5 Development and Implementation of Portable Biosensors in Microfluidic Point-of-Care Devices for Pathogen Detection

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Abstract

Massive pathological outbreaks have frequently impacted global risk, emphasizing the necessity for on-site sample analysis techniques that are rapid, reliable, and responsive to accelerate diagnoses and enable early action. The point-of-care examination makes it easier to quickly identify analytes close to patients, enabling improved identification, control, and treatment of the pathologic infection. It also provides fast medical decisions as the diseases can be diagnosed early, leading to better clinical outcomes for patients. The material used to manufacture the device is essential in microfluidic technology. Inorganic, polymeric, hydrogels, and paper are the four broad categories of materials utilized in microfluidic chips. Soft lithography, photolithography, conventional machining, and laser ablation are some of the technologies used to fabricate microfluidic devices. Electrochemical, electro-chemiluminescent, colorimetric, and enzyme-linked immunosorbent assay is the most common sensing technology integrated with microfluidics to detect microbes and biological analytes. This chapter will investigate how microfluidic technology has been utilized to develop portable biosensors and the current trends of these nanosensors for point-of-care diagnosis of various pathogenic diseases.

Keywords

Microfluidic · Biosensor · Point-of-care detection · Lab-on-a-chip

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5.1 Introduction

5.1.1 Microfluidics: Basic Concept

The definition of microfluidics depends upon two major characteristics: one of the characteristics is channel size, which has to be small in micrometer scale, and the other characteristic is the handling of volumes in nano-liter of liquids by microchannels for (10–100 μm) liquid flow, integrated with the other components such as microscale inlet valves, a specific system for pressure controller micro-pumps, and miniaturized outlet sinks along with various instruments for the study of liquids (Whitesides 2006; Park et al. 2011; Sin et al. 2013). The central parameter for describing the efficiency of fluids is the Reynolds number (Re), which is mainly influenced by the inert forces of viscous ratio. $Re > 10^3$ usually denotes systems for a macroscopic platform, whereas $Re < 10^2$ represents systems for a microfluidic platform. In microfluidic devices, the low Re number denotes laminar flow or nonturbulent flow, which is a key and common function of miniaturization of devices. In a nonturbulent or laminar flow system, diffusion at the interface between fluids is responsible for mixing of fluids. The diffusion time is reduced in microfluidics due to the small sizes, and the mixing process, which is diffusion-based, has now become an effective proposition to the mechanical parts. Based on the parameters mentioned above, the next generation of detection methods are being developed that reduce the individual steps such as sample preparation, reagent handling, bioreaction, and detection of biological analyte to a single-step procedure in a single platform based upon microfluidics (Nasseri et al. 2018; Demello 2006).

5.1.2 History

Researchers have been interested in the behavior of fluids confined to a tiny diameter since Hippocrates (400 BC), Galen (200 AD), and Theophilus (700 AD). The goal of this work was to decipher the functioning of the human body. The origin of microfluidic device fabrication with micromechanics is usually linked to Stanford University's position on gas chromatography and IBM's development of inkjet printer nozzles in the late 1960s (Castillo-León 2015) (Fig. 5.1).

The first commercially accessible continuous types of laser printers were developed by IBM researchers in 1976. Professor George Whitesides, one of the founding members of the microfluidics and lab-on-a-chip communities, wrote an essay in 1998 on the fast prototyping of microfluidic devices using polydimethylsiloxane (PDMS) (Verma et al. 2015). Due to its variable device shape, simple instrumentation, and ease of integration with other technologies, this novel technique greatly influences chemical and enzymatic processes, DNA-based applications, immunoassays, clinical diagnostics, and cell-based application proteomics (Choi et al. 2012). Studies involving cells and organs on chips provide an excellent example of the influence of 3D imprinting technology (Castillo-León 2015). Through the sophisticated sensor, microfluidic technology has taken the lead in

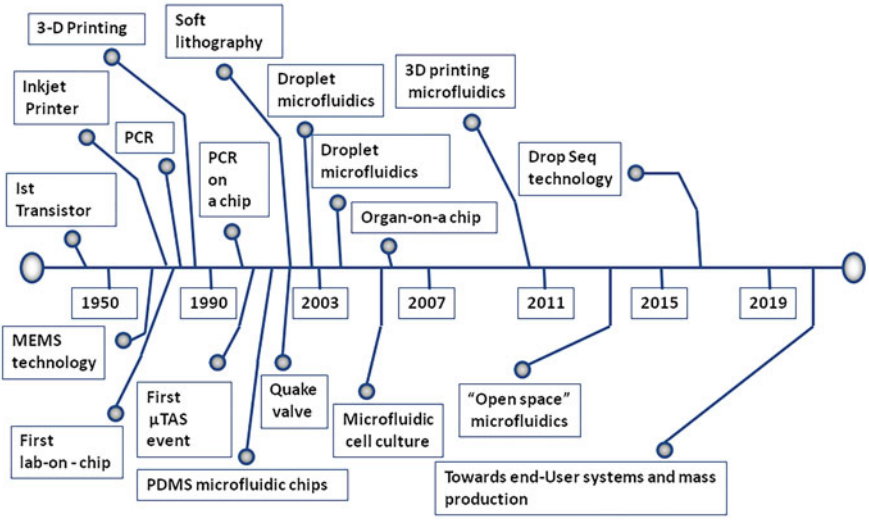

Fig. 5.1 Historical timeline of development in microfluidics technology

creating point-of-care diagnostics. In medicine, microfluidic chips shorten the time between diagnosis and therapeutic therapy, which is critical for patient safety (Chiu et al. 2017).

5.1.3 Role of Microfluidics in Biological Applications

The early diagnosis and surveillance of pathological infectious diseases are important for optimizing precise treatment, reducing mortality rate, and improving overall cost-effectiveness in the health sector. More precise and rapid diagnostic technologies are critical in the clinical diagnosis of pathogenic microorganisms such as viruses, prokaryotic bacteria, parasites, eukaryotic fungi, protozoa, and prions. Infectious diseases, for example, an acquired immunodeficiency syndrome (AIDS), Zika virus, malaria, tuberculosis (TB), and Ebola, kill millions of people per year (Na et al. 2018; Govindaraju et al. 2019). Microfluidics-based sensors have gained a lot of traction in the production of miniaturized biosensor devices for medical health purposes to address the existing issues. Advanced infrastructure, time-consuming methods, and expensive reagents are incompatible with resource-constrained settings in current diagnostic systems. While scalable material-based and paper platform technologies provide new ways to create point-of-care diagnostic assays for several medical applications, they face technological challenges when it comes to integrating with different detection systems. Microfluidic diagnostic portable devices have grown in popularity due to no need for expert operators and their rapid detection, high accuracy, responsive on-site clinical sample detection technique, ease of use, and disposable nature (Shafiee et al. 2015).

5.1.4 Point-of-Care (POC) Devices

Over the past two decades, modern generation biosensors have been gradually deployed for tracking and detecting biological analytes. Microfluidic systems' small size, high performance, and portability, combined with the ability to optically track and calculate the chips, have allowed the diagnostic approach that is integrated with significant advantages over traditional approaches**.** The use of miniaturized microfluidic platforms has facilitated developments in biochemistry and biomedical, medicine (biomedical devices), and chemistry (analytical), and biology. Microfluidic devices are used in many areas, such as pathogen and disease optical detection and surface chemistry science. Rapid detection of various diseases using convenient and robust "lab-on-a-chip" (LOC) devices have now become increasingly significant, especially in resource-poor areas (Reyes et al. 2002). Since they can offer critical input on health-related conditions to healthcare professionals and outpatients in remote environments and in real-time fashion, LOC diagnostic platforms are also considered a substitute to a centralized laboratory. LOC techniques may be used in combination with traditional diagnostic methods, specifically in the case of infectious diseases (Dittrich et al. 2006). LOC systems are mainly based on DNA, proteins, and cells that can assist physicians in diagnosing a broad range of pathogenic diseases and therefore selecting the appropriate treatment because of their quick, rapid, and highly precise detection. Microfluidic diagnostic technologies are evolving all the time in order to improve healthcare systems. In biological and therapeutic methods, these techniques utilize different ways of optical detection to classify and measure complex biomolecules. Microfluidic-based biosensors are thoroughly studied along with various detection techniques, depending on specimens (analytes), in the pursuit of fabricating on-demand (POC) devices. For manufacturing POC for clinical diagnostics, the WHO has set up certain standards that these devices should be inexpensive, specific, sensitive, rapid and robust, accurate, user-friendly, apparatus-free, and deliverable to end-users (ASSURED). Instant results and quick recording of disease status are necessary to build up diagnostic devices (Souf 2016; Nguyen et al. 2017). Nanoparticles such as gold nanoparticles (AuNPs), silver nanoparticles (AgNPs), magnetic nanoparticles, carbon nanoparticles, liposomes, and QDs have now been used combinedly with enzymes and other molecules to enhance the sensitivity of paper-based analytic devices (López-Marzo and Merkoçi 2016). As a detection test for viral pathogens such as H1N1 virus infection, researchers build up an RNA extraction technique, which is paper-based and would allow an in-situ amplification reaction from RNA. This amplification reaction can be done exactly after the RNA extraction that takes place on the paper extraction matrix and also eliminates the off-chip step for the elution process. Reverse transcription loop-mediated amplification assay (RT-LAMP) is fast and isothermal and does not involve a thermal cycler. Furthermore, the RT-LAMP was intended to include probes that facilitate direct downstream visual analysis on an immune chromatographic or lateral flow detection (LFD) test strip, similar to those platforms that are used in common pregnancy tests, obviating the need for detection equipment (Rodriguez et al. 2015).

5.1.5 Importance of Microfluidics-Based Sensors

Through microfluidics application, a large number of bio-chemical analytes, biomarkers, small molecules (DNA, RNA, and proteins), cells, and pathogens can be detected using different sensing analytic technologies, including chemiluminescent, electrochemical, and colorimetric methods, which have been developed for quantitative detection of the virus (Xing et al. 2020). In comparison to lab setups, the development, ease of production, and marketing of microfluidics-based devices are much simpler than those of other approaches. Furthermore, when introduced to the market, it requires less space and investment; thus, it is useful not only in the scientific or technical area but also in everyday life (Zhao and van den Berg 2008). Several low-resource settings lack the necessary equipment and infrastructure to conduct these diagnostic tests and analyses, forcing innovative workarounds to address this mostly unresolved issue in densely populated countries. Many diagnostic tests with built-in analytic capabilities are being developed using microfluidic technologies appropriate for low economical regions. Engineers and researchers developed innovative microchip manufacturing technologies that would be difficult or impossible to achieve using macroscale approaches. These microchips have found therapeutic applications since they need minimal biofluids for sample processing and can generally be performed quickly and efficiently (Sackmann et al. 2014). On a microchip, a new area of microfluidic devices has recently evolved to mimic in vivo organ function. This innovative "organ-on-a-chip" device integrates many well-known microfluidic components into a single in vitro device, helping developers to more accurately replicate in vivo operations. Scientists utilize microfluidics to solve this problem by developing potentially transformative ways to bring down the cost of prescribed drug development (Sackmann et al. 2014; Low et al. 2021).

5.2 Materials for Microfluidic Device Fabrication

In microfluidic technologies, the material used to fabricate the device is extremely important. A microfluidic chip is a molded or etched series of microchannels. Output holes are drilled into the chip to link the microchannel network to the macro-environment. Microfluidic chips, which also have valves for active flow control, can effectively accommodate fluids in a variety of applications. The materials used in microfluidic chips must be sufficient and possess the required properties**.** Chip materials have traditionally grown to reflect two significant microfluidic technology trends: effective microscale research platforms and low cost manageable (portable) analyses. The four broad categories of materials used in microfluidic chips are inorganic, polymeric, hydrogels, and paper.

5.2.1 Inorganic Material

5.2.1.1 Silicon

The first substance used in microfluidics was silicon, but it was soon replaced by glasses and then polymers. Silicon was initially chosen because of its high thermo-conductivity, ease of metal deposition, resistance to organic solvents, and stable electro-osmotic mobility. However, due to its stiffness, this material is difficult to treat, making it difficult to produce active microfluidic components such as valves and pumps. Dangerous materials used in the welding process necessitate the use of safety facilities as well. Both of these drawbacks, along with the high cost of silicon, make it an unappealing substrate for constructing microfluidic chips (Ren et al. 2013; Nge et al. 2013).

5.2.1.2 Glass

Following the preliminary reliance on silicon, the glass was chosen as a substrate for the construction of microfluidic chips. Prior to its incorporation into the microfluidic sector, quartz or glass capillaries for capillary electrophoresis (CE) and gas chromatography (GC) microchannels were used. For detection of glutathione from cellular supernatant, researchers used electrophoresis and chemiluminescence in every glass system (Zhao et al. 2009). Glass is an amorphous medium that is both optically translucent and electrically insulating. Standard photolithography or wet/dry etching methods are commonly used to process this material. The thermostability and solvent compatibility of glass/silicon systems contribute to additional essential applications (Mu et al. 2009). Glass is compliant with biological samples; it is also a gas-tight material with low nonspecific adsorption (Mellors et al. 2008).

5.2.1.3 Ceramic

Low-temperature cofired ceramic is usually used in ceramic microfluidic applications. Ceramic cofired at a low temperature (LTCC). This ceramic is made of aluminum oxide and prepared by laminate sheets that are assembled, patterned, and then heated at high temperatures. Because of its laminar structure, LTCC can be shaped into complex three-dimensional devices, and Fakunle and Fritsch demonstrated low nonspecific adsorption in an LTCC system using an enzyme-linked immunosorbent assay (ELISA) (Fakunle and Fritsch 2010). The electrical and mechanical properties of LTCC are outstanding, and it has high efficiency. LTCC technology is used to create a sophisticated micro-electromechanical system and micro-opto electromechanical system packages that incorporate electronic measuring, power, and signal conditioning circuits. Furthermore, electrical, optical, gas, and fluidic networks are implemented in a single box (Ren et al. 2013; Nge et al. 2013).

5.2.2 Polymers

Chips that are polymer-based were launched after several years of silicon/glass chips. A broad range of polymers are available, and this allows for considerable

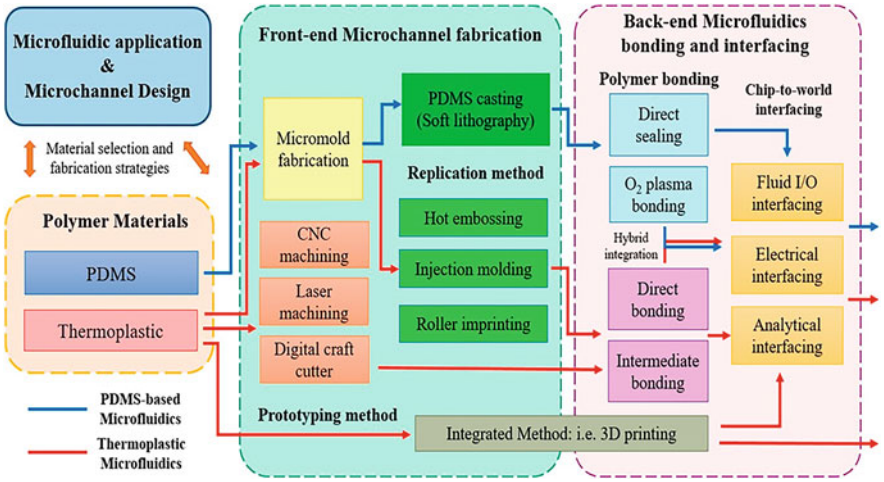

Fig. 5.2 Diagram of the fabrication process for polymer microfluidics. The PDMS-based microfluidics fabrication process is shown in blue, while the thermoplastic microfluidics fabrication procedure is shown in red. Figure source, reprinted with permission: ref. (Tsao 2016)

versatility in selecting a suitable material with unique properties. Polymers, which are less expensive and easier to produce than inorganic materials, have now become the most commonly used microchip materials. Polymers are divided into three categories depending on their physical properties: elastomers, thermosets, and thermoplastics (Fig. 5.2).

5.2.2.1 Elastomers

Elastomers are made up of cross-linked chains of polymers that are usually inter-twist; they can expand or constrict when subjected to extrinsic force and revert to their native form when the extrinsic force is removed. Polydimethylsiloxane (PDMS) is a broadly used elastomer in microfluidics (PDMS). Elastomers allow cost-effective and rapid prototyping as well as high-density valve integration on a microchip, enabling dynamic and parallel fluid processing as well as in-channel cell culture. Plastics are both simple and cheap to microfabricate, making them a suitable replacement for elastomers (McDonald and Whitesides 2002; Stroock and Whitesides 2003).

5.2.2.2 Thermoplastics

Thermoplastics are built up by densely cross-linked polymers, which are easily moldable when heated to their glass transition temperature but preserve their native form when they are cooled. These types of materials are usually more amenable and robust to micro-machining techniques, optically transparent, resistant toward small molecules, and more rigid than elastomers' permeation. Polycarbonate (PC), poly (ethylene glycol) diacrylate (PEGDA), poly(methyl methacrylate) (PMMA),

polyethylene terephthalate (PET), and polystyrene (PS) are among the thermoplastics that can be used to manufacture microfluidics-based products on a large scale (Nge et al. 2013).

5.2.3 Hydrogels

Hydrogels are formed by hydrophilic polymer chains that are organized in 3D networks, which span in an aqueous medium, and can contain up to 99% water. Hydrogels are extremely porous and have manageable pore sizes, which allow small molecules along with bioparticles to pass through. Hydrogels are useful for encapsulating the cells for 3D culture due to their aqueous nature and high permeability. Chemists may make use of hydrogels as an extremely porous structural medium, which facilitates molecules to diffuse without producing bulk fluid flows. Microfluidics has become particularly concerned in biological or medical research and bio-mimicking because of its rapid development (Ghaemmaghami et al. 2012).

5.2.4 Paper

For paper-based chip fabrication, a biosensor is an extremely cheap and user-friendly material. Clinical diagnosis, environmental monitoring, and food safety surveillance are among promising applications of microfluidic-based biosensors discussed. Paper-based sensors have gradually increased because of their broad availability, nature of hydrophilicity, and affordability. Paper-based biosensors are generally favored due to their high surface-to-volume ratio and lesser amount of volume requirement; these are the major reasons particularly observed in the development of the paper-based sensor (Wang et al. 2012). Paper is a highly porous cellulose matrix that is good at wicking liquids. As some areas of a paper are hydrophobically modified, the capillary effect specifically guides the aqueous solution added to the paper through the hydrophilic zone (Martinez et al. 2010). The fact that paper has a high surface-to-volume ratio, is structurally porous, and has a low-volume constraint, these are the primary explanation for the privilege in the construction of paper-based sensors (Wang et al. 2012). In the sensor production, the paper is chosen on the basis of its fabrication steps involved in designing the device as well as its application in specific areas. In the field research, different paper-based devices such as filter papers are primarily used in microfluidic platforms to make a device and sensor creation. A hydrophobic nitrocellulose membrane, on the other hand, is appropriate for nonbinding biomolecules, DNA, proteins, and other molecules (Liana et al. 2012).

Commonly utilized technologies for fabricating paper-based biosensing components include photolithography, cutting, laser, paper origami, and wax printing, all based on 1D, 2D, and 3D spatial and surface changes. Sealing the pores, cutting, flexographic printing, wax patterning, shaping, inkjet printing, and alkyl

ketene dimer printing for integrating the 2D features are the essential development procedures (Mahato et al. 2020).

5.3 Fabrication Technique for Microfluidics Devices

Microfluidics is rapidly growing in a research field that primarily emphasizes manipulation of small volume of fluid on the microscale level, and it is recognized most commonly by devices with critical dimensions of less than 1 mm. As the field is continuously growing, there are several different methods that have emerged for channel fabrications with the required dimensions. Different methods for microfluidic device fabrication include photolithography, soft lithography, conventional machining, and laser ablation (Fiorini and Chiu 2005) (Table 5.1).

5.3.1 Soft Lithography

In microfluidics field, there is remarkable work performed using soft lithography, which was introduced by Whitesides in 1998. Particularly, polydimethylsiloxane (PDMS) has been well documented in the soft lithography method. Soft lithography has facilitated a low-expertise way toward micro/nanofabrication, and it also plays an essential role in microfluidics, ranging from simple channel fabrication to the design of micropatterns onto a surface or within a microfluidic channel (Xia and Whitesides 1998; Kim et al. 2008).

The soft lithography protocol offers an overview of the methods, which primarily include printing, molding, and design using the stamp as an elastomeric. Soft lithography also gives a chance to make a structure with well-defined curves in a three-dimensional structure and tolerance of materials with a wide variety and generates controllable surface chemistries in a well-defined manner, which is usually companionable with medical/biological applications. Soft lithography is also experimentally convenient with lesser cost and has come up as a technology that gives access for a number of useful applications, including microfluidics, lab-on-a-chip for pathogen detection, cell biology, micro-electromechanical systems, and flexible electronics/photonics (Qin et al. 2010). Soft lithography is the collective name that consists of recently developed fabrication techniques for micro- and nano-structures. They have several applications, especially in biological sciences (Nur and Willander 2019). In the detection of pathogens, there are several applications of microfluidic biochips. There are several novel types of microfluidic systems, and new techniques that can be used for pathogen detection like viruses (e.g., HVB, HIV, ZIKA) have also been covered. Moreover, next-generation techniques relying on high sensitivity, specificity, and lower consumption of expensive reagents suggest that fast result generation can be achieved via optical-based bacterial cell detection. The introduction of smartphones in observation has replaced microscope-based observation, resulting in significantly improved detection of cell and other analytes and also

Table 5.1 Recent studies on microfluidics-based sensing technologies in pathogen detection

Substrate	Name of pathogen	Sensing technique integrated with microfludics	Analytical performances	References
PDMS	*Plasmodium vivax*	Electrochemical detection	The limit of detection of ~40 *vivax* infected RBCs (*Pv*-iRBCs)/10 μL blood sample in 5 min	Singh et al. (2021)
Silicon base and Pyer glass cover based channel	Detection of viruses	ELISA based	22 ng mL^{-1}; the limit of detection time was shortened from >3.25 to <30 min	Liu et al. (2005)
Paper, lateral flow test strip	Influenza (H1N1)	RT-LAMP	Detection limit of viral load of 106 copies/mL, contributing a tenfold enhancement over current prompt immune-assays	Rodriguez et al. (2015)
Cellulose paper	Human immunodeficiency virus-1	Electric and optical sensing	Can detect multiple biotargets selectively, with sensitivity and repeatability	Shafiee et al. (2015)
Lateral flow test strip	Human immunodeficiency virus-1	Nucleic acid lateral flow assay	LOD is 0.1 nM	Hu et al. (2013)
Silicon chip	Zika, Chikungunya, and dengue viruses	RT-LAMP	Clinically relevant sensitivity; detection of Zika virus as low as 1.56×10^5 PFU/mL from whole blood, low reagent consumption	Ganguli et al. (2017)

Transparent indium–tin–oxide (ITO) coated glass	Rubella virus	Electrochemical immunoassay	High sensitivity	Rackus et al. (2015)
Polycarbonate (PC) plate	HIV-1	Sandwich immunoassay	Low-cost, simple, and efficient operation, limits of detection (LODs) of 0.17 and 0.11 ng/mL for p24 antigen	Li et al. (2019)
Silicon, PDMS	*S. typhimurium*, *E. coli*	*Fluorescence* immunoassays	The detection limit of the sensor was 103 CFU/mL *Salmonella*	Kim et al. (2015)
Silicon wafer, a spin coater, UV aligner, PDMS, a photoresist (SU-82050)	Airborne pathogenic	ATP-bioluminescence	System can determine the existence of airborne microbes within 10 min	Lee et al. (2008)
Gold nanoparticles (AuNPs) attached on 1,6-hexanedithiol (HDT)	*Aspergillus fumigatus*	Electrochemical biosensor	In standard buffer and real sample, the biosensor detects glip-T with an extraordinary detection limit of $0.32 \pm 0.01 \times 10^{-14}$ M and $0.81 \pm 0.01 \times 10^{-14}$ M, respectively	Bhatnagar et al. (2018)

facilitating simplistic data processing, which helps in easy transfer of data for presentation purposes (Nasseri et al. 2018).

5.3.2 Photolithography

Microfabrication in microfluidics domain photolithography is one of the easiest and most essential methods used to design precise patterns in the material. In the photolithography system, a prototype or shape can be etched with critical exposure of a light-sensitive polymer to ultra-violet light (Ma et al. 2010). Photolithography has been used as a major method in the fabrication of microfluidic devices. It consists of exposing a substrate that is coated with photoresist to light in a manner that it inclusively developed regions that can be protected from (or subjected to) following fabrication processes such as deposition or etching (Lin et al. 2002) as the most obtainable light source in nature is sunlight, which consists of various types of light across a spectrum ranging from infrared, through visible light, to UV. At the ground level, UV-radiation light represents around 5% of solar energy, and the spectrum of radiation lies around 290 and 400 nm. Eventually, radiation is mostly used as a light source for the fabrication (Etzel and Balk 1999). One of the research articles explains FLASH (fast lithographic activation of sheets), which describes an instant method for laboratory prototyping of microfluidic devices in paper. Nowadays, paper-based microfluidic devices are growing as a new technology for various applications in diagnostics for the rising world, as simplicity and low cost are vital in the application in the biological field, specifically the diagnosis of pathogens (Martinez et al. 2008).

5.3.3 Wax Screen Printing

In the microfluidics segment, the fabrication is well established by using screen printing for the fabrication of chemical sensors and biosensors because there are various benefits such as versatility, miniaturization, low cost, and the opportunity of mass production (Renedo et al. 2007). There are numerous different forms of printing surfaces that can be used, such as ceramic, glass, cotton, and paper or similar fabrics. The type of ink used in the screen printing process is also determined by the printing surface and the intended usage. Characteristically, liquid inks and dyes are the printing materials. In one of the research, the printing material was used as a solid wax for screen-printing hydrophobic barriers on paper (wax screen printing method) (Tudorache and Bala 2007). Wax is environmentally friendly; moreover, it is extremely easy and cheap to attain than photoresist or PDMS. The fabrication method is well organized such that the process nowadays is accomplished without the use of a UV lamp, clean room, organic solvents, or sophisticated instrumentation. From previous reports, wax printing needs a wax printer ($2500 US), but recent progress in printing screen methods has made them inexpensive and broadly available around the world (Carrilho et al. 2009).

5.3.4 Laser Ablation

In the fabrication method, the laser ablation for machining the microflow channel has various advantages, mainly including trouble-free, rapid, and one-time ablation to complete the fabrication process. This technique is widely used, the method to machine main polymer materials is used, and the glasses can use the microflow channel on the surface. The glass-based materials are generally used because of their excellent surface stability, solvent compatibility, and optical properties due to the straightforward and well-understood fabrication system (Giridhar et al. 2004; Cheng et al. 2005; Li et al. 2011). Characteristically, in previous research, laser ablation is particularly referred to as the technique of ablating and a microflow channel machining on the surface of a polymer material by using a carbon dioxide laser with a wavelength of 10.6 μm (Nieto et al. 2010). There are various micro-machining processes for constructing microfluidic devices utilizing glass as a material that have been extensively established; the choice is based on how the materials will be handled, as well as the form and size of the key features. Hot embossing, injection molding, and further thermo-forming method provide high throughput and cost, but they are ineffective in the case of glass (Becker et al. 2000). Fabrication method for microchannels on glass by laser-ablation method has been explored and reported using carbon dioxide, UV, and ultra-short pulse lasers (Nieto et al. 2010; Stjernström and Roeraade 1998; Sohn et al. 2005; Flores-Arias et al. 2009). In the case of transparent materials, in the visible spectral range, laser ablation should preferably be executed with ultraviolet radiation since the linear optical absorption is in this wavelength range. Altogether, the laser ablation has provided a benchmark for machining channels of microfluidics devices.

5.4 Microfluidics-Based Sensing Technologies in Pathogen Detection

Pathogenic microorganisms include any microorganisms that have the capability of causing human or animal diseases, including viruses, bacteria, fungi, protozoa, helminths, etc. They can be easily transferrable from one host to another by air, body fluids, food, water, etc., causing national and international panic and economic losses (Yu et al. 2017a). There is a need for faster, portable diagnostic methods that have a more accurate result. The conventional identification method of pathogenic microorganisms includes large cell numbers of a pure cell culture, with time and labor consuming enrichment and pre-selection steps. For example, the developed world standards for target pathogen diagnosis, including culture, enzyme immunoassay, and polymerase chain reaction (PCR), often take between 2 to 4 days. Moreover, since most centralized laboratories are limited to large cities, nowadays near-patient testing using point-of-care (POC) devices has become increasingly important. Therefore, robust and portable diagnostic devices are capable of providing quick information on pathogens that is mainly helpful in reducing rates of mortality, hospitalization, and timely isolation if the pathogens are infectious.

Although, in the past two decades, many different biosensors have been developed, there is still a need for miniaturized, low-cost, disposable biosensors with the capability of rapid detection and precise identification of an extensive range of pathogens (Lazcka et al. 2007). For pathogen identification, microfluidic systems have a medium for RT-PCR, RT-LAMP, nested-PCR, nucleic acid hybridization, ELISA, fluorescence-based assays, sample preparation multiplexer (SPM), and CRISPR. Microfluidic devices coated with specific antibodies for capturing pathogens can be used to detect pathogens present in a solution. Using H1N1 virus as a model, researchers have developed a microfluidic chip that detects RNA-based viruses from throat swab samples (Ferguson et al. 2011). Microfluidics-based biochemical analysis enables quick detection of pathogenic microorganisms. With the help of the mass spectrometry technique, which can illuminate the molecular structure and molecular weight of analytes, matrix-assisted laser desorption ionization-time-of-flight mass spectrometry (MALDI-TOF MS) has been wildly used for bacterial identification since the 1990s (Zhang et al. 2018). Moreover, a simple and real-time paper microfluidic assay coupled with smartphone detection has been developed for detecting ZIKA from complex sample matrices through RT-LAMP (Ferguson et al. 2011). Using normal electrochemical methods, the biosensor was used to detect ZIKA by calculating variations in the electrical signal with changing virus concentrations in buffer and serum (Tancharoen et al. 2018). In a study, affinity-based biosensing mechanisms were developed with Quantum dots (QDs) examined in the domain of the microfluidic approach. For example, the combination of microfluidic technology and QD-based affinity biosensors is presented in order to build a stronger technical platform for COVID-19 virus diagnostics. Microfluidic devices offer a wide range of methods for detection of RNA viruses such as H1N1, H3N2, H9N2, Hendra, and influenza B viruses. These accurate methods of detecting RNA viruses might also have the potential for detecting the novel coronavirus that has caused a global issue of Covid-19 (Park et al. 2019). Microfluidics and lab-on-a-chip technologies have been fascinating for the miniaturization and integration of conventional laborious equipment. Various functions can be realized in small sensors, and microfluidic channels can be used to combine them into a single device. Many attempts have been made in this direction to miniaturize and integrate different functions for the identification of food-borne pathogens using PCR (Tancharoen et al. 2018) (Fig. 5.3).

5.4.1 Chemiluminescent Assay

Chemiluminescence immunoassay is an innovative approach focused on radiation immunity analysis and enzyme-linked immunoassay that is nonradioactive, free of carcinogenic compounds and does not pollute the atmosphere or humans. It is very useful in the early detection of many diseases because of its quick action, high sensitivity, and consistent outcomes (Nasseri et al. 2018). Chemiluminescent assay, combined with microfluidic-based apparatus, has been made for quick and easy-to-use recognition of antibodies and proteins against various pathogens. Antibody

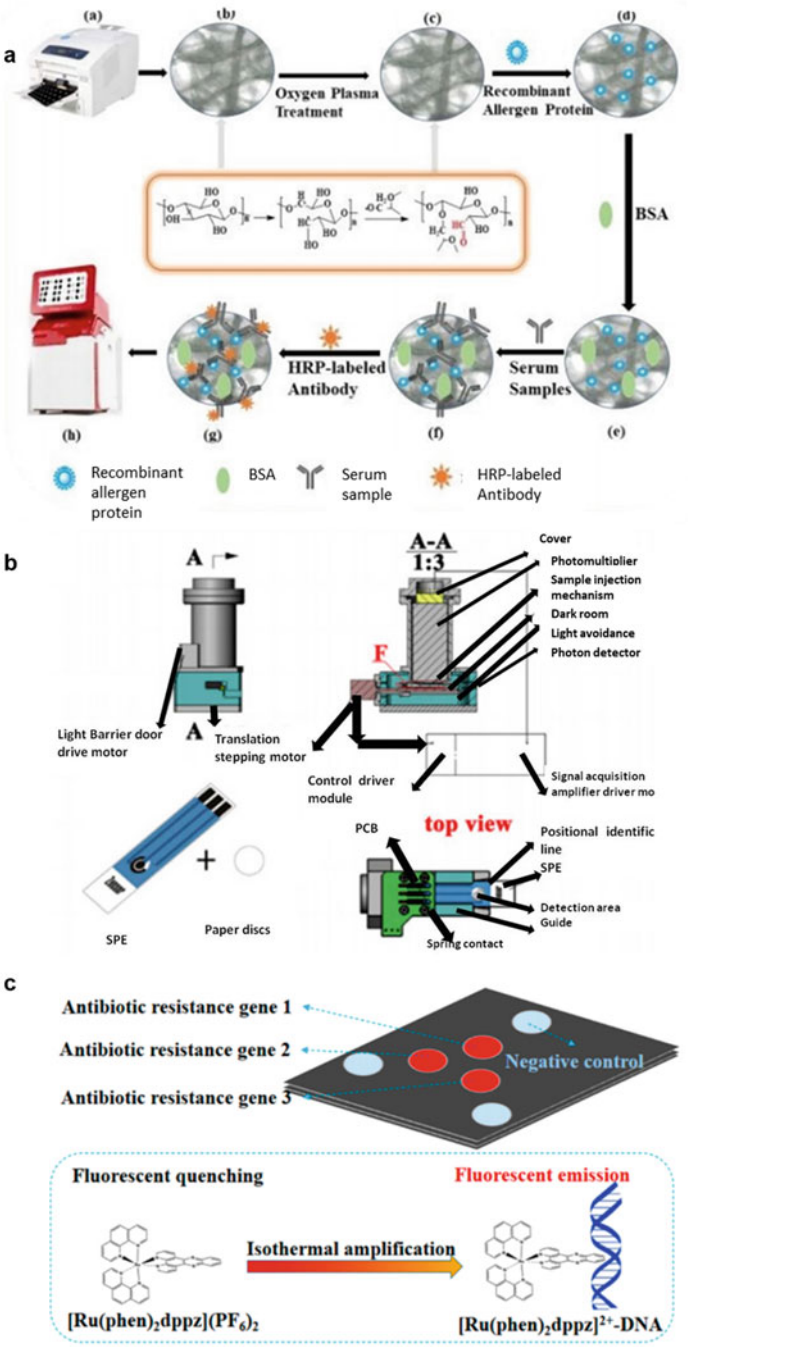

Fig. 5.3 Detection techniques and recent applications of μPADs. (**a**) Chemiluminescent detection of the paper-based immunoassay using horseradish peroxidase (HRP)-labeled antibody. (**b**) Electrochemiluminescent detection of the antigen of hepatitis B virus from clinical serum samples. (**c**) Antibiotic resistance gene detection via fluorescence sensing using a light source (Lim et al. 2019)

sensitivities in these assays are generally proportional to the sample's chemiluminescence. This method has recently been used to identify the nucleocapsid protein of the SARS coronavirus using RNA aptamers (Sayad et al. 2016). A new nanozyme-based chemiluminescence paper assay was developed for highly sensitive and specific recognition of SARS-CoV-2 spike antigen, combining nanozyme and enzymatic chemiluminescence immunoassay with a lateral flow strip (Ng et al. 2018). The focus of this research was to create a stable Co–Fe@hemin-peroxidase nanozyme that catalyzes chemiluminescence like natural peroxidase HRP, therefore amplifying the immunological response signal. The SARS-CoV-2 recombinant spike antigen had an identification limit of 0.1 ng/mL and a linear range of 0.2–100 ng/mL. Furthermore, the test's sensitivity for pseudovirus was equivalent to the ELISA technique, reaching 360 TCID50/mL. A multiplexed sandwich-chemiluminescent enzyme immunoassay for the simultaneous detection of *Salmonella typhimurium*, *Escherichia coli*, *Listeria monocytogenes*, and *Yersinia enterocolitis* was developed in one research. To accomplish multiplexed identification of the four diseases, a contemporary polystyrene 96-well microtiter plate format with each centre well having four subwells in the rim was created. Each subwell was immobilized with monoclonal antibodies appropriate for the microorganisms. After the samples were administered to the main wells, the bacteria that immediately responded to the accompanying monoclonal antibody were collected in one of the four subwells. The peroxidase activity of the bound polyclonal labeled antibodies in each well was ascertained using a low-light charge-coupled imaging system and an enhanced luminol-based chemiluminescent combination, after which a mixture of peroxidase-labeled polyclonal antibodies against the four bacteria was implemented. The test was straightforward and fast, with a limit of quantification of 10^4 to 10^5 CFU/mL for all bacterial species. The method's precision was adequate, with recovery values varying from 90 to 120% as compared to findings from a traditional culturing technique. This approach can be used as a screening tool to assess if these pathogenic bacteria are found in various foods (Basiri et al. 2021).

5.4.2 Electrochemical Assay

A transducer, also known as an electrode, is used in electrochemical biosensors for microbial bacterial identification. When target pathogens bind to an electrode using an electrochemical technique involving the electrode and a pathogen-containing electrolyte solution, the chemical energy associated with biorecognition is converted to electrical energy. In situ detection of pathogens on surfaces, rapid pathogen detection using low-cost platforms, sample preparation-free detection of pathogens in various matrices, multiplexed detection of pathogens in practical matrices, and pathogen detection via wireless actuation and data acquisition formats are all possible with electrochemical biosensors. As a result, pathogen detection electrochemical biosensors are now widely utilized in water safety, food, medical diagnostics, environmental monitoring, and bio-threat detection (Cesewski and Johnson 2020). Previously, a microfluidic RT-PCR device and an electrochemical

DNA sensor were used to achieve fast detection for initial viral infection screening. A paper-based microfluidic interface was also developed for multiplexed electrochemical detection of hepatitis C virus (HCV) and human immunodeficiency virus (HIV) antibody markers in serum samples. It was the first paper-based electrochemical immunosensing device with multiplexing and telemedicine capabilities for diagnosing HIV/HCV co-infection. An electrochemical microfluidic paper-based immunosensor array (E-lPIA) and a portable multichannel potentiostat make up the interface, which can conduct enzyme-linked immunosorbent tests on eight samples concurrently under 20 min (using a prepared E-lPIA). The platform's multiplexing capacity now allows it to create numerous measurement data for HIV and HCV markers from a single sprint, and the findings will be sent to a remote site for telemedicine. This device is small, low-cost, high-throughput, and user-friendly because of the unique convergence of paper-based microfluidics with mobile instrumentation. Researchers that have created electrochemical biosensors for pathogen detection explain the transduction components, electrochemical methods, biorecognition elements, and biosensor output in depth. The material of the electrode and the type factor of transduction elements are discussed (Reyes et al. 2002; Cesewski and Johnson 2020). Researchers who have created electrochemical biosensors for pathogen detection describe the transduction components, biorecognition elements, electrochemical methods, and biosensor output in depth. The electrode material and transduction element type factor are explored. The availability, processing, and immobilization techniques address aptamers, antibodies, and imprinted polymers, among other biorecognition components for pathogen detection (Dittrich et al. 2006; Housecroft and Constable 2010). A continuous-flow polydimethylsiloxane (PDMS) microfluidic RT-PCR chip and disposable electrical printed (DEP) chips were utilized in one of the experiments for fast amplification and sense of novel influenza (AH1pdm) virus of swine origin. There were four zones on the RT-PCR chip: an RT reaction zone, an initial denaturation zone, a heat cycle zone for PCR (two-step PCR), and a pressurizing-channel zone to prevent air bubbles from forming. To assess electrochemical signals, methylene blue was added to the RT-PCR mixture. The RT-PCR took only 15 min to complete, and the DEP chip detected the amplifiable reduction signals right away. The MB reduction current on the DEP chip with the amplicon was significantly lower than on nonamplified controls. The DEP chip for quick electrochemical sensing and this microfluidic technology for rapid RT-PCR are compatible and may create a portable diagnostic test device (Souf 2016; Yamanaka et al. 2011).

5.4.3 Calorimetric Assay

One of the earliest pathogen identification assays is the colorimetric assay. Colorimetry is a technique for utilizing colored compounds to determine the concentration of analyte in a sample. A colorimeter is an instrument that tests the absorbance of a given wavelength of light to determine the concentration of a solution (Wang et al. 2018). Respiratory tract infections are a widespread cause of disease and death

worldwide, and specific viral pathogens may cause them. For diagnosing various respiratory viruses, researchers developed an optimized microsystem focused on real-time colorimetry. This microsystem unit combines an eight-channel microfluidic array chip with a reverse transcriptase loop-mediated isothermal amplification (RT-LAMP) processor for point-of-care screening of viral infection in the respiratory tract. The entire detection procedure may be accomplished (sample aggregation, nucleic acid extraction, sample loading, real-time detection, and signal output). Furthermore, researchers have developed gold nanoparticles that are incredibly efficient in identifying pathogens due to their capability to change color rapidly and efficiently as their environment changes (Kaarj et al. 2018). The point-of-care device, utilizing the RT-LAMP amplification of nucleic acid (Choi et al. 2018), has been developed to tackle the recent outbreak of the Zika virus, leading to creating a point-of-care system for rapid virus identification in the resource-limiting region. Researchers created a wax-printed paper microfluidic chip that uses (Choi et al. 2018) undiluted tap water, human urine, and diluted (10%) human blood plasma to demonstrate the produced simple and responsive ZIKV assay. The paper pore size, shape, and channel dimension of different paper microfluidic chips were examined and adjusted to enable appropriate separation of direct-use biological samples (tap water, urine, and plasma) during capillary action-driven flow. Because of their versatility, practicality, and cost-effectiveness, colorimetric biosensors that detect target analytes with the naked eye have got a lot of coverage. Nanomaterials have recently been used for fast and sensitive identification of pathogenic viruses and bacteria as flexible signal transduction and amplification mechanism. Yu et al. published a paper that explored how nanomaterials and bioreceptors can be further combined to create a rapid and responsive colorimetric detection device for pathogen detection in the future. After the ZIKA virus RNA had flowed to the paper microfluidic chip detecting zone, it was excised and placed on a heated plate at 68 °C for the addition of an RT-LAMP combination, including a pH indicator. In 15 min, visible color variations from adequate amplification were detected and measured using smartphone visualization. The detection limit was as low as one copy per liter. The current system may be used to identify dengue virus (DENV), flaviviruses, and Chikungunya virus (CHIKV), as well as other readily spread microbial pathogens, potentially leading to field-based diagnostics (Yu et al. 2017b) (Fig. 5.4).

5.4.4 ELISA for Virus Detection

The enzyme-linked immunosorbent assay (ELISA) is a technique for detecting and quantifying molecules including proteins, antibodies, hormones, and peptides. It has been frequently employed in microfluidic devices recently, resulting in a quick and low-cost way to diagnose RNA viruses. Typical ELISA and fluorescence-based Luminex tests take three stages and many hours to complete, but combining this approach with the microfluidic technology has resulted in effective and quick diagnosis. Within 60 min, researchers developed an ELISA microfluidic device for

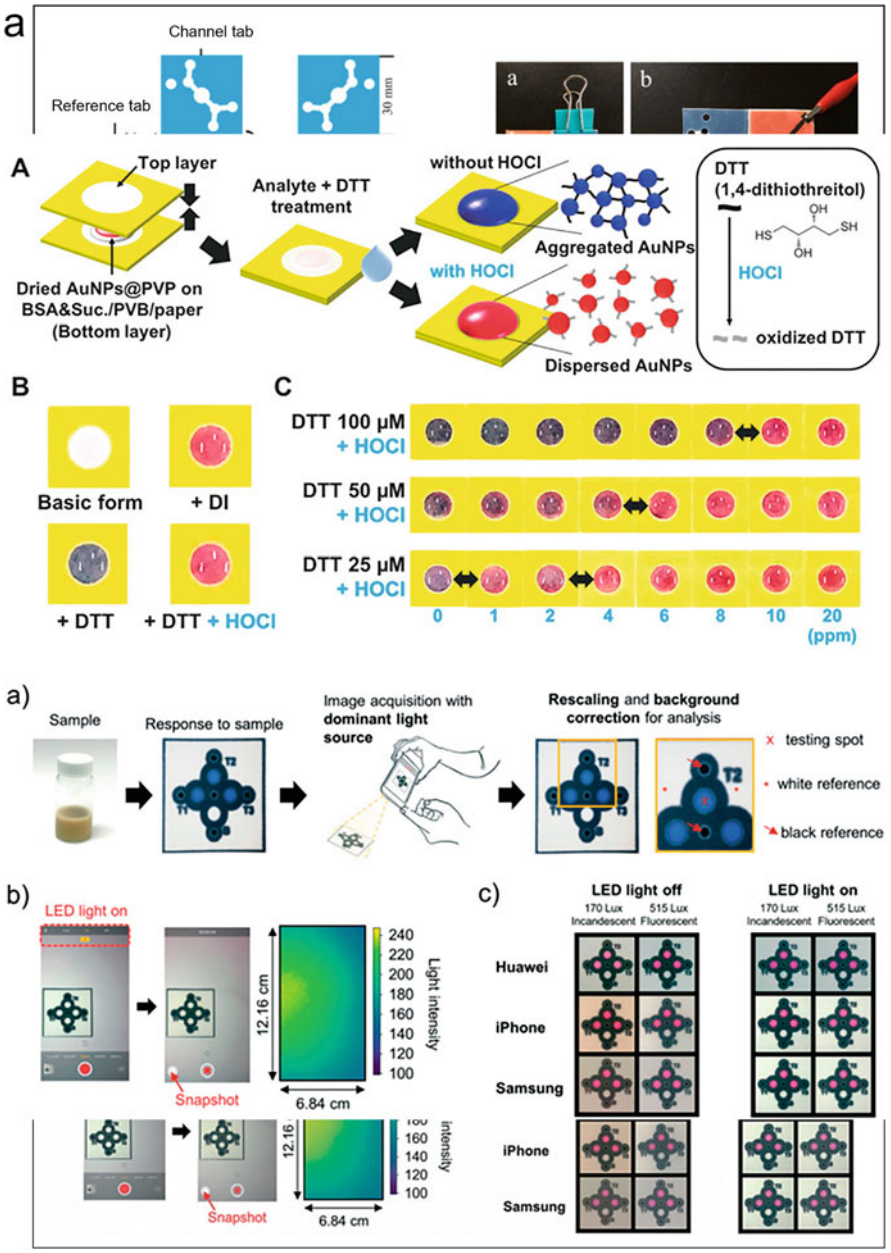

Fig. 5.4 Detection techniques and recent applications of μPADs. (**a**) Colorimetric sensing of HOCl via AuNPs by controlling the concentration of dithiothreitol. (**b**) Colorimetric sensing using a smartphone with an integrated light source. (**c**) Electrochemical detection of microRNA with chromogenic reaction (Lim et al. 2019)

detecting different pathogens as Hendra virus IgG antibody (Liu et al. 2005). One of the research used a bead-based microfluidic technology to produce a quick and responsive enzyme-linked immunosorbent test (ELISA) for pathogen detection with quantum dots as the labeled fluorophore. When compared to a standard ELISA on the same virus, the target virus's lowest detectable concentration was increased from 360 to 22 ng/mL, the detection time was cut in half from 3.25 to 30 min, and the quantity of antibody absorbed was reduced by 14.3 (Nguyen et al. 2020).

5.5 Conclusion

This chapter has covered the technological advancements of microfluidic devices toward the materials for device fabrication, fabrication technique, and sensing technologies in pathogen microorganism detection. We also discuss the primary methods for microorganism detection systems used in microfluidics: electrochemical, colorimetric, electrochemiluminescent, and enzyme-linked immunosorbent assay. POC biosensors are made of inorganic and organic materials such as glass, silicon, PDMS, PC, PMMA, PET, PS, and paper. Due to its wide availability, hydrophilic nature, portability, self-driven fluidic properties, and affordability, the paper has become one of the potential materials for manufacturing bioanalytical sensors. Stability, reproducibility, and mass manufacturing are critical criteria for clinical applications of microchips, necessitating the introduction of new fabrication methods and materials for microfluidic chips. Despite significant improvements in microfluidics, due to the requirements of external devices, there are still apparent issues and drawbacks in system miniaturization and integration, finding it challenging to implement in resource-constrained situations. Multiple parameter identification is necessary for clinical diagnosis, therapy, and prognosis to obtain a proper evaluation. Consequently, integrating several candidates such as proteins, nucleic acids, and other bioanalytes into a single platform fully automated may facilitate a better understanding of the disease and enhance the practical, specific application of inaccurate POC to improve detection credibility. In the future, several microfluidics-based technologies for bacterial extraction from blood, urine, and identification procedures may be combined, enabling fast, untargeted, and accurate detection of a broad spectrum of pathogens from patient clinical blood samples.

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