

Review

Microfluidic-integrated biosensors: Prospects for point-of-care diagnostics

Suveen Kumar^{1,*}, Saurabh Kumar^{1,*}, Md. Azahar Ali^{2,3,*}, Pinki Anand¹, Ved Varun Agrawal², Renu John³, Sagar Maji¹ and Bansi D. Malhotra^{1,4}

¹ Department of Biotechnology, Delhi Technological University, Shahbad Daulatpur, Delhi, India

² Department of Science and Technology Centre for Biomolecular Electronics, Biomedical Instrumentation Section, Materials Physics and Engineering Division, CSIR-National Physical Laboratory, New Delhi, India

³ Indian Institute Technology Hyderabad Ordnance Factory Estate, Yeddumailaram, Hyderabad, Andhra Pradesh, India

⁴ Centre for NanoBioengineering & SpinTronics, Chungnam National University, Yuseon-Gu, Deajeon, South Korea

There is a growing demand to integrate biosensors with microfluidics to provide miniaturized platforms with many favorable properties, such as reduced sample volume, decreased processing time, low cost analysis and low reagent consumption. These microfluidics-integrated biosensors would also have numerous advantages such as laminar flow, minimal handling of hazardous materials, multiple sample detection in parallel, portability and versatility in design. Microfluidics involves the science and technology of manipulation of fluids at the micro- to nano-liter level. It is predicted that combining biosensors with microfluidic chips will yield enhanced analytical capability, and widen the possibilities for applications in clinical diagnostics. The recent developments in microfluidics have helped researchers working in industries and educational institutes to adopt some of these platforms for point-of-care (POC) diagnostics. This review focuses on the latest advancements in the fields of microfluidic biosensing technologies, and on the challenges and possible solutions for translation of this technology for POC diagnostic applications. We also discuss the fabrication techniques required for developing microfluidic-integrated biosensors, recently reported biomarkers, and the prospects of POC diagnostics in the medical industry.

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Correspondence: Prof. Bansi D Malhotra, Department of Biotechnology, Delhi Technological University, Shahbad Daulatpur, Main Bawana Road, Delhi 110042, India

E-mail: bansi.malhotra@gmail.com

Additional correspondence: Dr. Pinki Anand, Department of Biotechnology, Delhi Technological University, Shahbad Daulatpur, Main Bawana Road, Delhi 110042, India

E-mail: pinkianand@gmail.com

Additional correspondence: Dr. Renu John, Indian Institute Technology Hyderabad Ordnance Factory Estate, Yeddumailaram, Hyderabad, Andhra Pradesh 502205, India

E-mail: renujohn@iith.ac.in

*These authors contributed equally to this work.

Abbreviations: DIC, differential interference contrast; EBL, electron beam lithography; HDL, high density lipoprotein; IDL, intermediate density lipoprotein; LDL, low density lipoprotein; LOC, lab-on-a-chip; MEMS, micro-electromechanical system; NADH, nicotinamide adenine dinucleotide; PDMS, polydimethylsiloxane; PMMA, polymethyl methacrylate; POC, point of care; SPR, surface plasmon resonance; VLDL, very low density lipoprotein

1 Introduction

The integration of biosensors with microfluidics has aroused much interest since there is a considerable demand for improved health care in both advanced and advancing societies. In this context, point-of-care (POC) devices can play a major role in rapid diagnosis, prevention and treatment of human diseases. However, outbreaks of new or re-emerging infectious diseases along with changing lifestyles have led to an urgent demand for a faster development of POC devices that can be used for effective therapeutic intervention [1]. In addition, even simple routine biochemical tests require well-trained technicians and an expensive laboratory infrastructure with a continuous supply of chemicals, all of which lead to increased costs. Diagnostic challenges have necessi-

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tated the development of new technologies that can take testing equipment nearer to the patients; these technologies are termed POC diagnostics [2].

POC diagnostics are analytical testing platforms that can be used both in a clinical laboratory and in the vicinity of a patient using portable equipment. Therefore, the entire clinical diagnosis and treatment community is witnessing a paradigm shift from conventional diagnostic devices to the miniaturized and POC devices. These devices can fulfill the increasing demand of the medical sector for fully automated instruments that can directly use unprocessed specimens and that require minimal electronic or mechanical maintenance. [3]. POC diagnostics have the potential to provide rapid and accurate results, at reasonable costs, for the biochemical tests required by the increasing number of patients in emergency rooms [4].

An ideal diagnostic device should provide a suitable environment for interaction of the analyte to be tested in a given sample with the bio-recognition molecule (e.g. protein, lipid, nucleic acid, whole cell), which is immobilized onto the sensor surface. Until recently, the development of such devices was considered unachievable due to the extensive variability in biological samples, resulting in unreliable clinical data. However, efforts are now being made to increase both the resolution and the accuracy of the analyte detection techniques in the integrated unit for sample pre-processing [5]. In this context, microfluidics devices have the potential to play a very influential role in diagnostics, and advances in microfluidics, arrays, sensors, and nanomaterials have paved the way for the development of “lab-on-a-chip” instruments [2, 6]. Microfluidics is an extremely attractive technology because of its ability to manipulate small volumes of fluid (micro- to nanoliters) within given spatial dimensions, and the ease of multiplexing and integrating this system into low-cost, compact and portable devices for on-chip testing [7].

Extensive research carried out over the past decade into the development of microfluidics technology reveals numerous opportunities in the realm of enzymatic, DNA and proteomic analysis involving immunoassays, microarrays, etc. [8]. In addition, a vast potential in the field of cell culture applications has recently evolved through the exploitation of the capacity of this technology to mimic *in vivo*-like conditions. This extensive area of research and development could lead to successful commercial POC diagnostics devices. In 2009, the global *in vitro*-diagnostics market was approximately \$ 37 billion, and it is estimated to reach \$ 49.8 billion by 2016 [9].

We present an overview relating to recent developments in microfluidic-based biosensing devices reported over the past five years. New biomarkers discovered recently for application in the medical industry are also discussed. We also look at the different types of microfluidics-based biosensors and the fabrication techniques used in the development of microfluidics devices for POC diagnostics.

2 Biomarkers

POC diagnostics are critically dependent on the combination of the analytical tools and the rapidly emerging knowledge on molecular biomarkers. Apart from being the cornerstone of preventive and personalized medicine of the future, new findings on biomarkers are leading to revolutions in drug discovery. They can be used to identify both physiological and biological entities associated with diseases, and have evolved from simple single physiological parameters to novel markers that span fields from genomics to metabolomics. Many of these biomarkers, identified after rigorous scientific studies, are now the basis for diagnosis and prognosis of disorders such as cancer, diabetes, AIDS, tuberculosis, Alzheimer's disease and other communicable diseases. However, the quest to discover new useful biomarkers in peripheral fluids, including blood and urine, still continues.

More than 160 types of biomarkers identified to date may be used to indicate the stage of a cancer. Although there are many techniques available for cancer diagnosis, expression of specific biomarkers, and their accurate estimation, can be helpful in the diagnosis, staging and effective treatment of a cancer at an early stage. Park et al. [10] reported that the concentration of miR-125a and miR-200a in saliva can be associated with the stage of the oral cancer. Wong et al. [11] have found that miRNA-184 increases in the tongue squamous cell carcinoma (SCC) cell lines and might represent a biomarker for SCC. Under normal conditions, shelf life and stability of mRNA in the salivary transcriptome has been found to be approximately 0.4–12.2 min. Analysis of mRNA requires a precisely controlled environment that would allow for its increased stability and shelf-life. Li et al. [12] reported seven different salivary RNA biomarkers (IL-8, IL-1B, DUSP1, HA3, OAZ1, S100P and SAT) that may be used for diagnosis of oral cancer. Pancreatic cancer is one of the most common causes of cancer-related deaths. The main reason is the lack of a pertinent diagnosis technique. Zhang et al. [13] discovered that changes in the concentration levels of 12 different mRNAs were associated with stages of pancreatic cancer. Sozzi et al. [14] reported that free circulating plasma DNA can be used as a biomarker for the diagnosis of lung cancer even at early stages. Recently, mechanical biomarkers have also been exploited in microfluidics devices. These markers have several advantages, such as being label-free, low cost, convenient maintenance, and reduced assay time. Bow et al. [15] used deformability of RBCs as a mechanical biomarker to develop a deformability-based, microfluidic flow cytometer for malaria detection. A crucial factor in biomarker discovery is access to patients who are willing to donate samples. In addition, a lack of standardization for the technologies and methodologies is currently a limiting factor for validation of biomarkers for clinical application.

3 Fabrication of microfluidics devices

Microfluidics devices can be integrated with scaled-down laboratory functions and processes to a miniaturized chip format known as “lab-on-a-chip”, and can provide many advantages over conventional techniques [16]. Several aspects need to be considered while designing a microfluidics device, such as selection of materials, dimensions of the microfluidics devices and fluidic control devices (such as pumps, valves, and mixers). Table 1 shows several fabrication techniques used to construct microfluidics devices for POC diagnostic applications, together with their potential merits.

Some of the important issues in designing microfluidics devices relate to fluid injection, mixing, separation, detection, size and geometry. UV-photolithography, X-ray photolithography, electron beam lithography, nano-imprinting, can be adopted for micro/nanofluidic fabrication. The photolithography technique essentially replicates a topographically defined structure on a master to a soft elastomer. A geometrical pattern is transferred to a photosensitive polymer (a photoresist) by exposure to a light source through an optical mask. In soft lithography, various fabrication techniques such as an elastomeric phase-shift mask, molds and micro-contact printing, as well as conformal photolithography are used. Soft lithog-

Table 1. Various fabrication techniques for development of microfluidics devices and their potential uses in POC diagnostics

Technique used for micro-fabrication	Materials used	Resolution of device	Analytes	Advantages	Limitations	Ref.
Photolithography	PDMS RTV 615, photoresist SU-8	200 μm	Anti-biotin Ab	– Small instrument size – Low reagent consumption and system automation – Excellent sensitivity and efficiency	– Low throughput	[45]
Photolithography	HMDS, photoresist AZ6612, PDMS	20 μm deep at reaction chamber and 180 μm deep at other zone	C-reactive protein (CRP)	– Autonomous – Requires only the addition of a drop of sample	– Air entrapment in horizontal channel delays the process – Lower concentration detection takes more time	[18]
Photolithography or wax printing	Paper, SU-8	100 μm	Glucose and heavy metal	– Low cost – Simple, portable, fast	–	[64]
Contact photolithography	SU-8, PDMS etc.	18 $\times$ 50 μm^2	Hepatitis C viral genome	– Label-free – Low-cost – Can detect multiple DNA oligonucleotide sequences	– Optical interference	[55]
Soft lithography Technique	PDMS, Teflon TM , SU8 photoresist	100 μm deep and 700 μm wide	<i>Escherichia coli</i>	– Rapid – Disposable – Low cost	– Concentrated sample required	[65]
Soft lithography	PDMS, SU-8	600 μm	Ochratoxin A	– High throughput – Highly sensitive – Portable	–	[66]
Soft lithography	PDMS, SU-8	200 μm	Cholesterol	– Highly efficient – Sensitive – Fast and rapid detection	– Single analyte detection	[40]
Lift-off	AZ P4620 photoresist, paraffin pellets	110 $\times$ 600 μm^2	Mycobacteria	– High specificity of culturing – Low cost – Rapid and easy-to-use fashion	– Low signal to noise	[67]
Nano-imprinting	PMMA	100 nm	–	– High resolution – Precise control	– Expensive – Low throughput	[68]
Electron beam lithography	SU-8 3010,	500 nm $\times$ 5 μm	–	– Good resolution – Can be precisely aligned	– Costly – Requires more time to fabricate	[25]

raphy offers high-throughput production and cost effectiveness [17]. Gervais et al. [18] fabricated a microfluidics device using photolithography and photo plotted a polymer mask for C-reactive protein monitoring via antigen-antibody interaction. In this, a patterned polydimethylsiloxane (PDMS) slab is sealed with the reaction chamber, collector, valves, deposition zone, etc. Sia et al. [17] fabricated microfluidic channels and other microstructures for enzymatic analysis, immunoassays, cell sorting, manipulation of cell, and mass spectroscopy using soft lithography.

Some of the materials used for fabrication of microfluidics devices for POC diagnostics include paper (nitrocellulose or cellulose), glass, silicon, plastics (cyclic olefin copolymers or polystyrene), and polycarbonate. Nie et al. [19] developed POC devices to detect glucose, cholesterol, lactate, and alcohol in blood or urine samples using chromatographic paper (Whatman). This technique allows reproducible conformal contact between the desired electrodes and the paper channels. It can be scaled to produce a large number of strips and requires low quantity of analytes. Silicon and glass are also being used for fabrication of lab-on-a-chip due to their surface stability, solvent compatibility, ease of fabrication, and good optical properties that can facilitate integration of on-chip electronics with the microfluidics network. However, silicon and glass are expensive and their etching is time consuming. Hence, polymers with improved optical, thermal and mechanical properties are required for the design and the fabrication of novel microfluidics devices.

The current efforts are focused on fabricating microfluidics devices using PDMS, polymethyl methacrylate, polycarbonate, etc., due to their excellent optical qualities, transparency (230–1100 nm) and infrared spectra response [20]. PDMS is presently the most favored candidate for microfluidics fabrication due to its ease of use, flexibility, non-toxicity, and low cost [21]. However, PDMS has many drawbacks for applications in microfluidics because its surface in an unprocessed state is hydrophobic, containing CH_3 groups. As a result, solid PDMS needs external pressure to maintain the fluid flow condition. To overcome this limitation, surface modification of PDMS using air/oxygen/argon plasma, UV/ozone or corona discharge, etc. are used to produce hydrophilic silanol groups (SiOH) on its surface. However, the hydrophilic PDMS produced only lasts for 20–30 min and is known to swell in organic solvents. In addition, PDMS adsorbs small molecules and may get deformed under pressure. Hence, it cannot be used for high-pressure operation. The use of wet chemical modification of the PDMS surface using piranha solution of H_2O_2 and H_2SO_4 is supposedly advantageous. Wu et al. [22] have developed a facile approach to modify PDMS using a vinyl-terminated initiator mixed with Sylgard 184 (PDMS oligomer and curing agent). However, the PDMS surface becomes unstable over a long

period. Bodas et al. [23] attempted to modify the hydrophobic surface of Sylgard 184 PDMS by introducing oxygen plus C_2F_6 and 2-hydroxyethyl methacrylate. However, these C_2F_6 - and 2-hydroxyethyl methacrylate-treated surfaces were found to be hydrophilic and only stable up to 2 and 10 days, respectively. Thus, urgent efforts towards tailoring a PDMS with improved characteristics for application in commercialized POC diagnostics are necessary.

The required patterned metallic microelectrodes can be fabricated using the lift-off photolithography technique [24]. In this method, popular photoresists, commonly known as Shipley 1827, 181, etc., are used as a sacrificial layer. For metal deposition, there must be a discontinuity in the metal layer that allows the solvent (acetone) to dissolve the photoresist underneath the gold layer during the lift-off step. After dissolving the photoresist, slight agitation selectively removes the metal, which now has the desired metal electrode pattern. Some metals, e.g. platinum and gold, can be etched using wet chemical etching via standard photolithography. These electrodes are used for electrokinetic manipulation and biomolecules immobilization via surface modification (using a cross-linker), and can be assembled with microchannels and a micropump. The size of these microelectrodes may positively affect mass transport of the ions to and from the electrode surface in the bulk solution as a result of spherical or radial diffusion, leading to improved mass transfer compared to that of a macroelectrode. However, UV-photolithography provides a resolution that is limited to approximately 1 μm . To achieve a higher resolution, nanoimprinting (<100 nm) and electron beam lithography (EBL) have been recently used. High-resolution (>10 nm) nanoscale structures can possibly be fabricated using both EBL and ion beam milling techniques. These methods, on the other hand, have a low throughput and are expensive. Koller et al. [25] reported the direct fabrication of precisely aligned 3D nanofluidic channels using the EBL technique, although it is known to be time consuming. Besides this, the handling/control of the fluids continues to be a challenge due to the high-aspect ratio of the nanochannels.

4 Microfluidics-based biosensors for POC diagnostics

Biosensors are widely used in POC diagnostics and their integration with microfluidics has led to increased diagnostic applications. The focus now is on the development of several types of microfluidics-based biosensors with improved diagnostics efficiency, e.g. enzymatic biosensors, immunosensors, optofluidics, microarrays and non-invasive biosensors (Fig. 1). In the next sections, we summarize the research and development of some of these biosensors reported over the past 5 years.



Figure 1. Application of microfluidics in POC diagnostics.

4.1 Enzymatic biosensors

Enzymatic biosensor provides a powerful platform for the POC diagnostics. In an enzymatic biosensor, enzymes are immobilized onto a suitable transducer that produces a specific signal on reaction with a given analyte. The main purpose is to convert an enzymatic reaction into a suitable analytical signal (electrochemical, colorimetric, optical property, etc.). The thrust is towards developing biosensors for rapid and accurate detection of the clinically important parameters such as blood sugar, urea and cholesterol that have been found to indicate the onset of a disease.

4.1.1 Glucose biosensor

Ever since the first glucose biosensor was proposed in 1962 [26], several devices for electrochemical biosensing of glucose have been developed. Most of these devices are based on enzyme-functionalized screen-printed disposable electrode strips that work on the principle of amperometric detection. Development of hand-held POC testing devices can be helpful in self-monitoring, as well as getting quick, reliable and accurate results using small volume of samples. Quantitative analysis of glucose via a biosensor primarily follows two mechanisms (shown in Figs. 2A, B) [27]. Many approaches have been followed to obtain improved characteristics of glucose-sensing devices by coupling them with microfluidics.

Hou et al. [28] reported a glucose microfluidic chip based on polymethyl methacrylate using a CO₂ laser and a hot plate press bonding fabrication technique. Compared to conventional detection methods, this chip has the added advantage of self-rotation, resulting in an improved mixing in the micromixer, and it can be used for rapid and low-cost detection of glucose. Recent developments in nanotechnology and engineering have enabled immobilization of enzymes on to nanoparticles and nanostructures with enzymes that in turn can be functionalized onto the given electrodes. Yu et al. [29] fabricated a microfluidic chip on which enzymes were immobilized onto the single-walled carbon nanotubes of the PDMS channel. This photolithographic fabricated microfluidics device shows high linear response up to 5×10^{-3} mol/L.

Whatman filter paper has also been used for the fabrication of a paper-based microfluidic glucose sensor using the photolithography technique. However, the poor shelf life and linearity need to be improved before commercialization of the paper-based glucose-sensing devices can take place. The different matrices used for the development of microfluidics-based glucose biosensor are summarized in Table 2.

4.1.2 Urea sensor

Accurate determination of urea level in a biological sample is considered very important as variation in blood urea

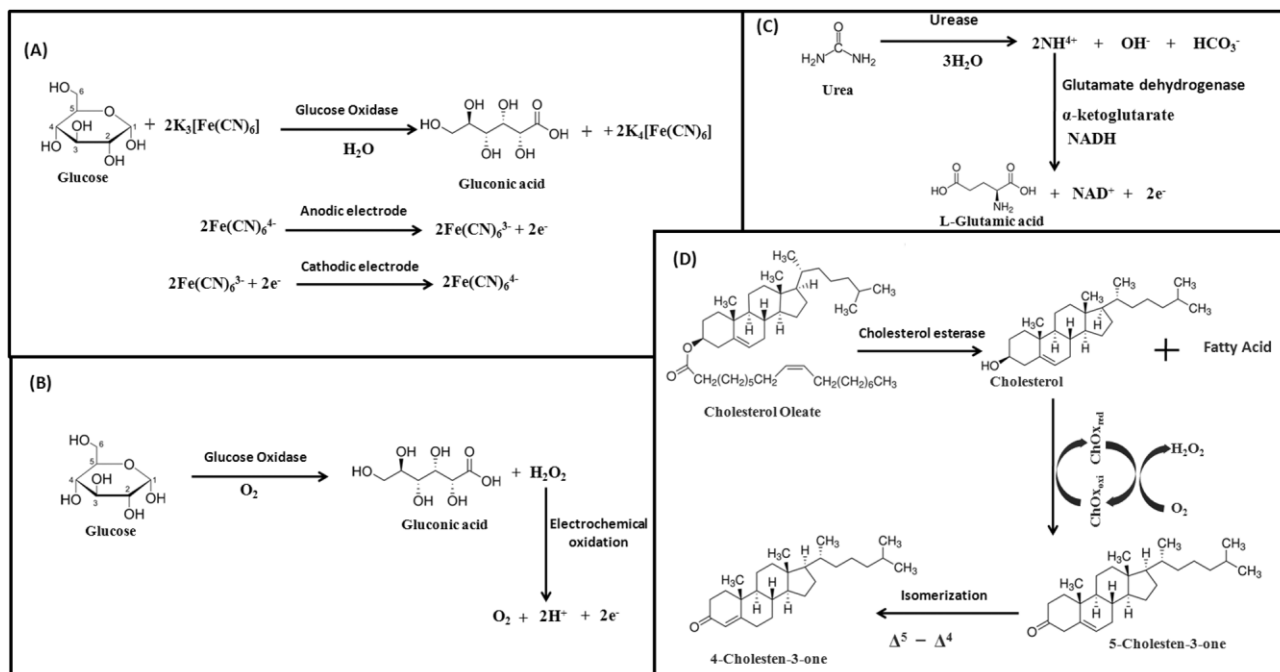


Figure 2. Schematic representation of biochemical reactions used in enzymatic biosensors. **(A)** Catalytic conversion of glucose into gluconic acid in the presence of potassium ferricyanide. At the surface of electrode, electrons are generated by conversion of Fe (II) to Fe (III). **(B)** Catalytic conversion of glucose into gluconic acid and H₂O₂, after which electrons are generated via electrochemical oxidation. **(C)** Biochemical reaction of urea biosensor. **(D)** Biochemical reaction of cholesterol biosensor.

concentration (8–20 mg/dL) is an indicator for several diseases, e.g. renal failure, hepatic failure, nephritic syndrome, cachexia, urinary tract obstruction, and gastrointestinal bleeding [30, 31]. Generally, the enzymes urease and glutamate dehydrogenase are used for the determination of the level of urea in biological samples. Urease converts urea to hydrogen bicarbonate and ammonia, while glutamate dehydrogenase converts the ammonium ions, α -ketoglutarate and nicotinamide adenine dinucleotide (NADH) to L-glutamate and NAD⁺, as shown in Fig. 2C. The current generated through the electrons

evolved after conversion of NADH to NAD⁺ is measured to estimate the urea concentration [32].

Microfluidics-based urea biosensors are in great demand in diagnosis because of their high sensitivity, low consumption of materials/reagents, fast response/reaction time and portability, with high accuracy and reusability. Nanomaterials can be used for the immobilization of urease. Srivastava et al. [31] immobilized enzymes covalently onto the gold electrode through a self-assembled monolayer of 10-carboxy-1-decanthiol. However, this urea sensor suffers from low sensitivity and low detection lim-

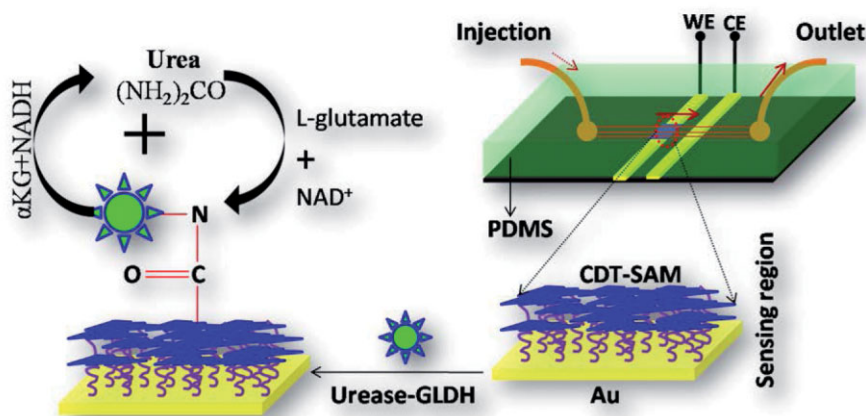


Figure 3. Microfluidics system based on a self-assembled monolayer (SAM) Au electrode for urea detection. CDT, 10-carboxy-1-decanthiol; GLDH, glutamate dehydrogenase; WE, working electrode; CE, counter electrode (Au).

Table 2. Various enzyme biosensors used for POC diagnostics

Analysts	Matrix	Techniques used	Linearity	Sensitivity	Response time	Detection Limit	Shelf life	Limitation	Advantages	Ref.
Glucose	Nanoporous platinum electrode in PDMS channel	Chemical process	1–10 mM	$1.65 \mu\text{A cm}^{-2} \text{ mM}^{-1}$	15 s	0.097 mM	---	Requires automatic calibration and pre-treatment unit for real sample analysis	Non-enzymatic amperometric detection Programmed electro-osmotic flow Fast response time	[70]
Glucose (in artificial urine)	Chromatography paper 1	Photolithography	0–5 mM	---	20 min	0.5 mM	30 days	High cost of fabrication Light condition affects calibration	Multianalyte detection Equipped with scanner and camera as a detector	[71]
Glucose (urine)	Chromatography paper, Carbon ink and Ag/AgCl ink	Photolithography and wax printing	0–22.2 mM	$0.43 \mu\text{A mM}^{-1} \text{ mm}^{-2}$	2 s	0.22 mM	---	Portable electro-chemical reader is required for field work	Simple, low cost, portable, fast response and disposable device	[65]
Urea	10-carboxy-1-decanthiol	Self-assembled monolayer	10–100 mg/dL	$7.5 \mu\text{A mM}^{-1} \text{ cm}^{-2}$	10 s	9 mg/dL	10 weeks	Poor detection limit Hydrophobic nature of PDMS	High shelf life Good linearity High sensitivity	[31]
Urea	Polydimethylsiloxane	Lithography, thin film deposition, lift-off process	3.16×10^{-4} – 3.16×10^{-2} M	$2.71 \pm 0.11 \text{ mV/log[M]}$	40 min	3.16×10^{-4} M	---	Poor detection limit Poor detection time	Good linearity High sensitivity	[72]
Urea	Titania–zirconia ($\text{TiO}_2\text{--ZrO}_2$) nanocomposite	Wet chemical etching process	5–100 mg/dL	$2.74 \text{ mA } [\log \text{ mM}]^{-1} \text{ cm}^{-2}$	---	0.07 mg/dL	4 weeks	Short shelf life	Good linearity High sensitivity with low detection limit Flow rate of $2 \mu\text{L min}^{-1}$	[33]
Free cholesterol	Carbon nanotubes	Chemical vapor deposition, photolithography	50–400 mg/dL	0.0512 nA/mg/dL	---	10 mg/dL	---	Poor sensitivity	Rapid detection High throughput Good stability and selectivity	[39]
Total cholesterol	Nickel oxide nanorods	Co-precipitation, photolithography	50–400 mg/dL	$0.12 \text{ mA/mMcm}^{-2}$	10 s	0.65 mM	2 months	Hydrophobic nature of PDMS	Higher sensitivity Fast response time Low reagent and power consumption	[41]
Total cholesterol	Aligned Au nanowires	Electroplating, photolithography	0.01–0.060 mM	0.85 mA/mM	---	0.1 mM	10 days	Poor detection range	Highly sensitive and selective	[73]

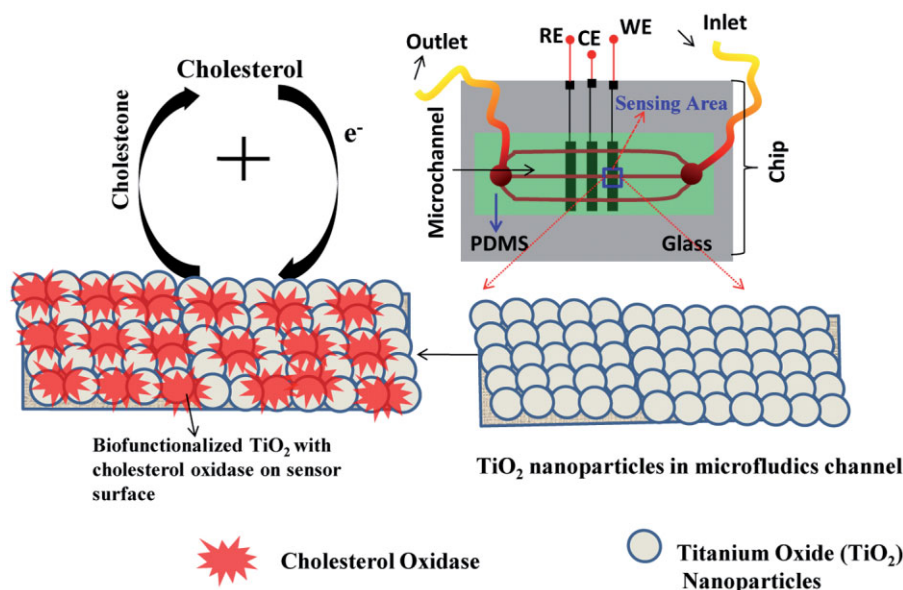


Figure 4. A microfluidics system-based anatase titanium dioxide nanoparticles immobilized with cholesterol oxidase for cholesterol detection. Pattern on indium tin oxide (ITO) has been fabricated using wet chemical etching technique. CE is the counter electrode and the reference electrode (RE) is Ag/AgCl.

it (Fig. 3). Sensitivity and detection range were improved when the enzymes were immobilized onto a titania-zirconia nanocomposite [33]. Table 2 summarizes the various matrices and fabrication technologies used with their limitations and advantages for the development of a urea biosensor. There is an urgent need to solve problem relating to the stability of urease and cost, etc.

4.1.3 Cholesterol/lipid biosensor

Cholesterol is an essential structural component of mammalian cell membranes, hormones, vitamin D, and cell signaling, and is also required for establishing proper membrane permeability and fluidity [34]. Cholesterol is carried in plasma by a series of protein-containing micelles known as lipoproteins, including the very low density lipoprotein (VLDL), low density lipoprotein (LDL), intermediate density lipoprotein (IDL) and high density lipoprotein (HDL) [35]. Cholesterol-rich LDL is considered as 'bad' because it helps to accumulate cholesterol in arterial walls, resulting in various heart and vascular diseases. As a result, it is a prerequisite to estimate cholesterol level to assess the general condition of a patient's health. The lipid content cannot be measured directly as LDL level, but can be estimated using the Friedewald equation [36]. Nuclear magnetic resonance spectroscopy and ultracentrifugation are the most commonly techniques used to quantify LDLs [37].

Fig. 2D shows a schematic relating to the details of cholesterol detection. Esterified cholesterol is converted to cholesterol and fatty acid in presence of cholesterol esterase. Cholesterol then produces Δ^4 -3-ketosteroid and H_2O_2 via isomerization of 3β -hydroxysteroids in presence of cholesterol oxidase. Ali et al. [38] have fabricated microfluidic biosensor for free cholesterol estimation

using nanostructured anatase titanium dioxide (Fig. 4). Wisitsoraat et al. [39] described a flow injection microfluidics device that uses functionalized carbon nanotubes for real-time cholesterol monitoring. Ruecha et al. [40] have developed a PDMS-based microsystem for rapid detection of cholesterol using an electrochemical technique. Ali et al. [41] developed a highly efficient microfluidic nano biochip based on nanostructured nickel oxide for total cholesterol monitoring. Although the use of macro/micro electrodes offers a high sensitivity as well as precise detection of cholesterol, it is a challenge to stabilize cholesterol oxidase and cholesterol esterase for a desired period onto an electrochemical transducer platform. Moreover, problems relating to fluid control in these microfluidic cholesterol chips equipped with syringe pump requires an urgent solution. Besides this, efforts should be made towards the technological development of microfluidic-integrated biosensor for LDL detection.

4.2 Immunosensor

Immunosensors are based on the immunochemical reaction comprising antigen or antibody as the recognition element, which is spatially in contact with a transducer surface [42]. Immunosensor-based devices can be used to detect immunocomplex formation by measuring changes in resistance, current, refractive index, capacitance, etc. A conventional immunoassay uses monoclonal antibodies that comprise of 'Y'-shaped structures containing two antigen-binding sites. The antigen binding to an antibody involves a highly specific interaction, making the immunoassay a highly reproducible and highly specific reaction format, suitable for a range of target analytes for biosensing applications. [43]. However, biological compo-

nents such as antibodies are expensive, and the conventional immunoassays are known to be time consuming. In this context, microfluidic-integrated immunosensors may perhaps yield: (i) sensitive detection, (ii) multianalytes analysis, (iii) reduced biological component cost by using very minute volumes, (iv) increased functionality for surface treatment, (v) decreased diffusion distance and fast detection time, (vi) integration of mixer, valve, etc., to improve the assay quality, (vii) automation with peripheral devices such as pump (control the flow of analyte), power supply, etc., and (viii) portability, thus making it viable for the POC diagnostics. The immunosensor-based POC devices can be fabricated for detection of food pathogens, bacteria and viruses due their high specificity and sensitivity, and both label and label-free rapid detection [44]. Many researchers have investigated label-free detection of different biological molecules, including aflatoxins, LDLs, etc. using integrated surface plasmon resonance (SPR) microfluidics devices [35]. Feltis et al. [45] developed a low-cost SPR-based immunosensor for toxin detection. Luo et al. [46] fabricated a multilayer PDMS array consisting of multiple gold spots for real-time observation of immunocomplex formation using SPR. However, label-free detection suffers from nonspecific binding on the sensor surface. Reliability and stability are other challenges, which could be addressed using a reference electrode. On the other hand, a labeled immunosensor measures a molecule attached to a receptor by optical, fluorescence, or amperometric detection. Fluorescence microscopy can be used to detect fluorophores at a surface density even below 1 fluorophore/ μm^2 with a corresponding concentration of analyte in solution in the picomolar range. The electrochemical detection of a given label via immunosensing has several advantages, such as high sensitivity and low cost. However, some of the disadvantages include separation of free from bound label, washing and separation steps, which increases the complexity of the immunoassay [47]. Despite these interesting developments, the microfluidic immunosensors are not yet commercially available. One of the major problems relates to the integration and optimization of peripheral and supporting accessories on a single platform.

4.3 Optofluidics

Advances in microfluidics technologies have provided an exciting opportunity to integrate photonics and microfluidics at the micrometer scale, resulting in a new area of research termed optofluidics. Non-contact label-free measurements and microscopic optical imaging based on microfluidics have been found to be a most effective method for fast, real-time and high-sensitivity detection of biomolecules [48]. Optofluidic elements that can be used to manipulate light inside a microchannel have been recently demonstrated [49]. The principles of optical microscopic imaging coupled with fluorescence tags and

SPR-based detection have been explored and found to be effective biosensing platforms for the detection and quantification of given biomolecules [50]. Microfluidics systems have been adapted for imaging intracellular events such as cell division and migration. Discovery of a wide variety of nanoparticulate imaging contrast agents has significantly contributed to the development of nanoparticle-based biosensors both in vitro and in vivo [51, 52]. Measor et al. [53] have used a liquid-core anti-resonant reflecting optical waveguides for on-chip detection of oligonucleotides with labeled fluorescence-tagged molecules (Cy3 and Cy5), using a specific optofluidic filter design at a concentration of 600 nM (detection limit ranging from 10 nM to 10 μM). Thus, the use of a microscope in combination with optofluidic channels may create new possibilities for POC applications.

4.4 Microarray

Microarrays are a powerful tool for parallel search and detection of biomolecules such as nucleic acids and proteins. Microarrays are microscopic slides that contain an ordered series of immobilized biomolecules that can be probed by a fluorescence-labeled target. Binding between probe and labeled target via non-covalent interactions can be detected by measuring the increase in fluorescence intensity [54]. The microarray thus provides an opportunity for a real-time detection of thousands of molecules simultaneously, and therefore has found many applications in proteomics, diagnostics and drug discovery [55]. The potential capabilities of microarray for POC diagnostic applications can be expanded by integrating this technology with microfluidics with its many advantages.

The combination of microarrays with microfluidics is likely to lead to improved technology, and add to a new dimension in diagnostics because sample pre-treatment, preparation, amplification, and purification, i.e. automated sample processing, are possible on a single platform. Sochol et al. [56] developed a microfluidics platform that utilizes an array of microbeads to increase the parallelization of a reaction. This is then conjugated with molecular beacons in the microfluidic channel to differentiate the mismatches by measuring the fluorescence after hybridization. To increase specific binding, Rupp et al. [57] developed a polymer-based low-cost microarray containing a micropump and a disposable hybridization chamber. The working principle of the micropump or microvalve is based on pneumatic control pressure present in the upper channel of micropump and hydraulic pressure present in a lower channel. This integrated system has advantages of accelerated reaction kinetics achieved by convective flow. Jeon et al. [58] reported an automated platform that does not require any additional flow control equipment. They used SPR to detect protein-protein interactions using single microfluidics devices

fabricated by a replica molding method that works on capillary forces. These authors obtained improved reproducibility, sensitivity and the ability to detect a very small signal. Roy et al. [59] fabricated a microarray platform for quantitative detection of microRNA in a microfluidic setting, using a gold nanoparticle-tagged signaling probe. Convection flow by periodically changing the direction in the microfluidics system minimizes the hybridization time, and hence direct visualization is achieved using differential interference contrast (DIC) microscopy, which creates a virtual 3D image of individual nanoparticles, conjugated to the signaling probes.

Many fabrication techniques (Table 1) can be used for developing bio-microarray-based detection systems in a microfluidic channel. Tan et al. [60] have fabricated a microfluidic channel based on the principle of hydrodynamic forces and an optical micro-bubble approach using lithography technique. This integrated device has the advantages of easier transport, immobilization, mixing, observation, and recovery of desired molecules on a single platform. The use of optical techniques simplifies the complicated circuit design, fabrication, packaging and control. They can be utilized in the cell-based diagnostics, drug testing, proteomics, etc. The principal challenge in the microfluidic microarray technology concerns the integration of the peripheral system into a single platform to eliminate manual handling of the desired fluids.

4.5 Non-invasive biosensors

Non-invasive biosensors have gained a lot of interest in the development of miniaturized biosensors due to their painless, continuous on-line patient monitoring, along with the analysis report ready to be used for immediate clinical assistance [61]. However, research and development in this area present a mixed bag of opportunities. Pain, irritation, chances of infection or even bruising caused by finger pricking continue to be the major concerns for the non-invasive approach. Although it has many advantages over invasive biosensors, reliability and calibration are major hurdles for its application. Non-invasive biosensors for glucose monitoring based on impedance spectroscopy have been reported [62]. This sensor measures glucose concentration by frequency variation of impedance. Impedance is measured using the skin-electrode contact of a resonant circuit. Kraitl et al. [63] developed a non-invasive sensor to detect blood components such as hemoglobin, oxygen saturation and pulse. This sensor is based on the measurement of the absorption coefficient of blood, which differs with wavelength. It is in the form of wearable finger clips, and measurement is done by the monochromatic light from a laser diode and a light emitting diode on finger. Szekely et al. [64] developed a module to control the flow of liquid in microfluidics systems without any disturbance. The system uses an algorithm that compares the actual flow with the required flow

rate, and suitably adjusts flow by non-invasive control of the electroosmotic flow. In the non-invasive method, various samples such as skin, saliva, sweat, and urine are used to measure desired biomolecules; however, here calibration is a serious drawback. To the best of our knowledge, non-invasive sensors for the detection of cholesterol and urea have not yet been reported.

5 Conclusions

The most attractive feature of microfluidics is the ease and accuracy with which it can deliver information on the clinically relevant parameters that a POC situation would demand. Recent developments in polymer materials and microfabrication technology have made it possible to integrate microfluidics with devices including microvalves, pumps, micromixers, micro reactors, incubators, separation columns, and various peripheral components. With the recent advances in biomarker discovery, immunosensors and microelectronic devices, there is a considerable potential for microfluidics in the development of real-world tools for POC diagnostics. Individualized diagnostic tools based on microfluidics and microarrays may emerge as personalized screening tools for diseases, genetic screening and critical care. Parallel and fast screening for simultaneous detection of several bio-analytes is one unique potential that has been demonstrated by integrated microfluidic microarray chip technology.

Many research groups have come up with novel strategies for fabrication and characterization of microfluidics devices. However, results from the academic world need to be analyzed in light of commercialization. Efforts should be made to speed up this transition and to bridge the gap between industry and academia. Appropriate and widely accepted standardization techniques should be one of the high priority areas for commercialization of microfluidics systems. Microfluidics technology is a growing field with unlimited promises and prospects, with a growth curve slowly acquiring the critical potential to launch itself beyond the research laboratory into real-world applications in healthcare and POC diagnostics.

Moving society's dependence away from the conventional biochemical assays to microfluidics-based clinical diagnostic devices may be regarded as a quintessential progression in the POC and effective disease management. The desired growth of POC diagnostics necessitates research and development in designing novel devices along with clinical evaluation and subsequent commercialization. Furthermore, immediate steps are required to meet the challenges presented by exhaustive collection and analysis of the vast amount of data obtained from a single patient. The first-ever biosensor proposed in 1962 by Clark [26] was just a starting point opening a gateway for enormous prospects in the near

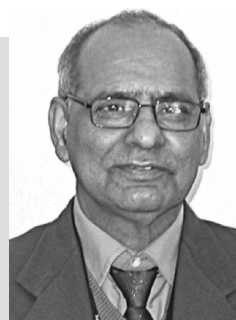
future. We believe that such high-throughput analysis offers the valuable opportunity for early, accurate and easy diagnosis with microfluidic-integrated biosensors followed by immediate therapeutic treatment.

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Prof. B. D. Malhotra received his PhD in physics from the University of Delhi, Delhi, India in 1980. He has published more than 230 papers, has filed 10 patents, has edited/co-edited books on biosensors and polymer electronics, and is currently Professor at Delhi Technological University, Delhi, India. He has 30 years of research experience in the field of biomolecular electronics

and has supervised 21 PhD students to date. He is a Fellow of both the Indian National Science Academy and the National Academy of Sciences, India. His current research activities include biosensors, conducting polymers, Langmuir-Blodgett films, self-assembled monolayers and nanomaterials.

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This issue is the first official special issue of *Biotechnology Journal* with the Asian Federation of Biotechnology (AFOB). In the cover, we use the grain as a symbol and analogy for biotechnology, as both the grain and biotechnology are common and uniting factors that link the member countries of the AFOB. Grain image credit: © Myimagine – Fotolia.com.

Biotechnology Journal – list of articles published in the November 2013 issue.

Editorial: *Biotechnology Journal* in Asia – the first AFOB special issue

Tai Hyun Park and George G.Q. Chen

<http://dx.doi.org/10.1002/biot.201300415>

Review

Organ-on-a-chip technology and microfluidic whole-body models for pharmacokinetic drug toxicity screening

Jong Bum Lee and Jong Hwan Sung

<http://dx.doi.org/10.1002/biot.201300086>

Review

Microfluidic-integrated biosensors: Prospects for point-of-care diagnostics

Suveen Kumar, Saurabh Kumar, Md. Azahar Ali, Pinki Anand, Ved Varun Agrawal, Renu John, Sagar Maji and Bansi D. Malhotra

<http://dx.doi.org/10.1002/biot.201200386>

Review

Imaging and tracing of intracellular metabolites utilizing genetically encoded fluorescent biosensors

Chang Zhang, Zi-Han Wei and Bang-Ce Ye

<http://dx.doi.org/10.1002/biot.201300001>

Review

Applications of cell-free protein synthesis in synthetic biology: Interfacing bio-machinery with synthetic environments

Kyung-Ho Lee and Dong-Myung Kim

<http://dx.doi.org/10.1002/biot.201200385>

Review

Current developments in high-throughput analysis for microalgae cellular contents

Tsung-Hua Lee, Jo-Shu Chang and Hsiang-Yu Wang

<http://dx.doi.org/10.1002/biot.201200391>

Research Article

Heterologous prime-boost immunization regimens using adenovirus vector and virus-like particles induce broadly neutralizing antibodies against H5N1 avian influenza viruses

Shih-Chang Lin, Wen-Chun Liu, Yu-Fen Lin, Yu-Hsuan Huang, Jin-Hwang Liu and Suh-Chin Wu

<http://dx.doi.org/10.1002/biot.201300116>

Research Article

Repetitive Arg-Gly-Asp peptide as a cell-stimulating agent on electrospun poly(ϵ -caprolactone) scaffold for tissue engineering

Pacharaporn Chaisri, Artit Chingsungnoen and Sineenat Siri

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Research Article

Global gene expression analysis of *Saccharomyces cerevisiae* grown under redox potential-controlled very-high-gravity conditions

Chen-Guang Liu, Yen-Han Lin and Feng-Wu Bai

<http://dx.doi.org/10.1002/biot.201300127>

Research Article

Automated formation of multicomponent-encapsulating vesosomes using continuous flow microcentrifugation

Huisoo Jang, Peichi C. Hu, Sungho Jung, Won Young Kim, Sun Min Kim, Noah Malmstadt and Tae-Joon Jeon

<http://dx.doi.org/10.1002/biot.201200388>

Research Article

Size and CT density of iodine-containing ethosomal vesicles obtained by membrane extrusion: Potential for use as CT contrast agents

Bomin Na, Byoung Wook Choi and Bumsang Kim

<http://dx.doi.org/10.1002/biot.201300110>

Research Article

Site-targeted non-viral gene delivery by direct DNA injection into the pancreatic parenchyma and subsequent in vivo electroporation in mice

Masahiro Sato, Emi Inada, Issei Saitoh, Masato Ohtsuka, Shingo Nakamura, Takayuki Sakurai and Satoshi Watanabe

<http://dx.doi.org/10.1002/biot.201300169>