Review

Microfluidics Based Point-of-Care Diagnostics†

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Abstract

Point-of-care (POC) diagnostic devices have been predicted to provide a boon in health care especially in the diagnosis and detection of diseases. POC devices have been found to have many advantages like a rapid and precise response, portability, low cost, and non-requirement of specialized equipment. The major objective of a POC diagnostic research is to develop a chip-based, self-containing miniaturized device that can be used to examine different analytes in complex samples. Further, the integration of microfluidics (MF) with advanced biosensor technologies is likely to result in improved POC diagnostics. This paper presents the overview of the different materials (glass, silicon, polymer, paper) and techniques for the fabrication of MF based POC devices along with their wide range of biosensor applications. Besides this, the authors have presented in brief the challenges that MF is currently facing along with possible solutions that may result in the availability of the accessible, reliable and cost-efficient technology. The development of these devices requires the combination of developed MF components into POC devices that are user-friendly, sensitive, stable, accurate, low cost and minimally invasive. These MF based POC devices have tremendous potential in providing improved healthcare including easy monitoring, early detection of disease, and increased personalization.

Abbreviations: [**L],**Linear Detection Range; [**LOD],**Lower Detection Limit; **[S],**Sensitivity;**2D**,Two dimensional; **3D**,Three dimensional; **cTnI**,Cardiac Troponin I; **DPV**, Differential pulse voltammetry; **EIS**,Electrochemical impedance spectroscopy; **IgG**,Immunoglobulin; **IL**,Interleukin; **K_2O,**PotassiumOxide; **MF**, Microfluidics; **MgO**,MagnesiumOxide; **MMP9**, metalloproteinase; **PDMS**, poly (dimethylsiloxane); **PETG,** Polyethylenetetraphthalate glycol; **pM,**Picomolar; **PMMA**, poly(methyl methacrylate); **POC**, Point of care; **PS**,Polystyrene; **PTFE** ,Polytetrafluoroethylene; **PVC**, Polyvinylchloride; **SAM**,Self Assembled Monolayer; **SiO_2**,Silicon Dioxide; **SO_3**,Sulfur Trioxide; **TGF-B1**,transforming growth factor-beta 1;**TiO_2**,Titatnium dioxide; **TSH**,Thyroid-stimulating hormone; **TSH**,Thyrotropin; **ZnO-CH**,Zinc Oxide –Chitosan;

1 Introduction

With considerable growth in human population, there is a rapid world-wide increase in the occurrence of both non-communicable (diabetes, cardiovascular, cancer) and communicable diseases [1] and the researchers are struggling to meet the challenges of the diseases that are treatable [2]. The diagnostics have been considered to play a vital role in healthcare by providing a suitable and timely care to patients, for both emergency public health interventions and long-term public health strategies [3]. However, most of the commercially available diagnostic tools for disease detection do not meet the pressing requirements such as limited-resource setting and basic healthcare infrastructure [4]. The shortcomings include prolonged time for testing which often results in delay in the availability of the data resulting in delayed diagnosis. Although the role of diagnostics is crucial, for field applications, there is an urgent need for the point-of-care (POC) diagnostics [5, 6].

POC testing is a requisite component for global healthcare, which should be affordable and allows rapid testing near a patient [1]. It can be utilized in limited-resource or non-existent healthcare settings, where the physical access to medical facilities is not available. The major prospects of using these rapid POC diagnostics include portability, user-friendliness, robustness, inexpensive and its ability to generate quick results [7, 8].

POC devices have been broadly classified into two classes, *i.e.,* hand-held and large bench-top devices. The small portable devices are being developed using state-of-the-art micro-fabrication techniques, which utilize automated sample preparation, analysis, assay steps, and detection of signals [9]. Large bench-top devices are miniaturized versions of mainframe central lab equipment, that have both reduced complexity and size. Moreover, the stringent requirement of POC diagnostics presents numerous challenges for biosensor fabrication. For example, the detection of target analytes with high sensitivity and specificity is an crucial aspect in POC diagnostics because there is a need for using small

sample volumes [7]. The other challenge is to combine the detection component with other fluid regulatory elements onto a single podium. These challenges can perhaps be overcome by the implementation of the microfluidics (MF) technology towards the development of POC devices [10].

MF based POC devices are widely used for molecular biology reactions at desired platforms for cell growth and analysis [11]. The beauty of MF lies in the precise control of quantities and the rate of flow of samples and reagents, which enable the separation and detection of analytes with high accuracy and sensitivity [12, 13]. MF based POC provide powerful platforms for desired biochemical and chemical analysis due to many advantages such as ease-of-fabrication, low reagent use, low response time, improved sensing parameters, and continuous monitoring of desired analytes [14].

An MF system requires a series of components including reagents, samples, channel designing, substrate selection, combining and mixing [15, 16]. Numerous materials such as silicon, glass, elastomers, plastics, hydrogels, paper, *etc.* are being used for fabrication of the MF devices [17]. Thus, depending on the specific application, microsystems with different properties of chemical compatibility, surface properties, thermal and electrical conductivity could be developed by selecting an appropriate material for their fabrication (Figure 1). This review provides an overview of the latest advancements in the field of MF-based POC devices, that are being integrated with the biorecognition systems, for the miniaturization of complex fluid handling steps, and ease of multiplexing.

2 Glass-based MF devices

The choice of materials for the fabrication of MF device depends on various factors such as the desired function, the extent of integration, buffer compatibility, excellent physiochemical properties and its potential applications [18]. In this context, glass has

been considered as a choice of substrate for efficient MF device development as it meets most of the mentioned prerequisite characteristics [12].

Glass is a non-crystalline amorphous solid that is often transparent and exhibits properties such as robustness, chemical inertness, transparency, surface stability and solvent compatibility. Also, it is known to be biocompatible, hydrophilic and provides a uniform coating, which renders its use in biomedical devices [19, 20]. In the next section, different techniques for fabrication of glass based MF device and its prospects in biosensing have been discussed.

2.1 Fabrication of glass based MF device

The development of glass based MF device can be carried out using the MEMS processes that include photolithography, thin film metallization and etching of the glass substrate. The fabrication of glass-based substrate depends on the composition of the substrate that further determines the selection of masking layer and the etching agent (Figure 2 i) [21]. The commonly used glass substrates are pyrex glass, borosilicate (BSG) and soda lime glass. Among these, BSG is the most widely used substrate for the fabrication of MF devices due to its excellent optical (transparent from ~350 nm through 700 nm) and physical properties (annealing temperature: 640 °C; resistant to most chemicals). The other advantage of using BSG is that it can quickly bond with PDMS, silicon, and BSG itself. For the design of MF parts, low coefficient of thermal expansion (α) is required and BSG offers this choice with α of about 3×10^{-6}/°C at 20 °C. Srivastava *et al.* fabricated an MF chip using indium tin oxide as a glass substrate based on the conventional three-electrode system [22]. Lima *et al.* recently demonstrated a new approach of SU-8 bonding, i.e., sacrificial adhesive bonding for the fabrication of glass microfluidic chip. This new technique relies on the conventional method of bonding two glass slides irreversibly with an additional step of removing the SU-8 from the microchannels only. Further , this method offers many advantages such as low cost, the feasibility of ultra large scale

integration, overcoming the challenges of material adherence, removal of bubbles with great adhesion strength **(Figure 2 ii)** [23].

2.2 Application of glass based MF Devices

Numerous applications of glass based MF in the detection of enzyme, antibody, and whole cell have been reported (Table 1) [24-26]. In this context, a 3D inter-digitated electrodes array for immuno-sensing of antigen IgG and cTnI (a specific biomarker for myocardial infarction) has been developed [27]. The fusion of glass substrate with other polymeric substrate has shown promising results for many biomedical applications [28]. Matharu *et al.* reported a cell culture/biosensor platform consisting of an aptamer modified Au electrode structured on a glass substrate with an MF channel and PDMS as a control/actuation channel [29]. This miniaturized aptamer modified MF device was used to monitor TGF-B1 release from hepatic cells. Wang *et al.* demonstrated a novel MF chip-based DNA biosensor for rapid and sequence-specific detection of DNA in saliva sample of oral cancer patients using a single T-MF chip (50 μm ×10 μm × 15 mm) on a glass substrate [30]. Chen *et al*. developed a multi-array LSPR chip assay, by MF patterning of CTAB coated AuNRs onto the O_2 plasma treated glass substrate through electrostatic interaction between the positively charged AuNRs and the negatively charged glass surface [31]. However, brittleness, cost, non-flexibility, and biocompatibility are some of the demerits that are currently heralding the development of glass–based MF devices.

3 Silicon-based MF devices

Silicon has emerged as a preferred substrate for the fabrication of MF channels because of its high tolerance to varying conditions and requirement of low bonding temperature [32][33]. Further, the use of silicon as a platform in MF not only offers miniaturization of the device but also provides flexibility in the designing part.

3.1 Fabrication of Silicon-based MF devices

The different techniques for the fabrication of silicon-based MF devices include bulk micromachining [34], buried-channel [35] or surface micromachining [36]. Among these techniques, the bulk micromachining is most prevalent, where channels are created on a silicon wafer by eradication of the material which is then enclosed with another wafer *via* chemical bonding or by physical adherence [34]. In the buried-channel technology, deep vertical channels are created by anisotropic deep reactive ion etching whereas side walls of the channels are passivated by chemical vapor deposition [35]. Surface micromachining can be achieved by deposition of a desired structural layer and etching of the sacrificial layer. This method is relatively complex and requires multiple steps (Figure 2 iii) [36, 37]. Nano-imprint lithography and electron beam irradiation could be deployed to fabricate even more precise patterns of a silicon substrate having a feature size of nanoscale level [38].

3.2 Applications of silicon-based MF devices

The incorporation of MF on silicon substrate significantly reduces the sample volume, assessment time, and enables precise regulated flow regime in biosensing. However, the fluid flow may destabilize the biomolecules attached to the surface of a substrate, thus the chemistry of surface functionalization, bioconjugation and stabilization are indispensably required to be assessed for the MF system. Different legends could be conjugated to the surface of MF channels by an appropriate immobilization method which further expanded the range of analytes detection [39]. Jenison *et al.* designed silicon-based biosensor to analyze nucleotide polymorphism in human cystic fibrosis transmembrane conductance regulator gene and an array of cytokines including interleukin (IL)-6, IL1-β and interferon-γ with the detection range of 4 ng/L, 31 ng/L and 437 ng/L, respectively [40].

To perform immunoassays using a silicon MF biosensor, initially, the antigens and corresponding antibodies can be incubated together followed by their filtration using a

biotin integrated nitrocellulose membrane. Yakovleva *et al.* developed MF enzyme immunoassay on silicon microchip of the size 13.1 × 3.2 mm having 42 flow channels of 25-μm wide and 235-μm deep to detect atrazine. Silica surface was further modified by different polymers viz. 3-aminopropyltriethoxysilane (APTES), linear polyethyleneimine (LPEI), 3-glycidoxypropyltrimethoxysilane (GOPS), or branched polyethyleneimine (BPEI). The atrazine detection limit of three different immunosensors was determined to be as 0.80, 3.8 and 45 ng/L for GOPS-BPEI-GL, LPEI-GL and APTES-GL, respectively. In this study, covalent coupling of GL with LPEI resulted in the highest stability of immobilized antibody [41]. Using portable and battery operated electronic circuit and a silicon microchannel obtained *via* screen printing technique, immunosensor was fabricated to detect *E. coli* in a blood sample with the detection limit of upto 10^3 CFU ml^{-1} [42, 43].

Cell-based biosensors have recently attracted much attention due to their ability to directly identify biochemicals effects expressed by a living cell. Biocompatibility of silicon substrate can perhaps be improved by attaching cell binding moieties on its surface. Further, these effects could be transformed towards digital electrical signals resulting in forming a bridge between electronics and living system [44]. Thus, silicon offers many advantages over the use of polymers such as high mechanical strength, high-temperature stability and high resistance towards chemicals [45] . The modified form of silicon viz. silicon nanowires [46] and porous silicon [47] have found many applications in biosensors. With the advancement in the field of cost-effective alternatives viz. polymers and flexible electronics, silicon-based MF devices are expensive alternatives [48].

4 Polymer-based MF devices

The application of polymeric materials to MF devices has greatly attracted the commercial manufacturers, due to their low cost and easy fabrication steps, as compared to glass and silicon. Poly(dimethylsiloxane) (PDMS) and poly(methyl methacrylate) (PMMA) are most widely used in MF devices since they exhibit excellent electrical/chemical resistivity and

transparency indicating significant potential for large-scale fabrication of MF devices [49]. The following is an overview of the important methods widely used for the fabrication of polymeric MF devices.

4.1 Fabrication of polymer-based MF devices

4.1.1 Imprinting and hot embossing

Silicon or metal stamp is widely utilized as an imprinting tool while fabricating polymer-based MF devices [50]. Hot embossing can be achieved at low pressure and high temperatures while imprinting can be achieved at room temperature with high pressure. The resulting microchannels of desired dimensions in the plastic substrate are exact replica of the stamp. Several parameters such as imprinting time, pressure as well as properties of the plastic are known to affect the dimensions of the room temperature imprinted microchannel [51]. The benefit of imprinting over hot embossing lies in the fact that the fabrication time is reduced and there is high reproducibility of the fabricated device. Figure 3 (i) shows the process of hot-embossing and UV-imprinting [52]**.**

4.1.2 Injection molding

In this process, the copolymer resin is inserted into a mold to create MF elements. The less viscous polymer solution enables superior contact with the mold leading to distinct characteristics of the device. By controlling the procedure, temperature and time, the injection molded components can be created with exceptional accuracy. The MF channels have been created with high precision in PMMA, and polycarbonate using injection molding [49]. Injection molding has been shown to have many advantages over imprinting and hot embossing as it can create 3D features, and the pre-formed elements can directly be implanted into the plastic.

4.1.3 Soft lithography

Soft lithography has been widely explored in the development of MF devices with distinct advantages [53]. A positive relief structure created in silicon is known as the master. When the elastomeric polymer is cured on the silicon stamp, it produces a well-defined replica of the master which is further peeled off the stamp. The huge benefits of soft lithography are that the elastomeric polymers can be simply attached to each other and to the other substrate such as glass or plastic via conformal contact. The different steps involved in soft lithography are shown in Figure 3 (ii) [54].

4.1.4 Laser photoablation

In this technique, the bond of the polymer backbone breaks down as a result of induction by pulsed UV laser irradiation [55]. Further, the particles are expelled from the polymer substrate as a result of wave pulse forming a void within. Polymers exhibiting significant absorption towards laser emission wavelength are more efficient for ablation. In the laser photoablation, light is passed through a mask which defines the ablated area into the polymer substrate.

4.1.5 3D printing

The 3D printing has recently aroused much interest because of its capability to create multifarious structures with high resolution and quick building time. Among the different approaches, stereolithography (SLA) and the fused deposition method (FDM) are most common. In SLA, UV laser is used to scan and trace the definite region to cure the liquid resin material. High power lasers or UV rays are used to harden the resin material, and the entire platform moves down in the z-direction through a single layer. The SLA method based 3D printing machines are most widely used and have been commercialized [56]. A major advantage of using SLA fabrication technique is its high precision. In FDM, thermoplastic substances are liquefied to the semisolid by heating. This semisolid is then forced out through the nozzle onto a staging layer by layer. When the first layer is

accomplished, the stage is shifted by one layer, and the procedure is repeated. The FDM method has been found to be simple and cost-effective [57].

4.2 Application of polymer-based MF devices in POC biosensors

Several electrochemical biosensor types, including immunoassays, DNA hybridization, and signal switching aptamer sensors have found applications in polymer-based MF systems. Real samples are often treated before they are introduced to a desired biosensor. Due to their specific binding capabilities; affinity-based biosensors are capable of handling complex sample matrices and samples. The recent advances in affinity-based biosensor developments for MF applications are summarized in Table 2 [58-65]. Further, the functionalization of electrodes with enzymes can be helpful to selectively catalyze chemical reactions. The generated or consumed electroactive species can be detected *via* change in the current. For example, enzyme-based electrodes have been intensively used for glucose determination [66, 67].

Polymer-based MF devices have advantages over the conventional glass/silicon MF devices since they do not require the complicated photolithographic process and are thus cost-effective. They have been found to have excellent electrical/chemical resistivity and optical transparency that make it suitable for electrochemical/optical biosensing devices. The disadvantage of polymer-based MF devices lies in their flexibility.

5 Paper-based MF devices

Paper is a cellulosic material that is flexible, low-cost, lightweight, recyclable and biocompatible. It is found to be a promising MF substrate because it does not require any external pumps or power supplies for the flow of fluid, needs small volumes of reagents and samples, provides rapid analysis, and are disposable [68]. The 3D hierarchical structure of cellulose paper and its interconnected porosity allows fast movement of liquid. However, its white background and fluidic properties provide an additional advantage in the qualitative/quantitative analysis of analytes. These characteristics of the

paper integrated MF devices have found application in clinical diagnostics, environmental monitoring, and food quality testing [69, 70].

5.1 Fabrication of paper MF devices

The main concern in the design of paper fluidics devices is to confine the flow of the liquid using fluidic channels. For this purpose, a hydrophobic barrier is patterned into the paper using several fabrication methods to create distinct channels that regulate the flow of fluids by capillary wicking.

5.1.1 Photolithography based paper MF

Photolithography is used in microfabrication for transferring patterns onto a paper substrate. A light-sensitive chemical called photoresist is initially spread onto paper, which is exposed to the desired pattern and is further, developed into a selective layer for additional processing. Using photolithography, extremely small patterns (a few tens of nanometers in size), with precise shape and size of the channels can be obtained. The major drawback is that it requires a flat substrate and clean room facility which include expensive photoresists, UV light sources, and oxygen plasma [71, 72] [71].

5.1.2 Plotting

In this method a computer integrated X, Y-plotter is used to print PDMS onto paper to make the desired pattern [73]. It is comparatively less expensive than photolithography but has a lower resolution up to 1mm, *i.e.,* the distance between two channels is 1 mm (lower than photolithography). Shangguan *et al.* fabricated paper microfluidics channels by PDMS patterning where the PDMS was adsorbed into the paper pores and forms a hydrophobic barrier that prevented access to aqueous solutions. To demonstrate the versatility of this design, biosensing application was performed for liver function markers, and serum protein [74].

5.1.3 Wax printing

In wax printing, a solid ink printer is used to pattern wax onto the paper which is further heated to melt the wax and form the hydrophilic barrier to direct the flow of fluids by capillary wicking. It is a simple and inexpensive technique and can be upscaled for mass production. Besides this, the wax is environment-friendly, accessible and stable up to 60^{o}C and hence is more convenient to use in low-resource setting [75]. The disadvantage of this technique is the low resolution due to additional melting step required in the process. Renault *et al.* fabricated hemichannels and fully enclosed channel within a paper and studied the wax transport properties in a paper [76]. These fully enclosed channels can be efficiently isolated from the external environment, thus results in decreasing contamination risks, simplifying the handling of the device, and slowing evaporation of solvents [76].

5.1.5 Inkjet printing

Inkjet printing can be used to print a digital image by propelling droplets of ink onto a user-defined position of a substrate. This technology has been extended, and the variety of inks developed comprise of various polymers to living cells for biosensing and tissue engineering application (Figure 3(iii)) [77, 78]. The possibility of depositing small volumes of material inks into precise patterns with high spatial resolution is unique and provides the unusual strength of inkjet printing. However, this technique requires an additional ink formulation steps that are limited by viscosity and surface tension of materials [79].

The knife cutting, etching and plasma can be used to manage the fluid flow in paper fluidics devices [70, 80]. Songok *et al*. deposited a thin, transparent, and highly hydrophobic surface layer using nanosized TiO_2 particles on the paper surface [81]. Photocatalytic property of TiO_2 was utilized to create hydrophilic channels by exposing UV

radiation through a photomask placed on the TiO_2-coated paper. The size of the fabricated channel has been found to be 0.5 mm in width and 60 mm in length.

5.2 Application of paper-based MF biosensor

For the fabrication of efficient paper MF biosensor, the device design and biomolecule immobilization onto a given surface play a crucial role. Morbioli *et al.* used a 3D design that enabled more uniform permeation of fluids along the cellulose matrix for fabrication of paper MF device for glucose detection [82]. For this purpose, a magnetic apparatus was used for layer stacking which facilitates fluidic dispersion and improved reproducibility of tests. It has been found to have many advantages such as uniform sample distribution, multiplexed assays, and individualized treatment of layers. Li *et al.* used ZnO nanowires modified paper to fabricate highly sensitive working electrode (WE) for detection of glucose [83]. Teengam *et al.* fabricated a paper fluidics based electrochemical DNA sensor for human papillomavirus detection [84]. A disposable paper-based MF immunosensor was developed using AuNPs decorated reduced GO-tetraethylenepentamine as electrode materials [85]. The different fabrication techniques that can be used to construct paper-based MF devices along with the sensing characteristics are given in Table S1 [86-93]. In spite of these interesting developments, there is a considerable scope for improvement towards enhancing the detection limits and sensitivities of paper-based MF devices by upgrading device design and modification of paper substrate with nanomaterials. The sensing characteristics of paper MF devices are significantly affected by humidity and temperature variation. Other issues such as complexity of design, different fabrication procedure, and integration onto a single chip are some of the factors that are hindering its growth for POC devices.

6 Microfluidics for non-invasive biosensors

Non-invasive detection is a procedure that does not require any break in the skin or the body orifice for diagnosis. For the fabrication of non-invasive biosensors, secreted

biofluids such as saliva, urine, sweat, tears, exhaled breath condensate, etc. can be used [94, 95]. The detection of a disease *via* non-invasive biofluids offers numerous advantages such as easiness, painless, safer to administer than serum sampling, availability of various specimens, easy collection and screening at home [96, 97].

The efforts are being made to integrate biosensors with MF for detection of several diseases. Gau *et al.* developed an MF electrochemical sensor that differentiates cancer patients from the healthy subject by salivary IL-8 protein and mRNA biomarker concentration [98]. Jokerst *et al.* developed quantum dots (QDs) labeled multi-analytes MF integrated fluorescence immunosensor for CEA, CA-125, and Her-2 salivary biomarkers detection. Due to tagging of the secondary antibodies with semiconductor QDs, the signal was amplified by 30 times, and the detection limit increased to nearly two orders of magnitude as compared to enzyme-linked immunosorbent assay technique [99]. MF integrated surface plasmon resonance biosensor was developed for detection of the cortisol in the saliva samples. The fabricated biosensor showed efficient detection of cortisol with a detection limit of 49 pg/mL and response time of 15 min [100]. Recently a soft, wearable colorimetric based MF biosensor was developed wherein sweat was used as an analytical, biological fluid. This four-chambered multi-analytes system had the ability to differentiate different concentration of glucose, lactate, chloride ions as well as pH of the sweat [101]. Efforts have also been made to distinguish diabetic from non-diabetic patients by investigating the glucose level in saliva, urine and tear samples [102-104]. Chitosan modified paper-based MF colorimetric biosensor was fabricated that could efficiently detect glucose in tears (2 μL) [104, 105]. Ji *et al.* fabricated MF integrated organic electrochemical transistors biosensor for multi-analytes (glucose and lactate) detection in saliva biofluid [106]. This MF device has a thickness of 100 nm and requires 30 μL of biofluid for analysis. The characteristics of the recently developed non-invasive

MF integrated biosensors that have been used in the diagnosis of different diseases are given in Table S2.

The detection of non-invasively secreted biomarkers *via* biosensor and its integration with MF is one of the new thrust areas of research and is still in its primeval time. There is an urgent requirement to explore more efficient biomarkers secreted in saliva and urine along with other noninvasive biofluids such as sweat, tear, nose and ear wax. However, the secretion of biomolecules at very less concentration is a major challenge in utilizing the noninvasive biofluids. More efforts are needed to explore the potential of using this proof of concepts of MF devices towards commercialization that can further be used in low-resource settings.

7 Conclusions and future perspective

This review has covered the technological advancements of MF devices towards the fabrication and application in POC developments. Although numerous materials such as glass, silicon, polymer, and paper are being used for the MF development, but the cost and high sensitivity of these devices is a major concern. Numerous biosensing applications of glass and silicon-based MF are being reported. However, some of the properties such as the brittleness, cost and lack of flexibility are being seen as demerits for the next stage of development of MF. Besides this, biocompatibility of material continues to be a major problem for its use in POC development. Further, efforts should be made for the fabrication of polymer or paper-based MF devices, which can be a promising alternative to these substrates. Paper holds great potential towards the development of flexible POC devices. Its low cost, light weight, portability, flexibility, self-driven fluidic properties, simple fabrication method without clean room facilities and small sample requirement are continuously attracting many researchers to this highly potential field. Moreover, recent development in nanomaterials, device design, and microfabrication technologies have made it possible to obtain MF devices with enhanced sensing characteristics.

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Conflict of interest

The authors declare no financial or commercial conflict of interest.

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Figure legends

Figure 1. Scheme showing the fabrication of MF based POC on A glass [31], B silicon [107], C polymer [108], and D paper [109]

Figure 2. i Scheme showing the basic steps of fabrication a glass microfludic channel. ii microfabrication steps for SAB. Glass slide (a), deposition of SU-8 over this wafer and then pre-bake (b), preliminary bonding against the glass substrate with the walls of the microchannel coated with a thin film of Al (golden in the drawings) (c), cure of the resist just around the microchannel after UV exposure and PEB (resist under the channel is not cured because it is protected from UV by thin film of Al that is deposited only inside the microchannel) (d), removal of the uncured SU-8 and, next, of the Al film by pumping specific solvents (blue in the drawing) (e), and final chip showing residual SU-8 in the bottom of the sidewalls (f) [23] and iii Schematic diagram of Bulk and Surface Micromachining depicting MF channels formation using silicon.

Figure 3. i Fabrication of the MF device using hot-embossing (left) and UV-imprinting (right), ii Scheme of soft lithography [52] and iii Technique to develop microfluidic channels on paper substrates: a) by simple cutting of paper and b) by modifying paper substrate with the hydrophobic material [77].

Analyte	Detection technique	Biological recognition element	LOD / Linear range	Reference
peptide	SWV	Matrix metalloproteinase 9, and polyethylene glycol hydrogel	60 pM / upto 50 nM.	[28]
TGF-β1	DPV	Aptamer modified Au Electrode	1 ng/mL upto 250 ng/mL	[29]]
IgG /cTnI	Chronocoulograms	IDA	0-100 ng/mL and 0-100 ng/mL	[27]

Table 1. Various techniques and application of glass based MF.

cytokine	LSPR	antibody-functionalized AuNR ensembles	5-20 pg/mL	[31]
Antibody	-	IgG loaded flow channel	1×10^{-5} RIU/ Upto 200 ng/mL^{-1}	[25]
parvovirus B19	CL	genotype specific B19 capture oligonucleotide probes	80 pmol/L and 650 pmol/L	[26]

Table 2. Characteristics of some enzymatic biosensors based on polymer MF.

Detection technique	Microfluidic chip features	Biological recognition element	LOD and/or Linear range	Reference
EIS	PDMS flow channel	antiapolipoprotein B functionalized carbon nanotubes–nickel oxide	0.63 mg dL^{-1}/ 5–120 mg dL^{-1}	[58]
		ssDNA modified Au electrodes	3.8 nM	[59]
		Antibody functionalized Au electrode	1000 liposomes μL^{-1}	[60]
		Aptamer modified magnetic beads	0.01–10 nM	[61]
Amperometry	Centrifugal MF	Antibody coated polystyrene beads	4.9 pg mL^{-1}	[62]
CV	PDMS flow cell	anti-CTB antibody modified magnetic beads	31.7 ng mL^{-1}	[63]
	Flow cell	MC1R-antibodies/amino-functionalized silica nanoparticles-polypyrrole nanocomposite/screen-printed electrode	20 cells mL^{-1}	[64]
DPV	Flow cell	anti-LF antibody modified paramagnetic beads	0.1 µg mL^{-1}	[65]

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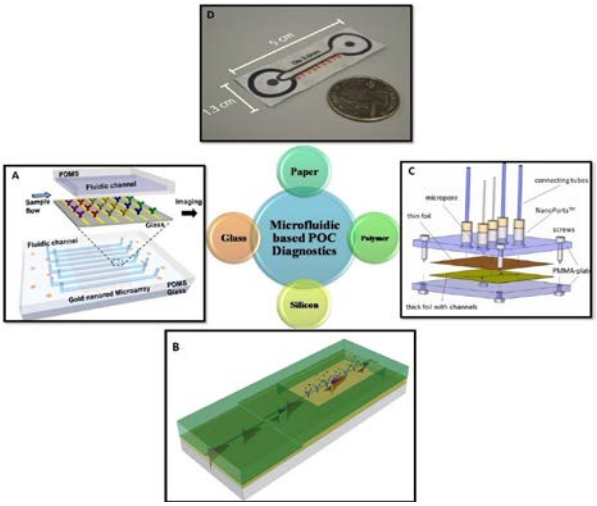

Figure 1

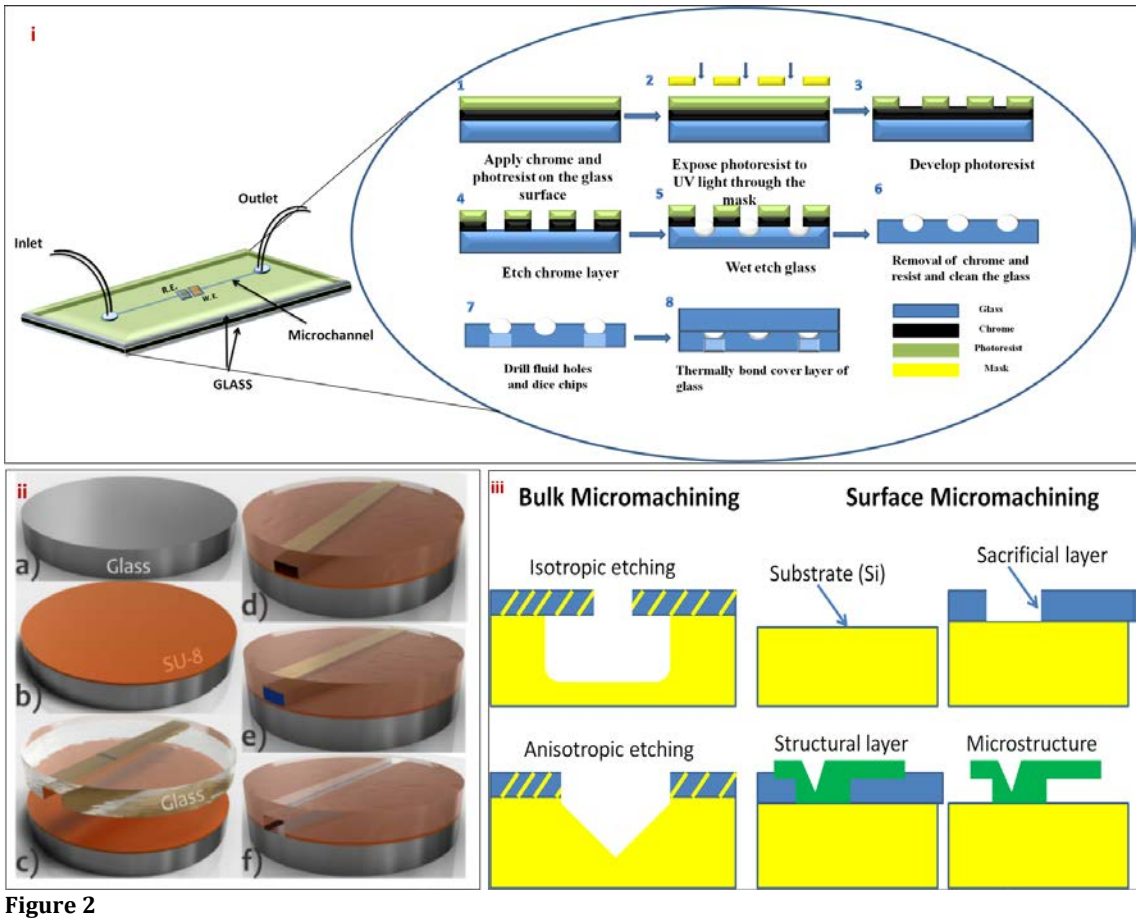

Figure 2

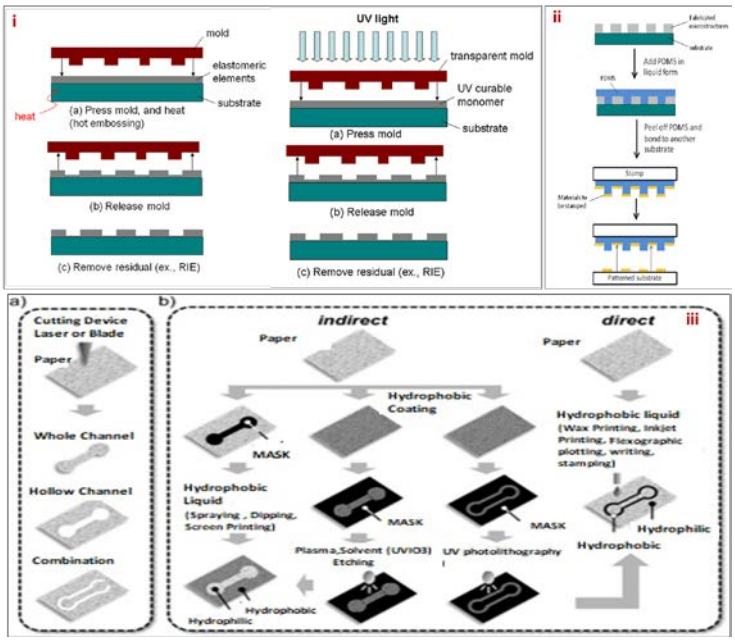

Figure 3