Chapter

Application of Microfluidics in Biosensors

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Abstract

This chapter reviews the up-to-date researches in the field of biosensors integrated with microfluidic techniques, most of which are publications within the last 5 years. The features of these biosensors, their applications, challenges, and possible future research interests in this field are also reviewed.

Keywords: microfluidics, biosensor, bioreceptor, lab-on-a-chip, bioaffinity, PDMS, μPAD, paper-based microfluidics, electrochemistry, optics, surface plasmon resonance, colorimetric, fluorescent, cell culture, food safety

1. Introduction

Biosensors are defined, by Tudos and Schasfoort [1], as "analytical devices comprised of a biological element (tissue, microorganism, organelle, cell receptor, enzyme, antibody) and a physicochemical transducer. Specific interaction between the target analyte and the biological material produces a physico-chemical change detected by the transducer. The transducer then yields an analog electronic signal proportional to the amount (concentration) of a specific analyte or group of analytes" [1]. The features of biosensors include the bio-recognition unit and the transduction mechanism from biological signals to measurable electronic signals, e.g., color, current, voltage, capacitance, light intensity, wavelength, and phase. Major parameters to assess the performances of biosensors include the following:

- High sensitivity. The sensitivity of a biosensor is always the first and one of the most important parameters in assessing its performance. The efficiency in capturing analytes, the specific characterization of the analyte, the capability of converting biological signals into electronic signals (or the response of the system), and the systematic and environmental noises both determine the sensitivity of a biosensor.

- High stability and repeatability. The stability of a biosensor refers to the capability of a biosensor in performing consistently and reliably under designated environments, and the stability of a biosensor is especially important when assessing portable or wearable biosensors that usually are applied in scenarios involving varied temperature, velocity, humidity, pressure, lighting conditions, etc. The repeatability of a biosensor mainly refers to the long-term performance of a biosensor under the same conditions and is usually tested regularly in commercial biosensors in order to recalibration.

- Quick response or real-time analysis and diagnosis. Real-time analysis usually delivers more information than providing a final result, e.g., binding rate, reaction time, kinetics, and saturation conditions, which can serve for the analysis in the applications of biological and chemical reactions and drug analysis. Response time required to bind sufficient molecules upon the sensing surface is typically determined by diffusion, which can extend to hours and even to days to generate a signal above the background noise level. This applies fundamentally to all sensors that accumulate and concentrate target molecules onto a transducer, including fluorophore-tagged molecules in microarray spots, label-free optical biosensors, and impedance-based sensors [2].

- Low consumption of sample volume. The samples for biosensors are usually with low volume due to the nature, e.g., tissue, antibody, and some biological samples are with low concentration or small molecular weight, and this enforces biosensors to perform with minimum sample consumption.

- Ease of operation. The application of biosensors goes from laboratory-based research to commercially available devices for at-home use. The operation of biosensors should eliminate professional operation or understanding of the device, but simply involves collecting samples and reading results.

- High throughput. Single-function biosensors are fading away from stage even with extremely low cost. Biosensors should be able to integrate all the good qualities mentioned in above bullet points together with the capability of multiple-tasking.

In traditional clinical healthcare, interests are in high-quality biosensor for the measurement of physiological indices. With the development in the requests of Internet of Things and self-service techniques, the interests and emphasis in healthcare transfers from clinical healthcare to family healthcare, e.g., long-term monitoring of chronic disease [3], disease prevention and early detection, reachable clinical services for remote districts, etc. Under the precondition of high quality, biosensors miniaturized as portable or wearable are emerging to meet the new trend of era, which promise a bright future in health management and digital health; researches on the biological and instrumental parts of point-of-care (POC) and lab-on-a-chip (LOC) techniques belong to this area. The potential applications of biosensors include real-time health monitoring, remotely synchronizing health data with medical personnel, patient management, POC disease diagnosis, big data statistics in health management, etc. [4]. Real-time health monitoring in domestic applications enables patients to monitor the health status by themselves at home without the assistance of professional personnel at minimum cost. Health data synchronized with medical personnel spares the patients from transporting and waiting for outpatient services, saving time and reducing medical expenses. Based on the collected health data, a potential service of clinical diagnosis is possible in an artificial intelligent health system in the near future [5]. Meanwhile, these new applications impose more requirements on the researches and developments of biosensors, as stated below.

- Low cost. For at-home applications, lower cost of biosensors is vital, so there are emerging researches on adopting cheap materials, simplifying sensing systems and adopting smart phones in data processing, etc.

- Noninvasive collection of samples. Biosensors for sensing human samples, noninvasive sensing, is preferable, especially for everyday or frequent

measurements. Researches using human samples as saliva, tear, sweat, and urine are one of the hottest topics.

- Miniaturization of the biosensing systems [4, 5], including sample pre-processing unit, sensing unit, data collection/processing system, and data displaying unit.

- The design of integrated sensor chip. A sensor chip with multiple functions is preferable especially for sensing human samples.

2. Challenges in biosensing technologies

All the qualities mentioned above, which both researchers and industry are seeking for, raise multiple challenges, and we try to summarize and list below:

- Specific binding. The recognition of analytes should be specific only to the analytes which is not affected by other chemicals, molecules, or cells. This is significantly more challenging when the sample components are complex and mixed with various kinds of molecules. For example, the detection of one specific antibody in human blood sample should eliminate the effects of all other antibodies, cells, electrolytes, etc., the detection reflects only the concentration of this antibody, and the detection of one specific heavy metal ion in a real polluted water sample should be able to distinguish the reaction induced by all other ions.

- Non-specific binding, i.e., biofouling, in some cases significantly introduces signal noise, drift, or delay in biosensors [6]. The most common method to reduce non-specific binding is to completely wash the binding surface with buffer after the binding processing is finished; thus the weak binding induced by non-specific binding could be eliminated to the minimum extent.

- Properties of bioreceptors, e.g., concentration and alignment. Plenty of papers [3, 7–10] presented the protocols of surface activation, modification, and functionalization, but the protocols greatly depend on operation and environment. Even following exactly the same protocol, the coverage rate of bioreceptors and the repeatability of operation may vary a lot, due to the immobilization of multiple layers on the sensor surface, which usually involves linking layers for stable sensor surfaces like gold and silicon, so the surface treatment protocols should be tested before operating the binding events. So far quite limited number of papers presented theoretical and/or experimental analysis on the effect of bioreceptor alignment/orientation on the performance of biosensors. The linking layer molecules are usually randomly polarized and oriented, which can induce destructive interference and dramatically reduce the collective charge polarization [11], and this means that even the surface treatment protocols could be repeated and the alignment of bioreceptors is another parameter that will highly affect the outcomes of the binding events. The detection becomes less sensitive. Chu [12] proposed a method to homogenize the orientation of the chemical linker on nanowire-based field-effect sensor by applying an external voltage on a metal plate about 1 mm above the chip surface at certain frequency while grounding the back gate electrode; thus the molecular conformation can be maintained for hours or longer, and this method has been tested and proven by the detection of DNA hybridization

reactions with poly-15 T ssDNA, showing that the alignment process promotes the sensitivity by 10-fold.

- Design of biosensor assay matrix. The effects of specific and non-specific binding on signal was tested and analyzed by Schneider [13, 14], which proves that for all the binding events, proper design of the sensor assay should be optimized, especially when the sample components is complex, for example, proper reference binding sites should be included in order to eliminate non-specific binding from different components. But this in another way increased the requirement in both the imaging capability of the biosensor and the data processing capability. Meanwhile, the surface treatment, modification, and functionalization [4, 7, 9, 10, 15, 16] will be more complicated and need to be tested and verified, and the complexity in sensor surface properties (e.g., various refractive indices of bioreceptors) requests better system compatibility and responsivity.

- Low concentration target molecule within a low-volume sample, i.e., extremely limited number of analytes available for detection. For the example, in the research of POC and LOC, 20–50 μL finger prick blood contains over 20,000 kinds of biomarkers of clinical interest at concentrations as low as 10 pg./mL, meaning that only 106–107 available biomolecules for one target [2].

3. What is microfluidics?

The definition of microfluidics, given by Whitesides from Harvard University, is:

It is the science and technology of systems that process or manipulate small amounts (10^{-9} to 10^{-18} liters) of fluids, using channels with dimensions of tens to hundreds of micrometers. It offers fundamentally new capabilities in the control of concentrations of molecules in space and time [17].

The material most commonly adopted for the fabrication of microfluidic structures is poly-dimethylsiloxane (PDMS), which is optically transparent and able to support important microfluidic components, e.g., pneumatic valves; meanwhile there are other materials with research interests, e.g., polycarbonate, polyolefin, silicon, and glass. Recently paper-based (reviews by [3, 18–21], and research by [22–28]) and cloth-based [29] microfluidics are drawing more attention because of the low cost, easy fabrication, and lightweight which are essential properties for POC applications. The major features of microfluidics are the small consumption of liquid sample and tiny dimensions of structures, which have significant impact on the development of biosensors, so the integration of microfluidics into biosensing techniques complies with the development of the era and generates unique features in biosensors, e.g., trace level of sample at high sensitivity.

4. Advantages of microfluidic-integrated biosensors

Microfluidics provide a closed and stable biosensing environment so to improve sensitivity. For on-site portable biosensors, the effect of an open environment on sensing results hugely lowers the biosensor performance. By integrating the microfluidic structures, sample processing and biosensing reactions are carried out within a closed and relatively stable environment, thus promising better sensitivity and reliability [30]. Taking the example of the application of solid-phase polymerase chain reaction (SP-PCR) in online molecular diagnosis, the

development of this technique is hindered by lack of sensitive and portable on-chip optical detection technology. Hung [24] proposed a LOC device which combined the solid-phase polymerase chain reaction with supercritical angle fluorescence (SAF) microlens array embedded in a microchip. He demonstrated a high sensitivity of 1.6 copies/μL and showed comparable detection limit and linear range to off-chip detection using conventional laser scanner, and he stated this device as an on-chip highly sensitive and multiplexed pathogen detection with low-cost and compact optical components.

Microfluidic channel can efficiently, accurately, and significantly reduce the sensing area. Simulations and experiment results have shown that reducing the sensing area could shorten sensing time and increase the sensitivity with smaller sample requirement, especially at lower target concentrations [7, 11]. An increase in sensitivity of two orders of magnitude has been reported by Li et al. [31]. Meanwhile, the distribution of binding events along the sensing surface could be heterogeneous, and this could be induced by the heterogeneous in bioreceptor coverage, different alignment of bioreceptors, nonuniform concentrations of targets within samples in laminar flow, and nonuniform temperature, pressure, or other physical parameters along the sensing surface, the heterogeneity can be eliminated to minimum. Reduced sensing area also means miniaturizing sample volume which is essentially valuable to low concentration targets and rare targets with limited access.

Microfluidic structures are capable of integrating multiple functions within one device without introducing extra equipment or tools. For example, by designing and optimizing microfluidic channels, sample injection, pretreatment, and processing can be easily realized. Usually for biosensing, the modification of the biosensing surface is compulsory for specific binding of targets, and this is doable in microfluidic structures which are even more stable and more promising than manual operations. For the biosensing events, the volume and speed control of sample are achievable which provides more valuable information, e.g., binding affinity, binding rate, kinetics, etc.

Microfluidic devices are capable of automation. With or without external pumping system/equipment/tools, microfluidics is capable of integrating sample pre- and post-processing, sensing, surface modification, temperature control, EM field control, etc. The automation of microfluidic-integrated sensors and structures is reviewed by [30, 32–34]. However, the automation of whole microfluidic-integrated biosensor as one device still seems quite challenging as the liquid handling in this field is usually more complex which could involve up to dozens of solutions and operations like filtering, centrifugation, etc., together with the activity of biological samples to be considered. Partially automized microfluidic-integrated DNA biosensors are reviewed by Ansari [35]. Joung [36] presented a novel lateral flow immunosensor (LFI) for microfluidic-integrated enzyme immunosorbent assay (EIA) in POC testing (POCT), a chemiluminescent LFI-based automatic EIA system, the operation of which does not require additional steps such as mechanical fluidic control, washing, or injecting. The key concept relies on a delayed-release effect of chemiluminescent substrates (luminol enhancer and hydrogen peroxide generator) by an asymmetric polysulfone membrane (ASPM). When the ASPM was placed between the nitrocellulose membrane and the substrate pad, substrates encapsulated in the substrate pad were released after 5.3 ± 0.3 min. As a proof of concept, the high-sensitivity C-reactive protein level in human serum was detected by this sensor.

Microfluidics enables both separate and mutual processing of multiple binding assays with single or multiple samples simultaneously. For the detection of single or multiple targets in a real or complex solution, the design of the binding assay usually involves more than one kind of bioreceptors, thus meaning

that the designed samples to flow over each bioreceptor spot could be different. The delivering of different kinds of samples in sequential orders can be realized by unique design of microfluidic channels, pneumatic valves [17, 37], and/or centrifugal forces [33, 34].

Microfluidic structures ensure the precise control over experimental conditions [38]. What can be precisely controlled by microfluidic structures include flow rate, sample volume, channel volume, channel height, reaction time, etc. Integration of sensors with microfluidic channels serves to reduce assay time by constraining the diffusion distance between the molecules in the sample and the sensor and to create laminar flow over the sensor to distribute target molecules broadly and uniformly [2].

5. The present of microfluidic-integrated biosensors

Biosensors can be classified based on target recognition events and transduction mechanisms [4]. Based on the target recognition events, biosensor receptors are included. Based on the transduction mechanisms, biosensors can be classified into optical biosensor (Raman scattering [39–44], surface plasmon resonance (SPR) [6, 45–47], fiber Bragg grating [48–53], fluorescent [54–58], chemiluminescence [36, 59]), electrochemical biosensor [60–64], calorimetric biosensor [6, 22, 65–69], and piezoelectric biosensor [70–74].

5.1 Target recognition

Biological targets to be detected by biosensors, especially for the detection of analytes holden by human beings/animals, could be divided into two kinds, i.e., physical parameters and physiological/biological targets. Physical parameters like the body temperature, blood pressure, heart rate, velocity, and location usually do not request a corresponding and unique bioreceptor on the biosensor, as these physical parameters usually can be detected directly by optical, electronic, and piezoelectric sensors. Analytes as physiological/biological targets, however, cannot be detected directly, because of the complex components in a real human sample, so bioreceptors are adopted for the specific recognition of these targets, including cell, antibodies, DNAs, aptamers, and molecularly imprinted polymers [4].

The most commonly adopted physiological fluids of human beings/animals are blood, which has to be collected in an invasive way, and fluids that can be collected in a noninvasive way, e.g., sweat, saliva, tears, and urine, can be used in the prediction and diagnosis of various diseases [75–77]. Comparing with other physiological fluids, saliva is the outstanding fluid with the advantages of easy accessing and large volume, but with a major disadvantage of large range of variability in components and concentrations depending on the extent of oral cleanliness; examples that have been experimentally verified are using human saliva for the detection of cytokine [78], dopamine [51], insulin [79], fetuin [80], bacterial load [81], cholesterol [25], and cortisol [82]; using tear for the detection of dopamine [83], proteomic, lipidomic, and metabolomic composition [77]; using sweat for the detection of cytokine [84] and proteomic [76]; and using urine for the detection of anticancer drugs [85], L-carnitine [86], *Chlamydia trachomatis*, and *Neisseria gonorrhoeae* [87]. Samples of sweat and tear have been significantly undeveloped until quite recent when flexible materials and flexible electronic techniques achieved some milestones [4]. Currently the most well-explored targets in human physiological fluids include electrolytes (e.g., K^+, Ca^{2+}, Na^+) and major metabolites (e.g., myocardial enzyme,

glucose, urea), which lack specification to diseases, indicating the general physiological conditions [4].

5.2 Transduction mechanism

So far, optical biosensors deliver the best sensitivity among the three other kinds of biosensors; electrochemical biosensors are the most popular choice as commercial-potential biosensors because of the compact size, low cost, and acceptable sensitivity; colorimetric biosensors are with a distinguished advantage of easy operation at extremely low cost but with a major disadvantage of low sensitivity; while the researches on piezoelectric biosensors are quite limited comparing with three other kinds of biosensors. Some most up-to-date researches on all fours kinds of biosensors are presented below.

5.2.1 Optical biosensors

Surface-enhanced Raman spectroscopy (SERS) and surface plasmon resonance are the two powerful optical biosensors with a unique feature of label-free sensing, as the analytes need no pre-processing to be labeled before sensing events and thus eliminate the false-positive or false-negative biosensing results induced by the labels. The first commercial product of SPR biosensor appeared in 1990 by the company of Pharmacia (named Biacore afterwards). Since then, more than 1000 papers were published annually using commercial SPR biosensors [88]. Most of these commercial SPR biosensors are bulky and only laboratory based. The development of plasmonic-based biosensors in the field of POC was reviewed in [43] together with recent advances in surface chemistry, substrate fabrication, and microfluidic integration. Here we explore a bit wider which is not limited to POC but microfluidic-integrated biosensors. In most of the researches mentioned below, the microfluidic structure usually serves as the sample handling unit.

Tunc I et al. [39] presented the molecular specificity of Raman spectroscopy together with self-assembled monolayer of metallic AuNPs to detect CA125 antibody–antigen molecules. Highly enhanced electromagnetic fields localized around neighboring AuNPs provide hot-spot construction due to the spatial distribution of SERS enhancement on the CA125 proteins at nM concentration level.

Carneiro M et al. [41] reported the detection of carcinoembryonic antigen (CEA) in SERS using two different bioreceptors for CEA, i.e., a molecularly imprinted polymer (MIP) and a natural antibody. The MIP acted as a pre-concentration scheme for the CEA, while the natural antibody signals the presence of CEA on the MIP platform. The MIP film was first incubated in the sample containing CEA and next incubated in SERS tag, which is gold nano-stars coupled to 4-aminothiophenol (4-ATP) as Raman reporter, so the MIP acted as a pre-concentration scheme for the CEA. Then the MIP was exposed to the natural CAE antibody. A sensitivity down to 1.0 ng/mL was reported.

Zhu JY et al. [89] presented a biosensor that can be used for clinical diagnosis. This biosensor is based on localized surface plasmon resonance integrated with a biomimetic microfluidic "adipose-tissue-on-chip" platform for an in situ label-free, high-throughput, and multiplexed cytokine secretion analysis of obese adipose tissue. It was stated that this system enables simultaneous measurements of pro-inflammatory (IL-6 and TNF-alpha) and anti-inflammatory (IL-10 and IL-4) cytokines secreted by the adipocytes and macrophages and identified stage-specific cytokine secretion profiles from a complex milieu during obesity progression.

In the research of [90], the plasmonic biosensor integrated the microfluidic unit for plasma separation, allows the in-line separation of plasma directly from the bloodstream without any pre-processing outside the device, and channels it to the active detection area, where inorganic cerium oxide nanoparticles function as local selective dopamine binding sites through strong surface redox reaction. A detection limit of dopamine was achieved at 100 fM concentration in simulated body fluid and 1 nM directly from blood without any prior sample preparation. This demonstration shows the feasibility of the practical implementation of the proposed plasmonic system in detection of a variety of biomarkers directly from the complex biological fluids. Li XK et al. [91] reported the plasmonic biosensor integrated a multifunctional microfluidic system with small-volume microchamber and regulation channels for reliable monitoring of cytokine secretion from individual cells for hours.

Besides the traditional plasmonic materials, graphene has recently received more and more attention in the field of both labeled and label-free sensing, because of its ability to harness electromagnetic fields, strong light-matter interaction of graphene layer, and its highly tunable optical properties [40]. Liu HP et al. [40] simulated the detection capacity of the graphene plasmonic biosensor using three-dimensional finite difference time domain method. Numerical results showed that the maximum sensitivity and figure of merit of the biosensor are 333.3 nm/RIU and 16.665 RIU, respectively.

Fluorescence is the other powerful optical biosensor which labels analytes and promises high sensitivity and specificity in target recognition. Raducanu VS et al. [56] reported a direct fluorescent signal transducer embedded in a DNA aptamer for versatile metal-ion detection. This sensor embedded with guanine-rich DNA aptamer internally coupled with Cy3 fluorescent dye that measures directly the DNA conformational changes upon metal-ion binding. Our signal transducer is environmentally sensitive that is internally coupled to the DNA aptamer. Potassium ion concentration was successfully measured in a variety of aqueous and biological test samples.

5.2.2 Electrochemical biosensors

There are plenty of researches on electrochemical biosensors, and majority of the commercialized biosensors belong to this category. Here we only present the electrochemical biosensors integrated with microfluidics that possesses both miniaturized structure and high sensitivity.

Electrode-based chemoelectrical biosensors are the most common ones. Usually a working electrode and a blank/reference electrode are designed in such biosensor, and the samples cover both electrodes and generate a measurable electrical signal. Mi SL et al. [92] reported a sensitivity up to 567 nA mM(−1) mm(−2), and the limit of detection was 4.5 M (vs. Ag/AgCl as the reference electrode) in the detection of metabolic lactate concentrations in HepG2 cells cultured with cancer drugs.

Evans D et al. [93] demonstrated a fully integrated microfluidic amperometric enzyme-linked immunosorbent assay prototype using a commercial interferon gamma release assay as a model antibody binding system. What is unique in this research is that the assay cell is based on a printed circuit board (PCB) and the microfluidic assay chemistry was engineered to take place on the Au-plated electrodes within the cell. All components were manufactured exclusively via standard commercial PCB fabrication processes. A detection limit comparable to high-end commercial systems and a short diagnosis time of 8 minutes were demonstrated.

Silicon nanowire field-effect transistor is one of the most sensitive biosensing techniques, but it is limited to analytes that carry charges. Weakly charged or uncharged analytes can hardly be detected [11]. Evans D et al. [31] presented a

method of immobilizing bioreceptors on the silicon nanowire sensing surface only, comparing with the traditional methods in which a large surrounding substrate is also covered with bioreceptors, and it was proven that restricting the surface modification substantially improves the sensitivity.

Besides silicon nanowire, copper nanowire is adopted in electrochemical biosensors [94]. In [94], microfluidic chip is coupled to copper nanowires for the fast diagnosis of galactosemia in precious newborn urine samples. Galactosemia is a rare disease that is diagnosed through the identification of different metabolite profiles. The specific detection of galactose 1-phosphate (Gal 1-P), galactose (Gal), and uridyl diphosphate galactose (UDP-Gal) confirms type I, II, and III galactosemia diseases. The detection is extremely fast which is less than 350 s, required negligible urine sample consumption, and displayed impressive signal-to-noise characteristics and excellent reproducibility.

Oliveira MC et al. [95] presented an amperometric biosensor using a screen-printed electrode modified with carbon nanotubes and nickel ions for the detection of glucose, which is characterized by the chemical oxidation of carbohydrate by NiOOH. Under optimized conditions, a limit of detection 3.9 μmol/L and a limit of quantification of 13 μmol/L were reported. The effect of concomitant species such as ascorbic acid, dopamine, and uric acid was investigated, and this method was successfully applied for the determination of glucose in a commercial blood serum human (original and spiked) sample. What is unique in this research is that the microfluidic system was assembled on a 3D-printed platform constructed with acrylonitrile butadiene styrene and integrated with nine cotton threads, providing a stable flow rate.

5.2.3 Colorimetric biosensors

Plenty of reports are available on colorimetric biosensors integrated with microfluidics, e.g., [27, 28, 96–98]; most of the reports highlighted the features of cost-effectiveness and miniaturization. Different from three other kinds of biosensors, the materials adopted for the integrated microfluidic structures are usually not PDMS, but paper for the majority and cloth in some researches. Currently majority of the researches focus on the applications in food safety [22, 27, 96] and heavy metal detection [67, 99, 100] in aqueous environment. The researches in the application of biological analytes are quite limited, due to the natures of analytes and bioreceptors and the environment conditions in order to keep the activities of both analytes and bioreceptors.

Fraser LA et al. [101] presented a malaria biosensor whereby aptamers are coated onto magnetic microbeads for magnet-guided capture, wash, and detection of the biomarker. A biosensor incorporating three separate microfluidic chambers was designed to enable such magnet-guided equipment-free colorimetric detection of PfLDH. The biosensor showed high sensitivity and specificity when detecting PfLDH using both in vitro cultured parasite samples and clinical samples from malaria patients.

5.2.4 Piezoelectric biosensors

The research of piezoelectric biosensor integrated with microfluidics is quite underdeveloped so far. Possible reasons could be the lower sensitivity, poor biocompatibility, and complicated fabrication.

Yamaguchi M. [82] proposed a mass sensor based on mechanical resonance that incorporates a disk-shaped mechanical resonator, a separate piezoelectric element used to excite vibrations in the resonator, and a microfluidic mechanism. Electrical

power is used to actuate the piezoelectric element, leaving the resonator free from power lines. This sensor was reported to be suitable to analyze the concentration of a salivary hormone, cortisol in human saliva samples.

6. Future research interests

Future possible research interests in the field of microfluidic-integrated biosensors are proposed as the following:

- Exploration of materials for both microfluidics and nanofluidics in different application scenarios. Besides PDMS, the exploration of other materials, e.g., engineering polymers, traditional glass, silicon, or metal, in special applications that requires high chemical stability, high thermal stability, unique optical properties, and/or special mechanical properties.

- Fabrication of microfluidics and nanofluidics and structures (e.g., valves, mixers).

- Microfluidics with high chemical and thermal stability for special applications.

- Integration of microfluidics and nanofluidics with sensors to form complete and functional systems that require no professional operations and ease in applications, e.g., LOC, etc.

- Integration of microfluidics with data processing algorithms. The application of machine learning in sensing data processing could enhance the performance of biosensors in specialized environments.

- Integration of microfluidics with communication techniques. Synchronization of sensing data with relevant users, remote control of the biosensors, and big data analysis of special sensing networks can be realized.

7. Conclusions

The state-of-the-art advances in biosensor development based on microfluidic technology have been reviewed in the book chapter with focus on the applications, challenges, and possible future research interests for each type of biosensor. It can be envisioned that microfluidic-based biosensors will remain a hot topic of investigations because of the ever-increasing demands in various applications ranging from industry to biomedical detection. The interests in microfluidic-integrated biosensors promise even more prospective future in these areas. It is applications in wearable biosensor; portable biosensor can be explored in the future with enhanced sensitivity, improved stability, and miniaturized structure.

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Conflict of interest

The authors declare no conflict of interest.

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