected and unaffected RBCs in a microfluidic channel (Banoth et al., 2015).

11.2.5 Microbiology

Antibiotic resistance is a concern worldwide in the treatment of bacterial infections and there is an increasing demand for antibiotic susceptibility screening. Such screening has been found to have an important effect on the patient treatments for bacterial infection.

Traditional methods of antibiotic susceptibility screening include phenotypic assays measuring bacterial activity in the presence of the antibiotic in consideration. Although sensitive and widely used, this process is slow and labor-intensive owing to

the need of incubation times and pure cultures. PCR-based approaches have been used to overcome these difficulties (Park et al., 2011; Pulido et al., 2013) and the implementation of microfluidic has been used in some of the following cases.

11.2.5.1 Antibiotic sensitivity microfluidics

Microfluidic flow microbial analyzers have been proposed that detect fluorescence and act as a microbial cell counter (Inatomi et al., 2006). This technique can be significantly cheaper and less resource demanding than conventional flow cytometers. A keen interest in microfluidic pathogen sensing has developed over the past few years, offering low cost and rapid POC diagnostics (Mairhofer et al., 2009; Saleh-Lakha and Trevors, 2010). Microfluidic approaches to antibiotic susceptibility screening make use of high surface-to-volume ratios to improve bacterial reproduction and oxygenation, and thereby reduce screening time to a couple of hours (Chen et al., 2010). Amperometric measurements of treated screen-printed carbon electrodes have been used to study antibiotic screening (Mann and Mikkelsen, 2008). The primary advantages of performing microbiological studies on a microfluidic platform are quick results, smaller quantities of reagents, low cost, and portability (Monaghan et al., 2000).

Beyond medical diagnostics, microfluidic platforms can be used for monitoring microbial pathogens in the environment, in which reagent volumes can be reduced by two orders of magnitude (Wen-Tso Liu, 2005). Microbial counting in freshwater from fluorescence measurements on-chip can reduce result acquisition times (Yamaguchi et al., 2011). Metabolic activity of microbes was studied and identified using on chip impedance-based techniques (Boehm et al., 2007; Gomez-Sjoberg et al., 2005).

11.2.5.2 Single cell/droplet isolation

Single cell isolation of microbial cells in microfabricated channels or traps can offer excellent control over the culture of these cells with respect to their structure, biochemical properties, and genetic variations. Droplet techniques, in which single cells have been confined in microscopic wells, can significantly reduce screening time by avoiding preincubation steps. Water-in-oil emulsion droplets were used to encapsulate and study growth kinetics and antibiotic resistance of microbial cultures (Baraban et al., 2011; Boedicker et al., 2008; Mazutis et al., 2013). Lipid/silica nanostructures have also been used to encapsulate bacteria by self-assembly processes (Baca et al., 2006; Hol and Dekker, 2014)

11.2.5.3 Sepsis urinary tract infection

Quick detection and identification of bacterial urinary Tract Infections have been performed by microfluidic isotachophoresis (Rapid detection of urinary tract infections using isotachophoresis and molecular beacons. Microfluidic Technologies Laboratory, n.d.). Bioluminescence of adenosine triphosphate (ATP) in a microfluidic lab-on-chip platforms have also been proposed for urinary tract infection (UTI) detection (Shilun Feng et al., 2014).

11.2.6 Integrated platforms

The vision of POC testing is not just to miniaturize the operations performed in a central lab but also to integrate the different kinds of instruments for different test categories into one platform. The Bill and Melinda Gates Foundation and partners like Grand Challenges Canada have been at the forefront of such efforts, as articulated by Bill Gates himself—"Can you create a new device that quickly diagnoses HIV, TB, malaria, and other diseases… accepts different samples, like blood, saliva, and sputum… is affordable… and reliable… and will work in a small clinic that has only a few hours of electricity a day?" (Gates, 2014). In a small health center or doctor's office, keeping track of several different platforms, their corresponding disposables, and calibration and service requirements can prove uneconomical and inefficient because of resource limitations. It is therefore an interesting opportunity for microfluidics to solve the integration challenge.

A common problem that is faced here is that groups of tests often cross categories. For example, a suspected case of dengue might require testing for both platelet counts and dengue antibody/antigen, which requires performing both hematology and an immunoassay. Platforms that are capable of performing different kinds of tests on one device are therefore particularly useful. Concepts of these sorts of instruments have been demonstrated in the past in the DxBox (Stevens et al., 2008) and more recently in the smartphone dongle which performs both an immunoassay and a clinical chemistry assay (Guo et al., 2015). The key challenge faced here is that different categories of tests often use fundamentally different workflows and detection systems.

11.2.7 Integration with smartphones

Smartphones can enable true POC testing, in which the capabilities of the smartphone are leveraged for multiple functions including detection through camera, powering of the sensor, data processing, transfer, and integration with other healthcare applications. There have been a number of reports beginning in 2008 (Martinez et al., 2008) on the use of smartphones for diagnostic applications. Although the earliest applications focused on using the cell phone camera for microscopy (Seo et al., 2009) and detecting stripe intensities on RDTs (Mudanyali et al., 2012), publications that are more recent have focused on integrating entire microfluidic devices with mobile phones. Smartphones can enable consumer-focused medical diagnostic testing at home which has the potential to revolutionize the field of medical testing and will have a huge positive impact on microfluidic device development.

11.3 Commercialization of platforms and challenges

Although the academic literature on microfluidics has expanded hugely over the past decade, the microfluidic chips that have successfully entered the market are few in number (Chin et al., 2012). In this section, we will outline some of the challenges associated with commercialization. A significant but often overlooked reason is in defining, prior to the development phase, the market segment and test profile one wishes to target.

11.3.1 Existing non-microfluidic technologies and their evolution

Understanding existing solutions and the competitive advantage one hopes to derive over existing solutions is an exercise that must be conducted before investing in the commercialization of a microfluidics platform. For instance, at the POC, RDTs based on lateral-flow technology have been immensely successful for the detection of infectious diseases, home pregnancy, drugs of abuse, and other applications. One of the major factors behind the success of these tests is the inherent simplicity of this platform enabled by capillary flow. Secondly, a mature manufacturing process for coating, cutting, and packaging these membranes exists which significantly reduces the technology risk in manufacturing such tests. On the other hand, lab automation technologies are becoming smaller and cheaper every year. They have also had a 40-year head start in which several issues such as the breadth of test menu, surface/stability properties, and manufacturing for scale have been successfully addressed. There are also several nonmicrofluidic POC technologies that have had varied degrees of success in the market. Microfluidics-based solutions have to be seen in this context as complementary to some of these existing technologies rather than necessarily replacing them. When a replacement is planned, it is important to define the advantages whether in terms of cost, multiplexing capability, ability to deal directly with complex specimens, ease of use, precalibration, etc.

11.3.2 Manufacturing challenges

Among the main challenges that microfluidic sensors for diagnostics face in getting to the market is the challenge of scalable manufacturing. Some of the key issues faced during this process are:

1. Lack of standardized components (van Heeren et al., 2015; Temiz et al., 2015), materials and manufacturing processes;
2. Expense involved in customized automation for cartridge assembly;
3. Underestimation of the true cost of goods including packaging, labor, tool costs, etc.

The lack of standardized components stems from the fact that no single microfluidics platform has yet established itself as the de facto standard. Further, polydimethylsiloxane (PDMS) which is a popular choice for academic research worldwide has proven to be problematic to scale into industrial use because of its ability to absorb organic molecules, its changing surface properties, and lack of mature manufacturing processes at the industrial scale (Berthier et al., 2012; Mukhopadhyay, 2007). Thermoplastic materials like polymethylmethacrylate (PMMA), cyclic olefin copolymer (COC), and other materials can be manufactured using injection molding, enabling industrial quantities of these devices to be produced (Becker and Gärtner, 2008). However, the cost of tooling can be prohibitive for early prototyping. For intermediate-scale manufacturing of small lots, hot embossing using low-cost soft tooling can be used to produce features down to the nanoscale in thermoplastic materials. One must bear in mind that the small features (<20 μm) possible in hot embossing may not

easily transfer to an injection molding process. The lack of standardized components and interfaces delays the rapid prototyping and iteration of designs while also making it hard to connect with other components like syringes, tubing, and detection systems. Recently some efforts have been made to address this issue through the setting up of a standards committee (van Heeren et al., 2015) for microfluidic cartridge design. In practice, individual microfluidic cartridges from companies tend to be highly customized to a particular application. There are now several companies such as Microfluidic Chip Shop, Dolomite, Micronit, Micrux, Elveflow, Thinxxs, Achira Labs, etc. that provide microfluidic fabrication services and components to help diagnostics companies prototype, make small batches, and eventually mass manufacture the microfluidic cartridges.

11.3.3 Regulatory barriers and trends

The lack of uniform regulation for medical devices has been an impediment to the rapid transfer of devices across geographies. This problem can be even more significant for microfluidic devices as they are not established platforms like ELISAs or lateral-flow assays. In the United States, the 510(k) route is the preferred route for the acceptance of a new device as it avoids the lengthy requirements of the Pre-Market Approval (PMA) process. However, for this, significant equivalence to an existing technology must be demonstrated. Many developing countries, including India and China, do not distinguish IVD from drugs or other medical devices, respectively. Particular care must be taken in each country that the country-specific requirements are adhered to while planning the introduction of a product. The International Organization for Standardization (ISO) 13485 guidelines are emerging as a unifying regulatory standard for IVD.

11.3.4 Outlook for the future

To take a microfluidic platform from academic proof of concept to a sellable product requires a median time of 7 years and funding in the range of $10–$50M (Chin et al., 2012). During the period of this transition, companies have to survive with little or no revenues and with significant risks on the technical and business side that are yet to be mitigated. Investor money may not always be available during this time as the risk is perceived to be too high. Nondilutive government funding has been very critical to the survival of a number of microfluidics companies during this period. This funding can also help companies find important clinical problems and provide a network to a young company. However, it also brings challenges of loss of focus and reporting requirements that are not always aligned with the priorities of the business. To attract more funding into such ventures, more examples of a clearly defined and successful business model being executed must be clearly seen. This means that the entire cycle from technology development to manufacturing, regulatory approval, and successful commercialization needs to be completed and a profitable company built. A clear focus on the final market is required which can vary from geography to geography. Although consumer-driven healthcare models might work in the developed world,

integration into the existing public healthcare system will be critical for success in the developing world. Several companies like Claros, Daktari Diagnostics, Cube, Scanadu, Theranos, Achira, iQuum (acquired by Roche), Genalyte, and many others have built microfluidics-based products for near-patient testing. The commercial success of a few of these companies will drive further investment and introduce a virtuous cycle into POC diagnostics.

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Electrochemical medical biosensors for POC applications

12

Ü. Anik
MSKU, Muğla, Turkey

12.1 Introduction

Detection of diseases at the early stage is important both for patient health and reduction of treatment costs. According to the Milken Institute, the total number of chronic disease-affected patients in the United States is about 162 million. In addition, as stated in the same report, $790 billion cost for the patient treatment is expected by 2023 (Vasan et al., 2013). For these reasons, it is important to have highly sensitive and diverse techniques that can be effective at early stages of diseases. Miniaturized, economic, and practical devices that have the capacity to replace time-consuming laboratory analyses are urgently needed for diagnostic processes. In this context, Point of Care (POC) technology is one of the technologies that fulfills these needs. POC covers the analyses that are conducted at the patient bedside by eliminating the laboratory step (Wang, 2006). POC also has the potential to bring faster and more economical diagnosis, which provides immediate results that may affect the patient's health status (Rasooly, 2006).

As well known, biosensors are small devices that provide selective analysis by monitoring biochemical molecular recognition. They are specific, fast, portable, and economical systems. On the other hand, utilization of electrochemistry brings practicality, sensitivity, accuracy, speed, and low cost to biosensor systems. POC technology needs practicality and portability together with sensitivity and accuracy. In this sense, electrochemical biosensors have the potential to become the core of POC technology. The further parts of this chapter will focus on definition and types of electrochemical biosensors, their potentials as POC systems, and their applications in medical areas.

12.2 General approach to electrochemical biosensors

Biosensors are integrated analytical devices, which use a recognition element, generally a biomolecule, to bind an analyte and some transduction mechanisms to detect this binding event (Fig. 12.1). Because biosensors are specific, economical, and practical, they provide advantages in many areas including clinical applications (Wang, 2006).

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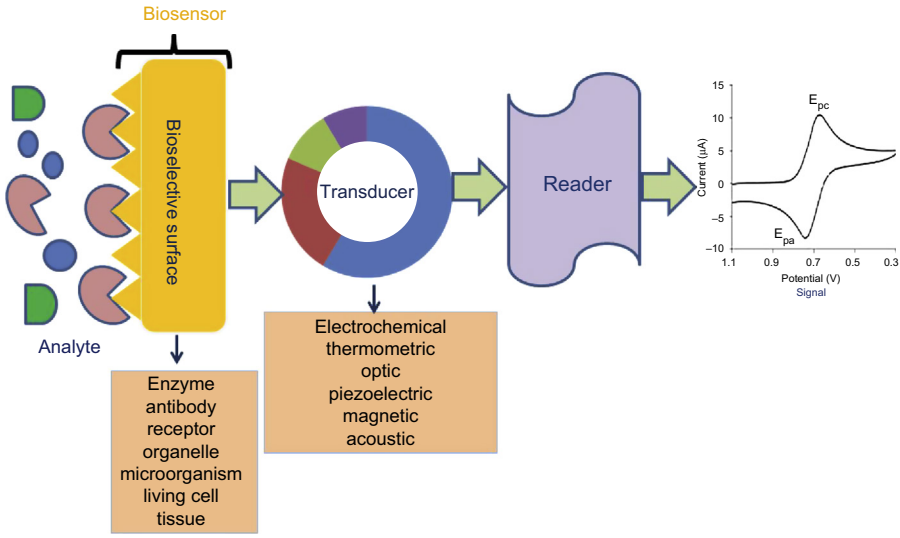

Figure 12.1 Schematic representation of biosensors.

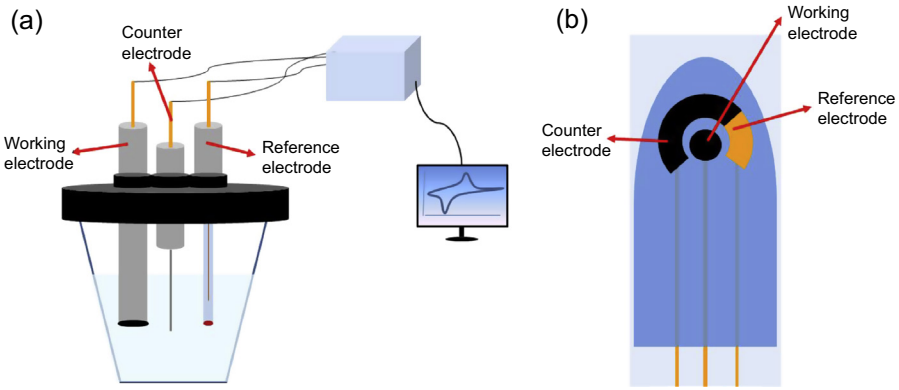

Figure 12.2 (a) An electrochemical working cell, (b) Disposable screen-printed electrode.

If an electrode system is used in the transduction mechanism of a biosensor as a transducer, then an electrochemical biosensor is obtained. Here, the suitable electrode, the transducer, converts the biological recognition event into an electrical signal.

12.2.1 Transduction in electrochemical biosensors

Conductometric, potentiometric, amperometric, voltammetric transistors together with impedimetric transistors have already been used in electrochemical biosensors. To apply these techniques, suitable electrodes are needed. Usually three types of electrodes, namely working, reference, and auxiliary electrodes, have been utilized for conducting these electrochemical techniques. Fig. 12.2(a) demonstrates the typical

electrochemical cell that is used in voltammetric methods. The working electrode is the electrode at which the concerning reactions take place. Considering electrochemical biosensors, these electrodes can be solid electrodes like carbon, gold, platinum (Pt), diamond, etc. The other types of electrodes, composite electrodes, are also frequently used as working electrodes. Carbon-based composite electrodes like the carbon paste electrode (CPE) and the glassy carbon paste electrode (GCPE) are two important examples of these electrodes. These paste electrodes can easily be prepared by hand mixing the proper amount of graphite (in the case of CPE) or glassy carbon microparticles (for GCPE) with the proper amount of mineral oil. The ease in renewal, preparation, modification, and miniaturization expands the uses of these types of electrodes. Recently, nanomaterials have been introduced in these electrodes to enhance their electrochemical performance. The types of nanomaterials that are combined with working electrodes will be given in more detail in the remainder of this chapter. Meanwhile, the reference electrode provides a constant against the changeable working electrode's potential. Silver/silver chloride and saturated calomel electrodes are the most convenient reference electrode types. The auxiliary electrode is generally utilized for voltammetric and impedimetric measurements and is known as current-carrying electrode. An inert solid electrode like Pt or graphite can serve as an auxiliary electrode. Besides these, in the view of POC, for portability and practicality, disposable electrodes are fabricated that contain these three electrodes in one (Fig. 12.2(b)).

Going back to the electrochemical methods, conductometry can be defined as a method that measures the conductivity difference of a medium. To process the method, charged species, ions, must be present in the medium. In conductometric biosensors, charged species are either produced or consumed which causes a definite conductivity change in the sample solution (Jaffrezic-Renault and Dzyadevych, 2008; Lawrence and Moores, 1972). Conductometric microbiosensors could be the strongest candidates in the application of these biosensors to POC systems. Conductometric microbiosensors have many advantages like suitability for miniaturization, not needing a reference or an auxiliary electrode, not being light sensitive, and applicable to a wide variety of samples. In addition, because of their differential mode of measurement, many types of interferences are eliminated (Saiapina et al., 2011).

On the other hand, in potentiometry, the potential difference between reference and working electrode is monitored under zero or very small current. Usually, ion-selective electrodes (ISE) have been used to observe conversion of the biorecognition process into a measurable potentiometric signal (Wang, 2006). For example, an enzyme can be immobilized on a pH-meter electrode. The bioelectrochemical reaction produces or absorbs H^+. The change in the H^+ concentration definitely causes the change in pH which can be easily monitored with the pH meter. Glass pH electrodes coated with a selective gas-permeable membrane for compounds like CO_2, NH_3, or H_2S, and AgS together with AgX-selective thin-membrane electrodes are most commonly used ISEs as potentiometric biosensor transducers (Bisen, 2014).

The other type of electrochemical biosensors, amperometric biosensors, measure the current under the influence of a constant potential that is applied between the reference and working electrodes. Many types of electrodes have been used in amperometric biosensors. However, considering the glucose biosensor, the Clark electrode has

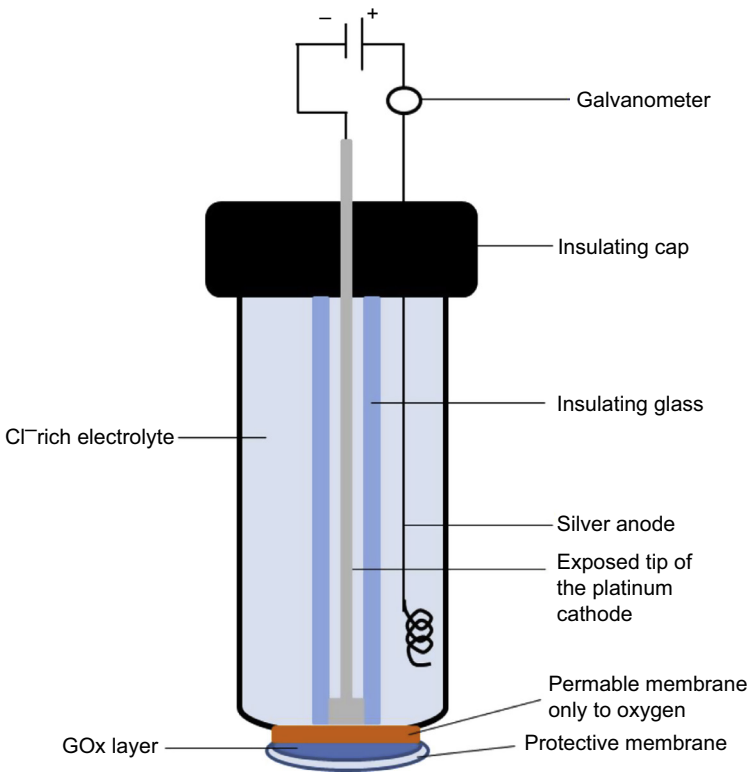

Figure 12.3 Schematic representation of Clark electrode.

to be mentioned specifically here. In the case of the Clark electrode, the combination of two electrodes is concerned (Fig. 12.3). There is a Pt cathode at which oxygen is reduced and a silver/silver chloride reference electrode. These two electrodes are combined by immersing them in saturated potassium chloride solution, and they contain an oxygen-permeable plastic membrane such as polytetrafluoroethylene (Teflon) (Fig. 12.3). The main reaction is as follows:

$$\text{Ag anode: } 4Ag^0 + 4Cl^- \rightarrow 4AgCl + 4e^-$$
$$\text{Pt cathode: } O_2 + 4H^+ + 4e^- \rightarrow 2H_2O$$

Oxygen is carried onto the cathode electrode surface from bulk solution and immediately reduced to water at the Pt cathode at which Ag^0 is oxidized at Ag^+ at the Ag anode. By monitoring the obtained current which leads to oxygen consumption, the analyte concentration is indirectly measured. As will be explained in the next sections, sometimes evolution of hydrogen peroxide is followed with this electrode (Dzyadevych et al., 2008).

Impedimetry, also called electrochemical impedance spectroscopy (EIS), has usually been used in affinity biosensors for monitoring the changes that happen on the electrode

surface. Upon binding of the new layer, the capacitance or charge-transfer resistance of the electrode has changed and this can be easily followed using EIS (Cecchetto et al., 2015) EIS provides the monitoring of a wide variety of molecules. In addition, it provides label-free detection which makes it practical compared to other techniques like the fluorescent-based or magnetic-based biosensors. However, the advantage of working with label-free biosensors decreases sensitivity compared to label-included biosensors. Also concerning POC systems, the complexity of required instrumentation should be taken into consideration in the case of usage of EIS (Manickam et al., 2012).

12.2.2 Types of electrochemical biosensors based on biological recognition elements

12.2.2.1 Enzymatic electrochemical biosensors

Enzymes can be described as biological catalysts that respond specifically to particular substrates. To obtain enzymatic electrochemical biosensors, a suitable enzyme has to be immobilized onto the electrode surface. Then the oxidation or reduction of a specific analyte is monitored via current change (Fig. 12.4). One of the oldest and the most remarkable enzymatic biosensor is glucose biosensor. Especially in the context of POC analysis, glucose strips that are used to monitor diabetes provide high sensitivity, real-time analysis, portability, and simplicity (Kerman et al., 2008; Lafleur et al., 2016; Ispas et al., 2012; Kimmel et al., 2012; Liu et al., 2012b). In the first commercial glucose biosensor, which was launched in 1973 by the Yellow Springs Instrument Company (Ohio, United States) glucose was measured based on the amperometric detection of hydrogen peroxide as shown in the following reaction:

$$\text{glucose} + O_2 \xrightarrow{\text{GOx}} \text{gluconolactone} + H_2O_2$$

For sensing glucose in the blood, glucose oxidase (GOx) enzyme is immobilized onto the membrane and attached on the Clark electrode's surface. GOx is a type of

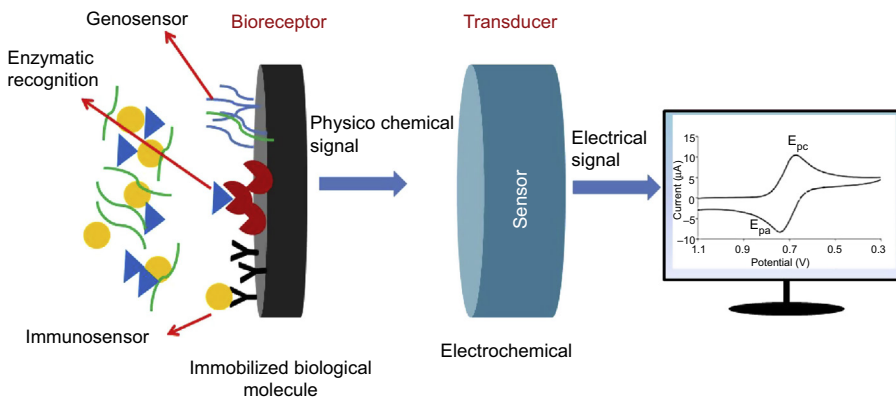

Figure 12.4 Types of electrochemical affinity biosensors.

oxidoreductase enzyme. These types of enzymes are the most common enzyme group that is used in amperometric biosensors in which O_2 consumption or H_2O_2 evolution is monitored. Although electrode types have changed, like usage of screen-printed electrodes instead of a Clark electrode for glucose stripes, similar reactions, meaning consumption of O_2 or evolution of H_2O_2, have been followed for monitoring the glucose level of diabetes (Wang, 2006; Newman and Turner, 2005).

12.2.2.2 Bioaffinity-based electrochemical biosensors

Compared to electrochemical enzymatic detection, it can be said that affinity-based interactions help us monitor assays that are more complex (Wang, 2006). Electrochemical techniques are very proper techniques to follow the biorecognition events in affinity-based sensors. Although amperometric detection is more practical relative to EIS, especially for monitoring the changes at the electrode surface, EIS is preferred (Lafleur et al., 2016).

Genosensors [deoxyribonucleic acid (DNA)-based biosensors], immunosensors, cytosensors, and aptamer-based biosensors (aptasensors) are types of affinity-based biosensors.

In electrochemical genosensors, usually a single-stranded (ss) oligonucleotide probe is immobilized onto the electrode surface. The transduction is completed with hybridization of this probe with its complementary DNA sequence via base pairing (Fig. 12.4). In terms of electrochemical detection, the hybridization of two DNA sequences is monitored via the increased-current signal of an indicator that binds to the DNA duplex or from other hybridization-based electrochemical changes like the current decrease of guanine base found in the DNA sequence. In one application of electrochemical genosensor, an 86-mer DNA peanut sequence encoding part of the allergen Ara h 2 (conglutin-homolog protein) was detected. Screen-printed gold electrodes were utilized as the working electrode and sandwich assay was used. The single line of captured DNA was thiolated and with this probe DNA together with mercaptohexanol, a self-assembly monolayer was formed. The working conditions were optimized, and under these optimum conditions, it was reported that a limit of detection (LOD) value of 10 mM and a linear range of 5×10^{-11} to 5×10^{-8} M was obtained (Lopez et al., 2014).

On the other hand, immunosensors rely on monitoring antibody–antigen interplay after the hybridization occurs onto the electrode surface. In some assays, antigen or antibody has been labeled with a substrate, usually with an enzyme that produces an electrochemically measurable signal. Besides enzymes, metal markers and redox tags have also been used for electrochemical monitoring of antigen–antibody interplay (Wang, 2006) (Fig. 12.4). Many electrochemical immunosensors have been used for medical applications. For instance, Hong et al. fabricated an electrochemical immunosensor which was reusable for the sequential detection of three tumor markers cancer antigen 125 (CA125), carcinoembryonic antigen (CEA), and prostate specific antigen (PSA). The immunosensor contained a temperature-responsive polymer, which facilitates the addition of biotin, streptavidin, and antibody on the electrode surface. Because of this polymer, when the temperature is changed, the adsorption and desorption of these biological substances are done without any damage. In the presence of

horseradish peroxidase and antibody-labeled nanoparticles, a range from 0.0064 to 256 units per milliliter (U/mL), 1 pg/mL to 100 ng/mL, and 10 pg/mL to 10 ng/mL with detection limits of 0.007 U/mL, 0.7 pg/mL, and 0.9 pg/mL of CA125, CEA, and PSA, respectively, were observed (Hong et al., 2016).

The third type of affinity-based biosensor is the cytosensor. Cytosensors can be defined as a kind of biosensor that evaluates the cells. In the fabrication process, the electrode surface is modified to be selective to significant cells. In the case of electrochemical cytosensors, the change in the current, impedance, and capacitance have been measured to obtain information about the cells (Liu et al., 2013). Detection of cancer cells has widely been conducted with cytosensors by capturing proper cell lines that are found in the sample solution. For example, our group developed an electrochemical cytosensor for detection of cervical cancer cells known as HeLa cells. In this study, GCPE was used as working electrode in which gold nanoparticles (AuNPs) cysteamine, glutaraldehyde, PAMAM, and folic acid were immobilized onto it successively. HeLa cells are selective to folate positive receptors. Based on this interaction, detection of HeLa cells were achieved by using EIS and voltammetric methods. Optimizations of experimental parameters were done and then linear range between 10^2 cells per mL and 10^6 cells per mL and LOD value as 100 cells per mL were obtained (Tepeli et al., 2015).

The last type of affinity-based biosensors that will be discussed here is aptasensors. Aptamers are short and stable single-stranded oligonucleic acid molecules, in another words single-stranded DNA or RNA molecules with high specificity to various ligands like amino acids, drugs, and proteins (He et al., 2013; Hianik and Wang, 2009). They are the cores of aptasensors (Fig. 12.4). It is easy to label aptamers with proper tags like fluorescence probes, quenchers, electrochemical indicators, nanoparticles, or enzymes. The ease in modification procedures makes it possible to immobilize aptamers onto different supports with high stability. Because of this variety, different detection methods can be used in aptasensors (Hianik and Wang, 2009).

As an example of application of electrochemical aptasensor to medical diagnosis, Sun et al. developed a label-free electrochemical aptasensor for thrombin detection in whole blood. For the fabrication of this aptamers, self-assembled multilayers of carboxymethyl-polyethylene glycol-carboxymethyl (CM-PEG-CM) and thrombin-binding aptamer (TBA) were used. The thrombin molecules in the medium bound onto the TBA which leads to current decrease. This current decrease was monitored by differential pulse voltammetry. A linear range of 1 pM to 160 nM was obtained for thrombin with LOD value of 1.56×10^{-14} M (Sun et al., 2013).

12.2.3 Nanomaterials in electrochemical biosensors

Nowadays, to increase sensitivity and selectivity of electrochemical applications, besides new techniques, chemical modification and functionalization of electrodes have also been conducted (Katz et al., 2004). In recent years, new electrode materials like GCPE and bismuth film electrode (BiFE) have been developed and applied to electrochemical biosensor systems (Anik et al., 2008; Timur and Anik, 2007; Wang et al., 2001). As mentioned earlier, nanomaterials like carbon-based nanomaterials, metallic nanoparticles, and nanoballs have been introduced into electrode structure

(Banks et al., 2005; Dai et al., 2004; Zhong et al., 2000). Providing suitable environment for biological molecules and facilitation of reachment of enzyme active center are two important properties that AuNP brings to this area (Cubukcu et al., 2007). On the other hand, carbon-based nanomaterials like carbon nanotubes (CNTs) and graphene have been widely used because they increase the electron-transfer rate and provide higher a surface area for the immobilization of biological molecules (Tepeli and Anik, 2015). In addition, decoration of these two carbon-based nanomaterials with metal nanoparticles is easy and produces robust material in terms of electrochemical catalysis (Tepeli and Anik, 2015). Besides decoration, sometimes nanohybrid or hybrid nanomaterials have been produced and used in electrochemical biosensors. For example, Peng et al. combined AuNP with multiwalled carbon nanotubes (MWCNTs) and, in this way, a nanohybrid was formed. They used AuNP–MWCNT hybrid in the layer-by-layer fabrication of glucose biosensor in which GOx was used as enzyme. By means of this nanohybrid layer, the group managed to immobilize large amount of GOx. In addition, they mentioned that because of this nanohybrid layer, electron transfer was facilitated and the charge-transfer resistance was decreased. As a result, a stable and sensitive biosensor with a wide linear range was obtained for glucose detection (Peng et al., 2011). The other recent works including nanomaterial-modified electrochemical biosensors for medical applications are given in Table 12.1.

12.3 Portable decentralized devices based on electrochemical biosensors for medical applications

12.3.1 Microfluidics platforms and labs on chips

As mentioned in the previous section, to integrate biosensors into the POC concept, portable, practical, fast, and sensitive biosensor systems must be developed. In this manner, microfluidic analytical platforms and LOCs are two systems that suit this approach. Systems like wearable biosensors and microarrays will not be discussed in this chapter Instead, application of medical electrochemical biosensors to LOC platforms will be discussed.

Microfluidic systems are based on Total Analysis System (TAS), which aims to diminish and accumulate all steps of analysis of a sample onto a single device (Guo et al., 2015). This system has to have driving equipment like pumps and reactors and necessary parts of the chemical processes like sample preparation, filtration, dilution, reaction, and detection (Guo et al., 2015; Connelly et al., 2012). Meanwhile, the microfluidic analytical platform, Micro Total Analysis System (μTAS), means a single micrometer chip that contains the whole laboratory (Guo et al., 2015; Dittrich et al., 2006; Kovarik et al., 2013).

As examples of microfluidic analytical platforms and μTAS, LOC systems can be described as a miniaturized lab that is proper for POC applications. Because of the microfluid's physics, LOCs can be designed properly with different functions like sample pretreatment including separation and enrichment before the sensing process (Guo et al., 2015).

Table 12.1 **Nanomaterial-modified electrochemical biosensors for medical applications**

Biorecognition element	Analyte	Measurement method	LOD/linear range	Medical application	References
P1/AuNPs/TB–GO/GCE	MDR 1 Multi-drug-resistance gene	DPV	2.95×10^{-12} M/10^{-11}–10^{-9} M	Investigation of DNA hybridization for early diagnosis of multidrug resistance which is a major obstacle in chemotherapy	Peng et al. (2015)
P/AuNPs/ITO	Apolipoprotein E gene	EIS	286 nM/–	Investigation of DNA hybridization related to a specific point mutation in Apolipoprotein E, which was related to the progression of Alzheimer's disease	Cheng et al. (2014)
Lipase/NPG/GCE	Triglycerides	Amperometry	–/–	Detection of triglycerides in human serum	Wu et al. (2014)
SNA/GA/BSA-incorporated Ag nanoflowers/GCE	DLD-1 human colon cancer cells	EIS	40 cells per mL/1.35×10^{2}–1.35×10^{7} cells per mL	Sensing DLD-1 human colon cancer cells for the early monitoring of tumor cells and convenient evaluation of sialic acid on living cells.	Cao et al. (2015)
Ab/Pd/GCE	Alpha-fetoprotein	SWV	4 pg per mL/0.01–75.0 ng per mL	Detection of cancer biomarker alpha-fetoprotein in human blood serum	Wang et al. (2014a,b)
ChOx/Pt–Au@ZnONRs/CS-MWCNTs/GCE	Cholesterol	Amperometry	0.1–759.3 μM	Determination of cholesterol in human blood serum	Wang et al. (2012)
CcR/CNT/PPy/Pt CcR/SAM-GNP/PPy/Pt	Cytochrome c	CV	1–1000 μM 5–600 μM	Quantification of the mitochondrial cytochrome c released in cytosol of human lung carcinoma cells A549 upon induction of apoptosis with doxorubicin	Pandiaraj et al. (2013)

Ab/Pd/GCE, alpha-fetoprotein antibody/palladium nanoplates/glassy carbon electrode; *CcR/CNT/PPy/Pt*, cytochrome c reductase/carbon nanotube/polypyrrole/platinum electrode; *CcR/SAM-GNP/PPy/Pt*, cytochrome c reductase/self-assembled monolayer gold nanoparticles/polypyrrole/platinum electrode; *ChOx/Pt–Au@ZnONRs/CS-MWCNTs/GCE*, cholesterol oxidase/platinum-gold@zinc oxide nanorods/chitosan-multiwalled carbon nanotubes/glassy carbon electrode; *CV*, cyclic voltammetry; *DPV*, differential pulse voltammetry; *EIS*, electrochemical impedance spectroscopy; *GOx/(AuNPs/MWCNT)$_5$/Au electrode*, glucose oxidase/multilayered (gold nanoparticles/multiwalled carbon nanotubes)/gold electrode; *Lipase/NPG/GCE*, lipase/nanoporous gold/glassy carbon electrode; *P/AuNPs/ITO*, DNA oligonucleotide probe/gold nanoparticles/indium tin oxide coated glass surface; *P1/AuNPs/TB–GO/GCE*, single-stranded DNA probe/gold nanoparticles/toluidine blue–graphene oxide/glassy carbon electrode; *SNA/GA/BSA-incorporated Ag nanoflowers/GCE*, sambucus nigra agglutinin/glutaraldehyde/bovine serum albumin-incorporated silver nanoflowers/glassy carbon electrode; *SWV*, square wave voltammetry.

Integration of biosensor systems to microfluidics platforms like LOC also provides improvements to the response of sensing systems. For example, in the case of LOC, because of these presensing procedures, interferences can be removed which increases the accuracy of the measurements, or analyte concentration may be increased in the detection volume which enhances the sensitivity of the measurement. Besides, because of diminished sizes and reduced volumes, only a little amount of sample, like a drop of blood, would be enough to achieve the analysis in LOCs. In addition, since the distance between analyte molecule and biorecognition element is reduced, diffusion-controlled electron-transfer yield is increased which reflects on sensor performance (Lafleur et al., 2016; Lynn et al., 2015; Lynn and Homola, 2015).

12.3.2 Types of detection in microfluidics and in LOCs based on electrochemical biosensor strategy

12.3.2.1 Enzymatic detection

Enzymatic detection means the combination of electrochemical enzymatic biosensor detecting principle with LOC systems. Considering the medical enzymatic electrochemical LOC systems, the following recent works could be illustrative. For instance, Ali et al. developed a nanoporous microfluidic biochip in which chitosan was modified with cholesterol esterase and cholesterol oxidase and immobilized onto an anatase titanium dioxide nanoparticle electrode. As a result, this electrode was integrated into a polydimethylsiloxane microchannel structure and the designed biochip was used in cholesterol detection. The detection mechanism was based on EIS measurements. By means of impedimetry and the integration of nanoporous anatase titanium dioxide and chitosan to the biochip, a sensitive, fast, and economic system was obtained. The developed microfluidic biochip has a linear range between 2 and 500 mg/dL cholesterol with detection limit of 0.2 mg/dL. In addition, the authors mentioned that the developed biochip has potential for application in clinical diagnostics (Ali et al., 2014).

The same group also developed another label-free impedimetric LOC by utilizing protein [bovine serum albumin (BSA)] and antiapolipoprotein B functionalized carbon nanotubes–nickel oxide (NiO) nanocomposite, and they used this chip for low-density lipoprotein (LDL) detection. They also tested the cytotoxicity of CNTs, NiO nanoparticles, and CNT–NiO nanocomposite, in the presence of lung epithelial cancer A549 cell line by using 3-(4,5-dimethylthiazol-2-yl)- 2,5-diphenyltetrazolium bromide tetrazolium salt (MTT) assay. They have been found that the toxicity increases with increasing CNT concentration. In terms of LDL detection, developed LOC was sensitive with detection limit of 0.63 mg/dL LDL and linear range of 5–120 mg/dL of LDL (Ali et al., 2015). The other recent developments about enzymatic medical LOC is presented in Table 12.2.

12.3.2.2 Electrochemical bioaffinity detection

Considering the integration of affinity-based biosensors to microfluidic platforms, it can be said that higher sensitivity is achieved due to faster analysis time and efficient collection of electroactive species because of improved transport provided by microfluidics (Hervás et al., 2012; Ben-Yoav et al., 2012; Zuzuarregui et al., 2015).

Table 12.2 Recent developments in electrochemical affinity and enzyme-based microfluidic systems for medical applications

Biorecognition unit	Analyte	Electrochemical method	LOD/linear range	References
CPE and GOx solution in reservoir of the electrochemical compact disk platform	Glucose	CV and amperometry	0.29 mM/1–10 mM	Rattanarat et al. (2015)
Secondary Ab/detection Ab with ALP/ConA/nanostructured Au electrode	NoV	CV	35 copies per mL/10^2–10^6 copies per mL	Hong et al. (2015)
Noninvasive label-free biosensor using (3-aminopropyl) triethoxysilane and GOx modified polysilicon nanogap	Glucose	Chronoamperometry	0.6 μM/5 μM–50 mM	Balakrishnan et al. (2014)
GOx/SPE	Glucose	Amperometry	–	Tan et al. (2012)
Anti-MPO coated magnetic beads/Au microband electrodes	MPO	Amperometry	0.200 ng/mL (MPO under flow) 0.004 ng/mL (MPO under stopped flow)	Moral Vico et al. (2015)
The surface of dendritic Au@PtPdNPs was linked with folic acid by click chemistry and folate receptor of cell surface determined with high affinity	K-562 cells	DPV, CV, and EIS	31 cells per mL/1.0×10^2–2.0×10^7 cells per mL	Ge et al. (2015)
Concentric double ring-single disk electrodes integrated with DC-biased AC electroosmotic stirring	DNA	EIS	0.5 aM/1 aM–10 pM	Wu et al. (2015)
Trp/MWCNT/AuSPE	Trp	Constant current potentiometric stripping analysis and EIS	4.9×10^{-12} mol per L/1.0×10^{-11}–1.0×10^{-4} mol per L	Majidi et al. (2016)
Gold microelectrode arrays fabricated on a glass substrate and functionalized with specific antibodies	*Candida albicans*, *Streptococcus agalactiae*, and *Chlamydia trachomatis*	EIS	–	Chiriacò et al. (2016)

Continued

Table 12.2 Continued

Biorecognition unit	Analyte	Electrochemical method	LOD/linear range	References
Lithographic gold electrodes were modified in two ways; (1) formation of a SAM of the bipodal alkanethiol DT2 followed by covalent linking of whole anti *F. tularensis* antibody and (2) direct chemisorption of *F. tularensis* antibody fragments	*Francisella tularensis*	Step and sweep amperometry	4.5 ng/mL for the lipopolysaccharide antigen isolated from *F. tularensis* and 31 bacteria/mL for the *F. tularensis* bacteria/–	Dulay et al. (2014)
Anti-EBV/Pt electrode	EBV	Stop-flow amperometry	–	Horak et al. (2014)
EA/anti-Cab/DTSP/Au	Cortisol	CV	–/10 pM–100 nM	Vasudev et al. (2013)
ssDNA probes/MWCNTs/SiO_2	ssDNA	DPV	1.36 μM/–	Kim et al. (2013a,b)
Thrombin-aptamer coated magnetic beads	Protein	EIS	0.01 nM/0.1–10 nM	Wang et al. (2014a,b)
Anti-Cab/ZnO nanostructures/Au electrode	Cortisol	EIS	1 pM/–	Vabbina et al. (2015)
MC1R-Abs were immobilized in (n-SiNPs)-PPy nanocomposite/SPE	CTC	CV	20 cells per mL/50–7500 cells 2.5 per mL	Seenivasan et al. (2015)
Thiolated probe ssDNA/Square Au thin-film electrodes	*Campylobacter* spp.	SWV/CV	90 pM/1–25 nM	Morant-Miñana and Elizalde (2015)

ALP, alkaline phosphatase (an electrochemical enzyme); *Anti-Cab*, anti-cortisol antibodies; *Anti-EBV*, Epstein–Barr virus antigen (viral capsid antigen); *Anti-MPO*, myeloperoxidase antibody; *Au*, Gold; *Au@PtPd NPs*, gold core platinum palladium shell trimetallic dendritic nanoparticles; *CNTs*, carbon nanotubes; *ConA*, concanavalin A; *CPE*, carbon paste electrode; *CTC*, circulating tumor cells; *CV*, cyclic voltammetry; *Detection Ab*, detection antibodies; *DPV*, differential pulse voltammetry; *DTSP*, dithiobis (succinimidyl propionate); *EA*, ethanolamine; *EBV*, Epstein–Barr virus; *GOx*, glucose oxidase; *IrOx NPs*, iridium oxide nanoparticles; *K-562 cells*, valuable markers for early human chronic myelogenous leukemia; *MC1R-Abs*, melanocortin one receptor antibodies; *MPO*, myeloperoxidase (a cardiovascular biomarker); *MWCNT*, multi-walled carbon nanotube; *NoV*, Norovirus; *n-SiNPs*, amino-functionalized silica nanoparticles; *PPy*, polypyrrole; *Pt*, platin; *Secondary Ab*, secondary antibodies; *SPE*, screen printed electrode; *ssDNA*, single-stranded DNA; *SWV*, square wave voltammetry; *Trp*, L-tryptophan; *Tyr*, tyrosinase.

For example, Seia et al. (2014) demonstrated that an electrochemical immunosensor combined with a microfluidic chip reduces the analysis time by a factor of 3.8 for thyrotropin (TSH) compared to an ELISA test, and improves the sensitivity. In addition, Ben-Yoav et al. developed an LOC device for electrochemical analysis of DNA hybridization events. This LOC contained 3 × 3 arrayed electrochemical sensors and a dual-layer microfluidic valved manipulation system. EIS was used in the transduction process and DNA hybridization was measured in terms of charge-transfer resistance. The detection limit was calculated as 1 nM in terms of ssDNA target (Ben-Yoav et al., 2015).

In another work, a centrifugal microfluidic device which contained a bead-based enzyme-linked immune-sorbent assay for electrochemical capture of target antigen from biological samples was prepared. This protocol was applied for C-reactive protein (CRP). LOD value of developed microfluidic was calculated as 4.9 pg/mL. Besides, this centrifugal microfluidic device completed a measurement less than 20 min (Kim et al., 2013a,b).

For aptamer-based microfluidics, micropatterned gold electrodes were functionalized with electroactive aptamers and used for detection of interferon (IFN)-ɣ and tumor necrosis factor (TNF)-α cytokines which are important in the diagnosis of diseases like tuberculosis. Anti-IFN-ɣ DNA aptamers and anti-TNF-α RNA aptamers were immobilized individually onto half-ring electrodes. For better immobilization on gold, aptamers were functionalized with thiols, and methylene-blue mediator was attached onto them for the production of electrochemical signal. Cell detection was achieved after the integration of electrode arrays into microfluidic devices and incubation with immune cells. The microfluidic platform contained two parallel microfluidic channels and each channel possessed four cell-sensing sites. In this microdevice, square wave voltammetry (SWV) was used as an electrochemical transduction method to obtain the necessary signal upon binding of anti-aptamers with target aptamers. For the future, the researchers expected to detect larger number of cytokines which can be used to understand the levels and behaviors of cytokine release in immune cells that leads to diagnosis and treatment of infectious diseases (Liu et al., 2012a).

The recent developments in bioaffinity-based microfluidic systems are presented in Table 12.2.

12.4 Conclusions and future prospects

Fast, practical, and economical diagnosis is very important in terms of the patient's health and government health policies. Since electrochemical techniques provide practicality and sensitivity together with low cost and simplicity, these techniques are the strongest candidates to be used in clinical applications. Fabrication of electrochemical POC devices based on enzymatic or bioaffinity assays could provide early detection and treatment of diseases that is important in terms of patient survival rates. Although many biosensor/microfluidic-integrated systems have not yet been vastly produced, it is obvious that there is a significant potential in combining LOCs with

electrochemical biosensors. Usage of microfluidics eliminates the interferences, concentrates the low amount proteins, and allows the labeling of molecules which, as a result, enhances selectivity in the measurement. For this reason, microfluidics in LOC design brings many advantages to LOC/biosensor systems. However, due to high cost and fouling of sensing material, together with the short lifetimes of biosensors, serious efforts must be consumed in future studies involving integration of these two systems. The other important point is the necessity of usage of these POC systems by untrained personnel. In addition, popular nanomaterials like graphene and conductive polymers could be very effective on the performances of LOC/biosensors.

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'*Note*: Page numbers followed by "f" indicate figures, "t" indicate tables, and "b" indicate boxes.'