Featuring work from the research group of Professor Utkan Demirci, Stanford University School of Medicine, California, USA.

Photonic crystals: emerging biosensors and their promise for point-of-care applications

Photonic crystals integrated with emerging technologies (such as smartphones, microfluidics, and wearable and flexible materials) hold great promise for biosensing applications at point-of-care (POC).

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Biosensors are extensively employed for diagnosing a broad array of diseases and disorders in clinical settings worldwide. The implementation of biosensors at the point-of-care (POC), such as at primary clinics or the bedside, faces impediments because they may require highly trained personnel, have long assay times, large sizes, and high instrumental cost. Thus, there exists a need to develop inexpensive, reliable, user-friendly, and compact biosensing systems at the POC. Biosensors incorporated with photonic crystal (PC) structures hold promise to address many of the aforementioned challenges facing the development of new POC diagnostics. Currently, PC-based biosensors have been employed for detecting a variety of biotargets, such as cells, pathogens, proteins, antibodies, and nucleic acids, with high efficiency and selectivity. In this review, we provide a broad overview of PCs by explaining their structures, fabrication techniques, and sensing principles. Furthermore, we discuss recent applications of PC-based biosensors incorporated with emerging technologies, including telemedicine, flexible and wearable sensing, smart materials and metamaterials. Finally, we discuss current challenges associated with existing biosensors, and provide an outlook for PC-based biosensors and their promise at the POC.

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1. Introduction

Biosensing is an emerging analytical field for the detection of biochemical interactions leveraging electrical, optical, calorimetric, and electrochemical transducing systems.\(^1,2\) These transduction mechanisms are employed to translate changes and variations within the biological domain into a readable and quantifiable signal (e.g., association, dissociation, and oxidation).\(^3\) Biosensors are most notably employed for detecting various biological targets,
such as cells,\(^4\) bacteria,\(^5,6\) viruses,\(^7\) proteins,\(^8\) hormones,\(^9\) enzymes,\(^10\) and nucleic acids,\(^11\) to facilitate the diagnosis and prognosis of diseases. Currently, state of the art clinical laboratories require trained personnel to perform sample collection, testing, and analysis using sophisticated biosensing devices in centralized clinical settings (Fig. 1). Staffing the necessary personnel to ensure accurate and reliable readings can be costly, and results are subject to operator error.\(^12,13\) Although certain automated instrumentation has been used to simultaneously process multiple patient samples at large volumes (e.g., hematology analyzers), technicians are still needed for device oversight and maintenance.\(^14,15\) Centralized laboratories also perform immunoassays and nucleic amplification strategies, but these methods are time consuming, labor intensive, and expensive. As an example, enzyme-linked immunosorbent assay (ELISA) requires several experimental steps, including antibody immobilization, target binding, labeling, substrate incubation, signal production, and multiple washing steps.\(^16,17\)

Recently, substantial research efforts have been devoted to the development of in vitro diagnostic tests including point-of-care (POC) devices with the market volume estimated to reach US$ 75.1 billion by 2020.\(^18\) One of the main drivers for these POC technologies is the detection of diseases in resource-limited countries.\(^19-25\) For example, commercial POC kits have been recently developed to detect human immunodeficiency virus (HIV) and tuberculosis in such settings.\(^26\) However, there are significant logistical, technical, and social barriers that need to be overcome when performing testing at these sites, and many of these technologies still require the recruitment and training of personnel (Fig. 1).\(^14,27-29,30\) Thus, there exists a need to develop affordable, sensitive, rapid, portable, label-free, and user-friendly POC diagnostic tools.\(^31-33\)

Incorporation of microfluidics and nanotechnology into biosensing platforms holds great promise to address the aforementioned challenges. Sensitive technologies, such as localized and surface plasmon resonance, electrical sensors, interferometric biosensors, and photonic crystal (PC)-based biosensors, have been employed as diagnostic devices (Table 1).\(^34-40\) PC-based biosensors hold many advantages over other existing competing biosensing technologies, including cost-effective fabrication and short assay time (Table 2). PC structures have been used to detect a wide array of biotargets in biological sample matrices, such as blood, urine, sweat, and tears,\(^41-43\) and can be fabricated using various inexpensive fabrication methods, such as colloidal self-assembly, hydrogels, and mold-based replica imprinting.\(^44-46\)

In this review, recent incorporation of PC structures within emerging label-free biosensing platforms is discussed, including their applications for detecting proteins, nucleic acids, allergens, pathogens, and cancer biomarkers.\(^47-50\) We will also provide a broad overview of PC structures and PC-based biosensors and their potential utilization as POC diagnostic tools. We describe various aspects of PC-based biosensors, including (i) PC structures and fabrication techniques, (ii) principles of PC-based biosensing, (iii) emerging technologies incorporating PC-based biosensors for potential POC applications, (iv) multi-target detection capability for PC-based biosensors, (v) surface chemistry approaches, (vi) current challenges and limitations for biosensors at the POC, and (vii) future outlook for PC-based biosensors at POC diagnostics.

### 2. Photonic crystal structures and fabrication techniques

PC structures consist of spatially arranged periodic dielectric materials that uniquely interact with light, providing high efficiency reflection at specific wavelengths. There are many examples of PC-type periodically nanostructured surfaces observed in nature.\(^51\) For instance, the bright iridescent color of the *Morpho rhetenor* butterfly,\(^52\) peacock,\(^53\) *Eupholus magnificus* insect,\(^54\) sea mouse\(^55\) and opals\(^56\) are all associated with the geometrical arrangement on their surface, where broadband light illuminates and reflects through PC structures (Fig. 2).\(^52\) In practice, PC structures can be fabricated in one-dimensional (1-D), two-dimensional (2-D) or three-dimensional (3-D) orientations incorporating microcavities,\(^57\) waveguides,\(^58\) slabs,\(^59\) multi-layered thin films,\(^60\) and porous geometries\(^61\) (Fig. 3). A diverse range of materials, such as silicon (Si),\(^62\) glass,\(^63\) polymers,\(^64\) colloids,\(^65-68\) and silk,\(^69-71\) are used in the fabrication of PC structures (Table 1).

PC structures are fabricated using various methods, including self-assembly and lithography techniques. For instance, colloids composed of hydrogel polymers,\(^72\) silica,\(^73\) or polystyrene\(^74\) are transferred from solution and self-assembled (via sedimentation, spin coating, or vertical deposition)\(^44,75\) onto a surface to create PC structures that reflect iridescent color.\(^75-77\) In addition, hydrogels are utilized in combination with colloidal particles in the fabrication of PC structures. While these self-assembly methods are inexpensive, precisely controlling the dimensions and geometry of the underlying PC structure is difficult. Top-down approaches, including electron beam lithography (e-beam), nanoimprint lithography (NIL), electrochemical etching, and thin film...
Overall, a wide range of materials and fabrication methods is available for the development of PC structures. Using PC structures for POC applications is highly feasible due to the availability of inexpensive fabrication materials such as hydrogels and colloidal particles and the scalable production method using NIL. The theoretical background behind the PC phenomenon and how these PC structures are used as biosensors are discussed in the following section.

### 3. Principles of PC-based biosensing

A periodic arrangement of dielectric materials creates a photonic band gap when a range of electromagnetic waves cannot propagate due to the destructive interference of incident light with reflections at dielectric boundaries.41 PC structures can be produced from a variety of geometries, including Bragg reflectors, slabs, opals, microcavities, and colloids. An optical phenomenon describing most of these structures can be deduced from understanding a simple Bragg structure. A typical Bragg reflector consists of alternating high and low refractive index dielectric deposition techniques 78,79 are alternatives to bottom-up self-assembly methods. Briefly, in the e-beam process, an electron beam is used to write a desired pattern onto a substrate (often silicon), which is previously coated with an electron-sensitive resist. The resist is then developed, and the electron-beam etching is transferred to the substrate via etching. Performing this method requires e-beam lithography devices, which are large, expensive, and require skilled operators. NIL is a rapid, simple, and scalable pattern transfer technique alternative to e-beam lithography.80 In NIL, a pattern is initially produced using deep UV/e-beam lithography on a master mold, which can be easily transferred to daughter replicas. The NIL method has been used to mass-produce PC structures rapidly and reliably; however, only a finite number of replicas can be generated from a single mold due to wear.79 Electrochemical etching can be used to fabricate porous Si structures that produce a photonic band gap due to formed periodic trenches. Electrochemical etching of Si is inexpensive and can be performed in research labs. Although trenches and channels provide a higher surface area for chemical interactions, large biomolecules may cause aggregation and blocking of the channels (e.g., cells) when using clinical samples.
Table 2 Comparison of PC-based biosensors with selected competing technologies

<table>
<thead>
<tr>
<th>Parameters</th>
<th>Electrical sensors</th>
<th>Plasmonic sensors</th>
<th>Nanomechanical sensors</th>
<th>Magneto-sensors</th>
<th>Photonic crystal sensors</th>
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<tr>
<td>Assay complexity</td>
<td>Low</td>
<td>Low</td>
<td>High</td>
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<td>Readout</td>
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<td>Possible, Temperature and vibration are controlled.</td>
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<td>Multi-target detection</td>
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<td>Clinical testing</td>
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<td>Possible, There are clinical validations with blood, urine, saliva, and serum.</td>
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d_{\text{high}} n_{\text{high}} = d_{\text{Low}} n_{\text{Low}} = \frac{\lambda}{4}
\]  

Another common PC structure is comprised of periodically modulated thin films, which are known as 1-D PC structures are commonly fabricated from a high refractive index coating layer over a periodically arranged low refractive index grating layer (Fig. 4d). In these PC gratings, only the zeroth order mode is allowed, while higher order modes are restricted at normal incidence, provided that the period of the grating (\(A\)) is smaller than the wavelength of the incident light (\(A < \lambda\)). Gratings of this type are also called subwavelength gratings, and exhibit efficient optical resonances. Subwavelength PC gratings can be designed to reflect a narrow band of wavelengths and produce a sharp peak in the reflection spectrum (Fig. 4e).

The resonance wavelength peak is determined by the period (\(A\)) of PC gratings and the effective refractive index \(n_{\text{eff}}\) under resonance conditions (eqn (2)).

\[
A_{\text{resonance}} = \frac{n_{\text{eff}} \lambda}{4}
\]

This resonance behavior of PC gratings is highly sensitive to the localized changes in dielectric permittivity on the crystal surface, which makes it suitable for sensing applications. In this regard, PC structures are widely utilized to develop sensing platforms for multiple applications of chemical sensing, environmental sensing, and more specifically, biosensing. Briefly, a biochemical interaction (e.g., binding) on the PC surface causes a change in the effective refractive index, which results in a shift of the resonance wavelength peak, which is proportional to the concentration of the biotarget (Fig. 5). PC structures have gained significant attention as sensitive transducers and have been incorporated into biosensors that capture, detect, and quantify various biological molecules, such as pathogens, DNA, proteins, enzymes, glucose, cells, toxins, and allergens.

4. Emerging technologies incorporating PC-based biosensors for potential POC applications

Recent advances in microfluidics, telemedicine, flexible materials, and wearable sensing technologies hold promise to provide compact and portable platforms in biosensing applications at POC for the rapid, reliable, accurate, on-site, and label-free detection of biotargets.
4.1 Microfluidics

Microfluidics technology offers considerable benefits to biosensing systems, particularly the POC devices. These advantages include (i) inexpensive fabrication materials (e.g., glass, paper and polymers), (ii) ability to control low sample volume, (iii) ease of integration with optical platforms, and (iv) flexibility in producing multiple channels to allow multiplexed testing platforms.119–121 PCs-integrated with microfluidic technologies are emerging as powerful biosensing diagnostic tools with the integration of these features.50,122 For instance, integration of 1-D PC slabs within a microfluidic channel network at the bottom of a 96-well plate was used to detect immunoglobulin gamma (IgG).46 This microfluidic-integrated platform enabled the concurrent multiplex detection of molecules using only 20 μL of the sample (Fig. 6). In another study using a colloidal polystyrene-based PC structure integrated with microfluidics, IgG molecules were captured and detected down to mg mL−1 levels.123 PC structures have also been incorporated with polymer microfluidic channels to detect proteins; for example, a slotted PC cavity fabricated from Si was shown to detect 15 nM of avidin protein.124,125

4.2 Telemedicine

Smartphones have been increasingly utilized in medical diagnostics and healthcare applications, such as cell counting from whole blood, immunoassay testing, and imaging.111,126,127 Smartphones will likely play an important role in the development of new biosensing platforms due to their wide availability, portability, compactness, capacity for data processing, ease of integration with microfluidic devices, and high-resolution optical components.111,128 Recently, camera and optical systems in cellphones have been integrated with microfluidic, microscopy, and photonic crystal technologies for the spectral analyses of biosensing applications.126,129–134 For instance, a 1-D PC slab was integrated with a smartphone to measure IgG concentration. The phone camera was used as a spectrometer to measure the transmission spectrum from the PC structures.135 Although the system produced a reliable dose–response curve, adsorption of
biomolecules could only be measured under dry conditions. Thus, further study with aqueous samples is required before this platform could be used to directly analyze clinical samples at the POC. In another study, a 1-D PC slab was integrated with a complementary metal–oxide–semiconductor (CMOS)-based smartphone camera to detect anti-recombinant human protein CD40 (Cluster of Differentiation-40), streptavidin, and anti-EGF antibody (Fig. 7). Smartphone-integrated platforms hold promise to address portability related issues at the POC, though their direct use in clinical applications is challenging because complex specimens, such as blood and tissue, need to be preprocessed before being brought into contact with the device.

4.3 Wearable and flexible sensors

Wearable sensors and flexible materials have recently gained attention for continuous and real-time monitoring of the physiological parameters and general health status of individuals. For instance, they have been employed to measure the heart rate, skin temperature, blood oxygen levels, and more recently glucose sensing from sweat. Wearable sensors are currently worn as wristbands, skin patches, and fabric patches. From a fabrication perspective, various nanotechnology-based techniques and materials are used for the production of these flexible and wearable sensors. In a recent study, a PC structure was designed with 2-D holes (with a diameter of ~100 nm) to evaluate strain changes. This flexible sensor could be bent without losing its optical properties (Fig. 8a and b), and provided a sensitivity that was independent of deformation. In another study, colloidal polystyrene spheres were deposited on a flexible polyimide film. A strain applied over this flexible film resulted in a blue shift in the reflection maxima (Fig. 8c and d).

3-D PC structures have also been incorporated into wearable sensors. For example, 3-D PC structures were investigated under pressure and may conceptually be used for detecting the severity of blast exposure to evaluate traumatic brain injury of soldiers in the battlefield. In this study, 3-D voids were fabricated in an SU-8 resist to create 3-D PC structures that exhibited a color in the visible spectrum. These structures were exposed to varying high pressures (410 to 1090 kPa) to measure blast strength (Fig. 8e), and it was determined that large external forces could be detected by visual inspection (Fig. 8f–h). The PC structure
that was exposed to high external forces underwent structural deformation, resulting in a color change. This change was used to estimate the degree of pressure on the PC structure. While this work is promising, using these detectors on soldiers’ uniforms is conceptual and their implementation in this field has not yet been evaluated.

4.4 Smart materials

Smart materials are an emerging class of responsive substances that can modify their physical or chemical properties, mostly reversibly, against external stimuli such as pH, temperature, electrical field, and light.\textsuperscript{150,151} Smart materials, such as hydrogels, polyionic liquids, graphene, and carbon nanotubes (CNTs), have been used for various applications, including biosensing. In particular, their incorporation into PC structures holds promise for rapid, sensitive, and reliable biosensing. Hydrogel materials are 3-D nanostructured polymers consisting mostly of water. Hydrogels may be responsive to external stimuli, such as temperature, pH, or bio-stimuli such as antigen–antibody interactions.\textsuperscript{45,72,152–155} For instance, PC structures comprised of hydrogel materials can be used as biosensors for the detection of DNA, proteins, antibodies and enzymes by monitoring the changes in lattice spacing or refractive indices.\textsuperscript{41,43,156–159} In this respect, hydrogel-based PC structures provide either quantitative spectral results or qualitative naked-eye detection of biotarget concentrations.\textsuperscript{41} Hydrogel-based PC structures hold great promise for POC applications owing to their cost-effective fabrication and simple optical detection systems. In a recent study, a hydrogel-based nanoporous PC structure was employed for label-free detection of rotavirus with concentrations ranging from 6.35 μg mL\textsuperscript{-1} to 1.27 mg mL\textsuperscript{-1} (Fig. 9a and b).\textsuperscript{160} Polyionic liquids (PLs) are a class of polymeric materials containing repeating ionic monomeric units, which have recently been demonstrated for sensing applications.\textsuperscript{161,162} In one such study, PIL was used to fabricate a 3-D macroporous PC structure, that exhibited Bragg reflection in the visible wavelength range, to detect a variety of ions.\textsuperscript{163} Hydrogels can also be used in combination with other materials including graphene or carbon nanotubes (CNTs) to produce PC structures. In one such study, graphene oxide was deposited on a silicon wafer and embedded into a hydrogel matrix to detect beta-glucan.\textsuperscript{164} Graphene based-PC structures have also been investigated for enhanced sensitivity biosensing.\textsuperscript{165} In addition, CNTs were incorporated into PC structures that provided a photonic band gap in the visible light spectrum.\textsuperscript{166} Recently, CNT-based PC structures were investigated for optical applications.\textsuperscript{167–169} Smart materials have been studied extensively and have the potential to be utilized as biosensors due to the unique properties of each material. However, they require further validation using clinical matrices.

4.5 Metamaterials

Recently, PC structures based on metamaterials have been investigated for various applications, including imaging and biosensing.\textsuperscript{169–172} For instance, a PC metamaterial with a 3-D woodpile geometry was proposed to excite plasmons with high spectral sensitivity.\textsuperscript{170} The proposed structure was a silver-coated
woodpile crystal providing a high surface-to-volume ratio with a sensitivity more than 2600 nm per refractive index unit (RIU) (Fig. 9c and d). In another study, a hyperbolic metamaterial biosensor consisting of 16 alternating layers of thin Al$_2$O$_3$ (aluminum oxide) and gold layers was demonstrated to detect biotin (Fig. 9e) with very high sensitivity up to 30 000 nm per RIU. This 1-D multilayer structure supported guided modes ranging from visible to near infrared, enabled optical biosensing at different spectral regions with ultra-high spectral sensitivity, and detected 10 pM biotin in phosphate buffered saline (Fig. 9f). Light coupling was achieved with a 2-D gold diffraction grating on top of the multilayer films, eliminating the need for additional optical elements (e.g., prism). Although metamaterial-based biosensors enable label-free detection with high sensitivity, they require multiple fabrication steps and may not be compatible with clinically relevant matrices (i.e., whole blood, urine, and saliva).

Overall, the integration of PC structures with emerging technologies is promising for biosensing applications at POC owing to compact, flexible, and easy-to-use platforms. In particular, PC-based biosensors composed of smart materials may create a new class of flexible and wearable POC sensors with high sensitivity.

5. Multi-target detection capability for PC-based biosensors

PC-based biosensors have been employed to detect multiple biological targets, such as pathogens, proteins, nucleic acids, and glucose, for the diagnosis of a broad range of diseases, including diabetes and cancer. Here, we provide a broad perspective of using PC structures to quantify various molecular interactions ranging from biotin–streptavidin to cancer biomarkers.

5.1 Protein detection

PC structures have been used to capture and detect numerous proteins, such as protein A, Immunoglobulin Gamma (IgG),
bovine serum albumin (BSA), and Protein G. Streptavidin is often used in conjugation with biotin in experiments to validate the sensitivity and detection limit of new PC geometries due to the extraordinary affinity of streptavidin for biotin. PC structures have been employed to investigate the substrate specificity and catalytic activity of certain enzymes, such as

![Fig. 6](image_url)

**Fig. 6** PC biosensors integrated with microfluidic platforms for POC applications. (a) Multi-well plate integrated with a network of microfluidic channels with PC-based biosensors at the bottom. Reproduced from ref. 46 with permission from The Royal Society of Chemistry. A varying color difference was observed within the plate as the concentration of the analyte was changing. (b) A multi-valve microfluidic platform integrated with PCs for biosensing applications. Reproduced from ref. 119 with permission from The Royal Society of Chemistry. (c) Drawing of a microfluidic channel integrated with multiple PC (black rectangles) for optofluidic biosensing applications. Reproduced from ref. 124 with permission from Elsevier, copyright (2011). (d) PWS graph on a PC integrated with microfluidic channel. (e) PWS values as against an increasing concentration of IgG protein in the same microfluidic channel. Reproduced from ref. 243 with permission from The Royal Society of Chemistry.

![Fig. 7](image_url)

**Fig. 7** PC structure integrated with a smartphone for biosensing applications at the POC. (a) Drawing representing a general scheme of a PC incorporated smartphone. The CCD camera of the phone was utilized as an optical sensing element. (b) Actual image of the PC smartphone platform. (c) The PC cartridge fits in a cradle to facilitate the light interaction with PC surfaces. (d) Drawing of another smartphone that employs a PC biosensor. An LED light source was collimated and directed to the PC surface and the transmitted light was captured by the smartphone camera. Reproduced with permission from ref. 136. (e) The spectrum of the PC surface measured with a smartphone CCD camera. Subfigures a, b, c, and e were reproduced from ref. 135 with permission from The Royal Society of Chemistry.
acetyl cholinesterase, pepsin and other proteases. In one study, a porous Si-based PC structure was developed to evaluate proteolytic activities of pepsin and subtilisin proteases down to 7 pmol and 0.37 pM, respectively. When coupled with a fluorescence assay, a PC surface can significantly amplify the fluorophore intensity, increase the signal-to-noise ratio and reduce the detection limits. For example, a PC structure was coupled with fluorescence-labeled secondary antibody to detect...
TNF-α concentrations at pg mL⁻¹ levels. The ability of an assay to detect disease targets at low concentrations at an early stage is very important. In this research, imaging of the PC spots was performed for the multiplex detection of different proteins.

Colloidal PC structures have also been widely employed for protein detection. For instance, arranged colloidal nanoparticles embedded inside a hydrogel were used to visually monitor a reflectance shift in response to protein concentration. In this study, silica nanoparticles were embedded within a poly(ethylene glycol)-diacrylate hydrogel to generate a PC structure. This system was able to observe IgG proteins bound to protein A on the surfaces of the embedded nanoparticles. A color change from orange to green was observed after exposure to 10 mg mL⁻¹ IgG, and the detection limit in the color shift was at the concentration of 0.5 mg mL⁻¹ IgG (Fig. 10). Since this procedure uses a self-assembly deposition method and does not require advanced manufacturing technology, it is cost-effective; however, the concentrations necessary to observe a visual change are high, and thus, may not be compatible with sensitive detection applications.

By coupling with fluorescence-labeled secondary antibodies, PC-based biosensors have also been utilized to capture allergen-specific immunoglobulin (IgE) antibodies. PC structures can enhance fluorescence signals when the optical resonance of the PC surface overlaps with either the excitation or emission spectra of a fluorophore. This enhanced excitation and emission yielded ~7500-fold increase in fluorescence signals. In a recent study, a PC-enhanced fluorescence (PCEF) microarray platform was used to detect low concentrations of IgE in human sera with a limit of detection of 0.02 kU L⁻¹, which was comparable to current blood-based IgE detection methods. However, current PC-based allergen platforms rely on fluorescence detection, which limits their use at the POC due to the requirements for labeling, additional instrumentation, and multiple assay preparation steps.

### 5.2 Nucleic acid detection

Biosensing of DNA, RNA, and DNA–protein interactions using PC-based platforms has been studied for various applications, including the determination of infectious agents, identification of genetic disorders, and monitoring of DNA–protein interactions. For instance, DNA and protein interactions were evaluated using a 1-D PC slab structure with a TiO₂ layer over a low index material, and DNA was detected down to nanomolar concentrations. In this study, a panel of 1000 compounds were screened on a microplate-integrated PC-based biosensing platform (Fig. 11a–c). This platform uses multiple fibers, a motorized stage, and a coupled readout system (SRU Biosystems Bind Reader) capable of recording simultaneous readings from 384-wells. This platform has significant potential for drug-screening studies at the POC in resource-constrained settings since it incorporates a disposable and inexpensive 384-well microplate. The platform can further be utilized for the detection of RNA–protein and protein–protein interactions, and may shed light on gene expression at the cellular and molecular levels. In addition to 1-D PC slab structures, colloid PCs have also been utilized for nucleic acid detection. In this study, self-assembled polystyrene beads were utilized to fabricate a colloidal PC structure that could detect...
hybridized DNA down to 13.5 fM. In another study, a planar waveguide was employed for the detection of single-stranded DNA at a concentration of 19.8 nM. The use of PC structures is a promising alternative to the conventional polymerase chain reaction (PCR) techniques for nucleic acid detection due to their low cost, ease-of-use, rapid response, and high detection capacities.

5.3 Applications in cancer

Biosensors are widely employed in the detection of biomarkers for diagnosis and prognosis of cancer. Currently, various biomarkers, such as epidermal growth factor receptor (EGFR), human epidermal growth factor receptor 2 (HER2), prostate-specific antigen (PSA), carcinoembryonic antigen (CEA), tumor necrosis factor-α (TNF-α), and calreticulin (CRT), are under clinical study for diagnosis of cancer. Detecting these biomarkers at an early stage of malignancy can contribute to better treatment outcomes and significantly increase the quality of life for cancer patients.

Recently, PC-based biosensors have been employed in diagnosis and early detection of cancer. In one study, a waveguide integrated with a cavity was employed for the detection of CEA protein for the diagnosis of colon cancer (Fig. 11d–f). This platform provided a detection limit for CEA protein down to the 0.1 pg mL⁻¹ level. In another study, a cavity and a line defect were fabricated on the surface of a silicon substrate to capture lung cancer cells. In another study, 1-D PC slabs obtained from quartz materials were fabricated via NIL. This PC-based platform was used to detect 21 different cancer biomarkers, including HER2, EGFR, and prostate-specific antigen (PSA) with a detection range from 2.1 pg mL⁻¹ to 41 pg mL⁻¹. This multiplexed cancer biomarker platform can function in both fluorescence and non-fluorescence modes, providing flexibility to work with labelled and non-labelled biotarget sensing.

5.4 Pathogen detection

Rapid identification and quantification of pathogens, such as bacteria and viruses, is important for diagnosis and prognosis in the POC environments at resource-constrained settings. Recently, PC structures have been deployed at the POC for diagnoses of infectious diseases caused by pathogenic agents and toxins. For instance, 2-D PC pillars, fabricated on polymer substrates...
using NIL, were used for the detection of human influenza virus (H1N1) in human saliva. This platform can detect H1N1 antigens at a concentration as low as 1 ng mL\(^{-1}\).\(^{93}\) In another study, polymer-2-D pillar PC structures were used to detect \(L\). pneumophila bacteria down to 200 cells per mL.\(^{96}\) PC-based platforms can also be used for the detection of viruses such as rotavirus, HIV-1, and human papilloma virus-like particles.\(^{92,94,195}\) To detect HIV-1, a PC surface was functionalized with anti-gp120 antibody for capturing HIV-1 ranging from \(10^4\) copies per mL to \(10^8\) copies per mL (Fig. 11g–i). In another study, silica microspheres were used to fabricate colloidal PC structures for the detection of multiple mycotoxins in cereal samples.\(^{201}\) Although microspheres are fabricated inexpensively as droplets in water–oil two-phase flow, this system still depends on fluorescence measurements and may be subject to undesirable background variation due to the inherent labelling procedure.\(^{196}\)

5.5 Glucose sensing

Detection of glucose holds significant importance in POC diagnostics for diabetics.\(^{197,198}\) Although glucose sensors are globally available as POC tools, there is still a need for non-invasive glucose biosensing using new and advanced technology sensing platforms, including PC-based biosensors.\(^{199,200}\) Non-invasive monitoring can be achieved by collecting samples other than blood such as sweat, tear fluid, and urine. For instance, a hybrid photonic structure (1-D Bragg gratings) was fabricated from silver nanoparticles and a hydrogel to detect glucose, fructose, and lactate. This platform was tested with urine samples from diabetes patients with a detection limit of 90 \(\mu\)M.\(^{41}\) In another study, the poly(hydroxyethyl methacrylate)-based (pHEMA) matrix was UV cross-linked, and silver nanoparticles were dispersed in this hydrogel. A pulse laser was then used to align the silver particles in confined regions creating a periodic structure, which ultimately provided PC properties.\(^{201}\) Furthermore, the platform was also tested in artificial tear fluid for accurate glucose sensing (Fig. 12). This platform is unique because it employs inexpensive hydrogels and can be linked to biomolecules by easy conjugation with carboxylic groups. In this study, PC structures were fabricated from polystyrene colloidal spheres integrated with hydrogel for glucose sensing at 50 \(\mu\)M.

Overall, although PC-based platforms have been employed for the detection of glucose with encouraging results, their widespread utilization for glucose sensing and diabetes diagnosis needs to be evaluated for reliable and accurate sensing.

6. Surface chemistry approaches in PC-based biosensing applications

PC-based biosensing platforms consist of an optically active layer and immobilized binder molecules, such as affibodies, nanobodies, peptides, antibodies, and antibody fragments to ensure biotarget capture.\(^{157,158,202}\) Depending on the material type used for the optically active layer, binder molecules can be immobilized using various functionalization strategies, including physical adsorption (physisorption), covalent binding, and affinity-assisted coupling. Furthermore, anti-fouling agents play...
an important role in reducing the non-specific interactions and improving the sensitivity and specificity. In this section, we discuss surface chemistry approaches for TiO$_2$, Si$, and SiO$_2$-based PC sensors, as well as anti-fouling agents to minimize non-specific binding.

Physical adsorption strategies are used to accumulate biotargets onto optically active layers via hydrogen bonds and van der Waals interactions. By applying plasma techniques, the net charge on a surface can be changed to increase the surface coverage of a biotarget. For instance, PC waveguide structures with a Si layer were employed to monitor the physisorption of bovine serum albumin (BSA). In this study, a BSA solution was directly applied to the PC waveguide surface and non-specific physical adsorption of BSA molecules was monitored. Although physisorption is simple, easy-to-apply, and does not require any wet-chemistry or laborious modification steps, it can interfere with other biomolecules in the detection buffer. Furthermore, physisorption is based on weak interactions between the surface and the biotarget, and is therefore not stable and can easily detach when surface charge is altered by changes in pH, ionic content, and temperature.

Covalent binding is one of the standard methods for immobilization approaches using the strong chemical linkage that forms between a sensor surface and binder molecules. TiO$_2$ and SiO$_2$ surfaces are common substrates for optical sensors; however, performing coupling on these surfaces is laborious since it requires layer-by-layer surface functionalization including surface activation, functional group generation, and binder immobilization. Silane-based molecules with a variety of functional groups are commonly used to immobilize biomolecules onto glass surfaces. A standard protocol for silanizing a surface begins with cleaning the surface using a strong oxidizing agent, such as piranha solution (a mixture of H$_2$O$_2$ and H$_2$SO$_4$) to increase the density of silanol groups exposed on a surface, which also increases the hydrophilicity of the sensor surface. Then, a silanization agent, such as (3-aminopropyl)triethoxysilane (APTES) or (3-aminopropyl)trimethoxysilane–tetramethoxysilane (MPTMS or 3-MPS), is applied to generate a self-assembled monolayer (SAM), which consists of hydroxyl groups, alkyll backbone chains, and functional tail groups. Alkyl chains enable the height of captured biotargets to be adjusted from the sensor surface, and can also contain active tail groups, such as amine, carboxyl, and succinimide esters to tether binder molecules [Fig. 13].

The latter surface functionalization approach provides affinity-based interactions at specific regions on binders and anchor molecules. However, clinical samples have a complex composition including proteins, lipids, and sugar units that can non-specifically adhere to a sensor surface. Non-specific binding can occur at active, passivated, and untreated areas on the sensor. Anti-fouling agents, including chemical modifiers, proteins, and polymeric substances, serve to prevent non-specific binding and increase the detection accuracy of target molecules. Furthermore, working with biospecimens requires sample preparation steps to avoid signal fluctuations and inaccuracies, considerably increasing the complexity of biosensing assays.

7. Current challenges and limitations for biosensors at the POC

In this section, we discuss a number of emerging technologies with respect to challenges associated with current biosensors at the POC. These criteria include label-free sensing, assay complexity, assay time, multi-target detection, read-out mechanisms, fabrication methods, and applicability for clinical testing. We compare PC-based biosensing platforms with up-to-date biosensing technologies: nanomechanical sensors, plasmonics tools, electrical sensing platforms, and magnetosensors (Table 2).

7.1 Label-free biosensing

Labeling of biotargets, often with fluorescence molecules, has been extensively utilized in biosensing applications to enhance signal readout for improving measurements. However, introducing a label potentially adds complexity, increases experimental errors, and presents additional inefficiencies and uncertainties, such as quenching effects and photobleaching. Additionally, labeling a biomolecule can significantly alter its characteristic properties (conformation, solubility, and affinity). Considering the challenges associated with labeling, label-free assays can reduce cost, complexity, and time for POC tests by eliminating the use of labels, dyes, and high-volume of reagents. Therefore, there is a demand for label-free, rapid, sensitive and accurate biosensing platforms at the POC, which will address the challenges associated with current label-based biosensor strategies. In this regard, PC structures represent a new class of biosensors that hold promise for label-free biosensing with potential applications at the POC.

7.2 Assay time

To be sustainable, emerging technologies need to provide rapid, inexpensive, and multiplexed solutions over existing assays and methods. Some platforms require filtration-type sample preparation steps to concentrate targets in the sample, which also increases assay complexity and time. From a POC perspective, biosensing platforms need to be fabricated with inexpensive materials and methods using simple and inexpensive production techniques. For instance, some of the biosensing platforms require clean room facilities and multiple chemical etches for their fabrication, which may significantly increase the total assay cost.

The read-out mechanism is another pivotal criterion to obtain reliable measurements at the POC. For instance, nanomechanical platforms, including quartz crystal microbalance and piezoelectric sensors, are affected by multiple external parameters such as temperature and vibration and require additional equipment (e.g., vibration insulation and temperature control systems) to minimize these external interferences to ensure reliable measurements. This additional equipment limits the portability and may also increase the cost, thus not satisfying some of the key requirements for a POC device.

7.3 Multiplexing capability

An ideal biosensing platform needs to detect multiple targets. This feature will provide a wide window to evaluate different
targets on a single platform, increasing its applicability for versatile POC testing. To immobilize various antibodies/binders onto a single sensor surface, PC-based biosensor platforms can benefit from antibody printing technologies (Table 2).193

7.4 Clinical validation

Biological specimens, such as blood, saliva, urine, and sweat, have distinct characteristics. These matrices have various ionic content, ionic strength, pH, and a diverse makeup comprised of cells, proteins, and lipids. Detecting biotargets in biological matrices constitutes one of the major challenges for biosensing. For instance, electrical-based sensing platforms measure electrical potential via different modalities, such as amperometry, potentiometry, and capacitance read-outs. Most of these platforms require replacing the biological matrix with non-ionic fluids, and therefore multi-step flow or centrifugation is required to minimize or eliminate interfering factors for read-out.115,216

Ultimately, biosensors need to undergo extensive clinical validation before they can be used at the POC.

8. Future outlook for PC-based biosensors at POC diagnostics

The global biosensor market is valued at approximately US$ 13 billion in 2013 and projected to grow substantially to US$ 22 billion by 2020.217 On-site (bedside) biosensors at the POC are poised to transform the healthcare industry as invaluable tools for the diagnosis and monitoring of diseases, infections, and pandemics worldwide. Advances in flexible, wearable, and implantable sensing technologies integrated with responsive materials can potentially connect patients to the clinic, thus providing continuous monitoring, such as glucose sensing for the patients with diabetes at the point-of-need.218,219 Due to their

Fig. 13 Surface chemistry approaches for PC-based biosensors. Initially, the PC surface (i.e., TiO₂) is treated with piranha solution and/or oxygen plasma to increase the hydrophilicity by exposing polar molecules on the surface. The surface is then immersed in a silane solution (such as 3-mercaptopropyltrimethoxysilane (3-MPTS)). The other end of bound silane is conjugated to a linker molecule (4-maleimidobutyric acid N-hydroxysuccinimide ester (GMBS)) containing a succinimide end-group. An anchor protein such as neutravidin can be immobilized using a GMBS linker molecule. A biotin-conjugated antibody can interact with neutravidin and then specifically bind to a desired biotarget molecule.
characteristics including flexibility (e.g., hydrogels) and integration capability with smart materials (e.g., CNTs and graphene), PC-based sensors will be an asset to the current wearable continuous monitoring tools and sensors.

A color shift that can be observed with the naked eye or with the help of a color legend is valuable at the POC. One interesting potential application for PC-based structures is to dynamically change the optical properties in response to environmental parameters, such as geometry, pH, and temperature. An example can be found in nature as suggested by a recent study on chameleon skin, which revealed the presence of guanine pillar-like nanocrystal PC structures.220 When relaxed, crystals were randomly distributed, but changed to a square or hexagonally-packed lattice geometry when excited, thereby changing the skin’s visible colors (Fig. 14). Inspired by this example, PC structures could also be fabricated as simple diagnostic tools to produce a color shift against an external stimulus with a subsequent change in geometry. This method may potentially eliminate the need for large and expensive optical devices for biosensors in the POC applications.

PC structures with more complicated geometries, such as 2-D PCs, are sensitive to changes in the refractive index in nano- and micro-scale volumes. Large wavelength shifts were experimentally observed after binding single sub-micron sized metallic and polymeric nanoparticles.122,221–224 Detection of virus particles using these structures are highly promising, since viruses strongly interact with light, and can be easily captured on top of or inside photonic crystals.34,194 However, biological detection of viral particles using 2-D PC structures has been difficult due to the low refractive index contrast between water and biological targets. Recent work with human papillomavirus-like particles spiked into serum has suggested that the detection of biologically relevant particles is possible, with a detection limit in the nanomolar range.92

Fig. 14 Spatial arrangements of PC structures in chameleon’s body. (a) The color change of two male chameleons. The left column indicates the relaxed state; the right column indicates the excited state. (b) TEM images of these two states. In the relaxed state guanine PC structures are closer to each other, while in the excited state they attain a square lattice structure, which results in a shift in the reflected color of the body.220
9. Conclusion

Detection of biomolecules at the POC faces multiple challenges, including the lack of centralized labs, limited technical capabilities, the absence of skilled staff, and poor health care management systems (particularly in resource-limited settings). PC-based biosensors represent a novel class of advanced optical biosensors that readily address these drawbacks. PC structures are used as biosensors for cells, bacteria, viruses, and numerous biomolecules, such as proteins, cancer biomarkers, allergens, DNAs, RNAs, glucose, and toxins. These structures can be manufactured with metals, oxides, plastics, polymers, and glass in mass quantities using NIL technology or wet chemical synthesis of colloidal and polymer structures. Recently, PC structures have been integrated with emerging technologies such as smartphones, flexible materials, and wearable sensors to enhance their utilization as potential diagnostic tools at the POC. However, clinical specimens may require sample preparation steps such as filtration, which may limit the use of PC-based biosensors at the POC. Additionally, complex biological fluids comprising cells and tissues may interfere with the transistor of biosensors and some of the delicate PC structures might experience challenges with the sensing mechanism including read-out systems. In addition, PC structures have been translated to a few products in biosensing, chemical and humidity sensing. PC-based biosensors represent a new class of advanced technology products that can be good candidates for a wide array of applications at the POC.

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