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Investigating Molecular-based Micro-systems for Point of Care Applications in Food and Medicine

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ABSTRACT

Considering the importance of early diagnosis of diseases as a challenge in medicine, which can be significantly effective in increasing the health level of society via preventing the progress and spread of infectious diseases, especially in developing and underprivileged countries with insufficient medical facilities. On the other hand, medical diagnosis methods that require advanced equipment and tools with expert staff limit the use of these tests. Along with the continuous development of technology, microfluidic systems have shown great potential to advance biomedical research that was previously unattainable using conventional techniques. For point-of-care applications, these systems can quickly detect diseases at low cost. This study discusses the challenges in the field of medical diagnosis and the importance of microfluidic systems as the best candidate to answer this need. Also, it describes the components of the microfluidic system, their manufacturing methods, and some of their most important applications in the field of health.

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Introduction

Access to the correct diagnostic tools is one of the most essential components of global health assessment and improvement. Clinical diagnosis has undergone significant changes in recent decades and will soon enter a new field of molecular diagnosis that has occurred simultaneously in the fields of technology, proteomics, and genomics [1]. Molecular diagnostics use powerful tools, such as deoxyribonucleic acid (DNA) sequence analysis, gene expression profiling, and biomarker identification to prognosis and determine the susceptibility of each individual to a specific disease or to identify disease stages [2]. Rapid DNA sequencing and identification of single nucleotide polymorphisms leads to disease control and prevention in each person specifically. DNA microarray has provided a good opportunity to collect and simultaneously analyze rare genetic changes and various biomarkers were also identified as the main confirming molecules for the diagnosis of early stages of cancer [3]. These developments in the field of technology provide an opportunity for medical diagnosis laboratories to be directed toward conducting molecular diagnosis tests with high accuracy and specificity. In addition, such developments provide the basis for the expansion of a new field of individual medicine, which is based on the three principles of prevention, early diagnosis, and specific treatment based on the individual characteristics of each person [4-6].

Most of the available diagnostic tools which are designed to accelerate laboratory tests, were targeted for use in developed countries and they do not meet the needs of the health sector in developing countries and when they are presented as a solution to global health problems, they seem expensive and complicated [7, 8]. In addition, many diagnostic methods require a moderate level of facilities and training, which are unavailable in large areas of developing countries. For this reason, the entry of biotechnology and nanotechnology into the field of diagnosis to design accurate, fast and affordable diagnostic methods based on molecular principles can be an important step toward improving global health conditions [9, 10]. Although most of molecular detection methods provide the necessary sensitivity and specificity to diagnose a wide range of infectious diseases; however, these methods have their complexities and require efficient trained forces, tools and complex techniques [11, 12].

The concept of on-site diagnostic tests, that diagnostics assay is performed near the patient by a person with no level of expertise, is not a new issue and its origin is in the 15th century when urine was inspected and tested at the field, patient's home [13]. However, these tests have been of interest in the past four decades due to the high demand for the clinical diagnostic market and the tremendous development of new devices and technologies for biomedical applications [14].

On the other hand, the outbreaks of emerging human viral infections pandemics in the 21st century (MERS-CoV, Ebola, SARS-CoV-2). They demonstrated the importance of fast, accurate, and rapid diagnostic technologies for point-of-care testing in emerging and re-emerging epidemics [15, 16].

The lack of efficient and expert personnel and lack of access to technical diagnostic facilities due to their high cost in developing countries and international emergency times at epidemics, it is necessary to design diagnostic systems that are simple, fast and affordable. For this reason, diagnosis systems at the point of care (POC) or diagnosis at the patient's bedside have been proposed as a response to this need (Figure 1) [17].

In the path of progress in the field of clinical diagnosis, microsystems were developed as the best candidate to answer these demands. One of the most important applications of microsystems is the miniaturization of molecular and clinical diagnostic methods for POC assays [18]. The importance of POC diagnostic tests is significantly observed where time plays an important role in early diagnosis or there is no laboratory equipment, such as in military areas, outside the earth's atmosphere or economic conditions are unavailable. In such conditions, these tools provide appropriate, correct and immediate diagnostic services and can cause tremendous progress in diagnostic sciences [19].

Scientists confirm that the key to solving challenges that have been expressed in the field of molecular diagnosis is the creation of lab-on-a-chip (LOC) technology. LOC is the miniaturization and integration of conventional and specialized molecular diagnosis reactions in the form of a microfluidic system with the brand name Microchip [20]. The biggest advantage of this achievement is increasing the speed of the reaction, reducing the manufacturing cost, the ability to perform several reactions simultaneously, and automatic operation. In a microfluidic device, several detection steps can be performed simultaneously on a microchip. As a result, diagnostic processes are significantly easier and faster [21].

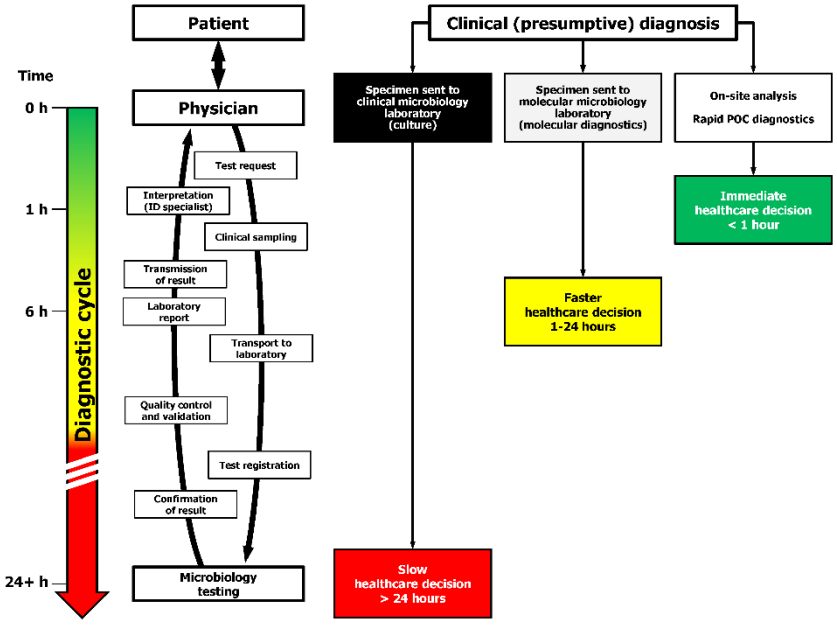

Figure 1. The importance of using point-of-care diagnostic tests in reducing the diagnosis time of infectious diseases

Note: Initiating if necessary antimicrobial therapy against microbes, and controlling host reactions to infection. In clinical microbiology, the turnaround time of the diagnostic cycle (>24 hours).

One of the most important points in the design of practical tools for POC diagnosis is the country's industrial and economic situation and its position in the world market [22]. Thus, the main priority in the design of POC diagnostic systems in developed countries is to improve the performance and simple use of these systems with the help of the latest achievements in electromechanical engineering and without considering the cost of construction [23]. This priority in developing countries changes to cost reduction and economic efficiency along with simple use [24]. In addition, clinical and medical health needs are different in these countries; accordingly, the design and construction of POC diagnostic systems in developing countries are directed toward the diagnosis of common infectious diseases, which are often contagious [25]. In developed societies, the priority of the diagnostic system is the production and supply of POC equipment to diagnose heart diseases and cancer [26].

The design basis of POC tests can be divided into two categories as follows: 1) Design and manufacture of tools and equipment that are a miniaturized form of laboratory equipment; 2) Miniaturization or downscaling of traditional tests in the field of molecular diagnostics, such as polymerase chain reaction (PCR) and isothermal methods of DNA replication or microarray in the dimensions of chips. The first category is called chip-in-a-lab and the second category is LOC [27-30].

To design and select a POC diagnostic system to introduce to the field of medical diagnosis, the following three steps are suggested [31]:

1) Determining the diseases that are the main priority in the medical health of the society and identifying the major obstacles in the diagnosis of these diseases. At this stage, only methods are selected that directly and effectively respond strongly and completely to the needs of the society; 2) Finding the answer of question, whether the selected diagnostic method is suitable for design and presence in the field of POC diagnosis, or can it be miniaturized and done on a smaller scale? This is because these methods must have sufficient accuracy and precision, be easy to use and be economical for the manufacturing company and users. At this stage, only the methods that have the necessary qualifications to enter the field of diagnosis are selected; 3) Finally, taking all conditions of developing countries to enter the field of POC diagnosis, a suitable diagnostic system should be selected and designed [32].

Manufacturing of POC diagnosis systems includes high-tech and low-tech methods. Depending on the societies in which this new field of medical health is introduced, the manufacturing and design methods of these diagnostic systems can be high-tech for developed societies and low-tech for developing societies [33].

The development of new molecular diagnosis technologies based on POC diagnosis requires finding new solutions for deficiencies in the molecular diagnosis field. POC systems must meet all or at least most of the following requirements in molecular diagnosis, including the following items: Volume and type of sample (less than 30 µL blood); accelerating the decision-making time for disease diagnosis due to the high speed of analysis of the test, which has led to a reduction in the duration of the analysis and obtaining the results of the tests (5 to 10 min); easy to use (no need for an expert to do the analysis or user training); efficiency (determining the accuracy, correctness, specificity, and sensitivity of the test); no need for quality control tests; reducing the cost of materials and tools; ability to measure various parameters from one sample; no need for expert personnel to perform diagnostic tests; reducing the waste volume of each reaction; and no need to collect and store samples in laboratory systems [34, 35].

POC analyses that are commonly used in developing countries include the following items: Measurement of blood glucose; determination of blood gas concentration; analysis of the concentration of electrolytes in blood; rapid conglutination tests; rapid diagnoses of heart disease biomarkers; drug abuse screening; diagnosis of infectious diseases, such as typhoid and malaria [36].

POC diagnostics are often made through the use of tools and equipment that are light and portable due to their miniature scales and are made in a small chip the size of a human palm, which provides the ability to carry them by hand [37].

An interesting point advantage of POC molecular diagnosis methods is that there is no need to collect and store the sample; hence, there is no need to provide biological safety systems for the transfer, collection, and storage of the sample [38].

Small, miniature, faster, specialized and cheaper POC tools have attracted significant attention for using the achievements of this field to create inexpensive and low-cost diagnostic tests for various types of diseases, such as diabetes, microbial diagnoses, etc. which is critical in the medical health system of developing countries [39, 40].

Lab-on-a-chip (LOC)

LOC is another term for micro total analysis system or µ-TAS, which is used to describe micro/nanodevices with some evolution in performance. These tools offer advantages in sample handling, preparation, mixing, isolation, cell disruption, and biomarker identification. Many of these designed systems are capable of performing more than one analysis step [41].

The major question about the LOC revolution is why the uptake and replacement of this achievement has not yet been observed despite the benefits it has provided to the field of diagnostics [42]. The cause is not the lack of interest and acceptance of this achievement by the scientific community, it is because the estimation of the results of conferences and scientific articles in reputable journals confirms the opposite opinion [43]. The main problem is designing the implementation phase of miniaturized reactions on the surface of these chips and studying how to make these tools so that they can be used several times without imposing additional costs on the user. Therefore, creating a system that is a combination of biological, chemical, mechanical, and electronic reactions and placing it at an acceptable level in terms of cost is certainly not a simple matter [44].

LOC systems are created by integrating the following components (Figure 2):

1) Microfluidic systems that provide fluid flow control, which are the reagents involved in the reaction and include channels, microvalves, and micropumps to create fluid flow in the desired path, wells as bio-microreactors, exhausting systems to dispose of consumables, heating and cooling systems to provide suitable temperature conditions and cleaning systems; 2) Measurement and detection systems that are usually based on electronic tools, such as fluorescence optical detection systems, photography systems, electrochemical detection systems and converters, magnetic detection tools, and electromechanical and photometer tools; 3) Electronic systems for capturing received signals, such as high-powered computers; 4) Bioinformatics software or systems for processing received signals and analyzing system data [45].

The technological application of microfluidic systems includes a wide range in many fields. Among the main reasons and motives for using these tools in the research field, the following items can be mentioned [46]:

1) The volume of solutions or fluids used in diagnostic microfluidic systems is smaller compared to their common types in laboratories due to the small scale of these systems as a new reactor for the reaction; 2) Due to the change in the volume of the reaction and the reactants that are effective in the occurrence of the reaction, from large volumes on a laboratory scale to volumes in the mi-

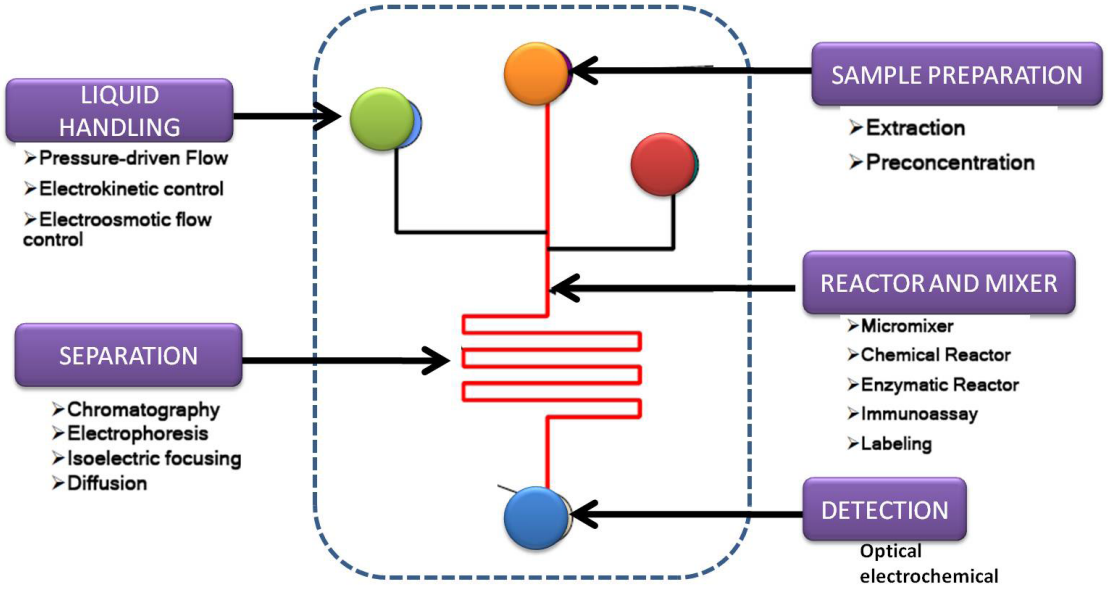

Figure 2. Components of diagnostic devices with a lab-on-a-chip approach

croliter or nanoliter scale in the scale of microfluidic systems, and subsequently changing the physical properties affecting the fluid flow. It is easier to control fluids and this has made microfluidic systems a suitable and ideal tool for use in various fields of medical sciences. The use of these tools does not require specialized skills, it is affordable and cheap in terms of construction cost, and analysis in these systems does not require a high sample volume and specialized interpretation [47].

Microfluidic devices provide the ability to perform reactions automatically with a smaller volume of reagents in less time and perform several reactions simultaneously. Microfluidics is a branch of study related to the phenomenon of fluid transfer on a micrometer scale and the design of a system to control this flow. The main difference between microfluidic reactions and bench-top reactions is the scale, which is defined by the difference in fluid volume [48].

Microfluidics includes fluid analysis on a micrometer scale and these fluids are the same reagents used in biological studies to perform the reaction. The most important features of microfluidic systems, which are essential in the design of these systems include use in biomedicine, changing the physical properties of fluids, such as laminar flow, diffusion, fluid resistance, surface-to-volume ratio, and surface tension on a micrometer scale [49].

By exploiting physical phenomena at the micrometer scale, microfluidics can be used to perform experiments in new conditions that are not applicable at the micrometer scale. Microfluidics is the transfer and analysis of fluids in microliter volumes in structures or substrates on a micrometer scale [50]. Microfluidic systems are recognized as an emerging technology for realizing on-site or bedside diagnostics to address health issues on a global scale. The miniaturization and flexibility of these systems meet the criteria set by the World Health Organization (WHO) in terms of flexibility and practicality [51, 52]. Therefore, it is considered a cost-effective, specific, sensitive, fast, and strong diagnostic method, user-friendly and not dependent on equipment with the ability to be delivered to end users [52].

According to the WHO, such devices should be used in areas where there are limited facilities, such as places with non-sterile conditions, unreliable electricity and a lack of trained personnel. It is also based on the duties that are usually considered for health department employees and nurses [53].

Fabrication methods for microfluidic systems

Capillary tubes are the first and simple microreactor as a role of microfluidic systems that can simulate the occurrence of reactions in modern microfluidic systems. According to studies, transferring According to the WHO, such devices should be used in areas where there are limited facilities, such as places with non-sterile conditions, unreliable electricity and a lack of trained personnel. It is also based on the duties that are usually considered for health department employees and nurses reduces the duration of the reaction and affects the opti-

mum temperature of the reaction. This shows the importance of microfluidic systems in improving molecular reactions [54].

Laser ablation

Laser ablation is one of the conventional techniques that are suitable for manufacturing microfluidic systems based on plex glass, polymethyl methacrylate, and polycarbonate plastic substrates. However, this method also has limitations due to the precision and size required in manufacturing [9]. The weaknesses of this method include low reproducibility due to poor control over laser focus, adverse surface effects, un-controllable power and change in product quality between different types of lasers [55, 56].

One of the rapid prototyping methods of microfluidic systems is the laser engraving method [57, 58]. By focusing a high-intensity laser beam on the substrate and based on the energy of the beam, the substrate is vaporized at the point. Using a mask as a covering of the substrate and moving the laser beam or the substrate in any direction to create a pattern, provides the appropriate form of mask pattern on the substrate surface. The interesting feature of laser ablation is the surface chemistry and charge of the material compared to the bulk by the interaction of the material with the laser beam changes [59, 60]. Another alternative method is to use continuous beam CO_2 lasers and cutting materials [59, 60] based on femtosecond lasers, which enables the machining of transparent materials [61-64].

3D-printing

3D printing technology provides a unique opportunity to improve the performance of POC devices and build complex microfluidic systems based on low-cost and one-step manufacturing and the ability to not require complex equipment [65]. Compared to conventional production techniques, 3D printing has various advantages, such as unlimited design, freedom in complex geometries, and waste reduction [66]. Issues, such as the transparent materials' lake of variety, the need for polishing and smoothing the surface, the low precision of z-axis printing, and the limitation in the accurate manufacturing of hollow parts are some faints that limit the widespread implementation of 3D printing for the production of microfluidic systems [67]. Therefore, it can make the manufacturing of low-cost prototypes for proof-of-concept studies possible [68]. However, 3D printing is limited due to the resolution for dimensions less than 100 µm and the choice of materials [69]. 3D printing technique has been used to develop immunosensors and microfluidic systems [70].

Soft lithography

Soft lithography is a valuable fabrication method for an integrated microfluidic system. Soft lithography was first introduced by Whitesides et al. and includes a group of techniques in which a soft polymer template, such as polydimethylsiloxane (PDMS) is copied from a hard original composition [71, 72]. Master molds are usually made by photolithography to create a stamp-like pattern.

Soft lithography has created a low-cost path toward the fabrication of micro/nanostructures and it plays an important role including making a simple channel and creating micropatterns on the surface or inside a microfluidic system channel [73]. Microfluidic systems based on PDMS using a modified soft lithography process consist of two main parts as follows: A mixing part and a distribution part (or reaction and detection areas). Creating a hard mixture, pouring the liquid polymer into the mold, thermal curing and separating the polymer form the four steps of soft lithography. Considering that a molded stamp is created from a flexible material, molding and highlighting structures on a micrometer and nanometer scale are also used for printing [72, 73].

Chemical processes

There are several methods based on chemical manufacturing used to fabricate glass and silicon microfluidic systems, such as dry and wet etching or electrochemical discharge [74].

The fast speed and simultaneous processing of many wafers made wet etching common [75]. In this method, strong chemicals are needed to remove materials, and hydrofluoric acid is the choice. Although its safety and environmental risks are considered a challenge for using this material [76], the equality of the channels created on all sides is a disadvantages of this method [77].

Etching based on reactive ions is one of the dry etching approaches and has solved some of the challenges of wet etching as an alternative. Directional ion bombardment in this method made it possible to create uneven profiles on all sides and precise microfluidic channels [78]. This method is reported to be more suitable for creating transparent substrates, although it has a lower speed compared to the wet etching method due to less selection of masks [78].

In the electrochemical discharge machining method, the spark produced electrochemically exposed to the surface of the tool is used. By applying a voltage between two electrodes immersed in the electrolyte, a spark is formed. Its high temperature removes undesirable substances by thermal or chemical methods. This method can be used for ceramics and glass as non-conductive materials [79].

Mechanical processes

Micromachining as a routine method for making microfluidic systems is based on the physics of semiconductors [80]. It is possible to produce surfaces without cracks by using mechanical processes to maintain dimensional accuracy and surface roughness [81]. These techniques are suitable for equipment based on polymer materials based on the master replication manufacturing method and silicon and glass processing [80, 81].

One of the common methods for making various polymer objects is injection molding. The ability of this method for mass production, cost-effectiveness, and high precision for making microfluidic systems has made it practical [81]. Micro injection molding is similar to this method. Then the melted materials are placed under pressure inside the hot mold. By maintaining the pressure for some time and reducing the temperature below the transition temperature of the polymer glass, the solid material is obtained from the mold [80, 81].

Materials for microfluidic systems fabrication

Inorganic materials

The development of the design of microfluidic systems is one of the advanced consequences of material science. Despite of a wide range of materials, surface properties, such as porosity are important for fluid dynamics, hydrophobicity, or reaction kinetics. Therefore, accuracy in the construction of these types of diagnostic systems is inevitable. In microelectromechanical systems, it is recommended to use mineral materials with high stability, changeable thermal conductivity, and solvent adaptability.

Silica and glass

Among the first materials that were used in the construction of microchannels were silica and glass, which are easy to mass produce, available, and easy microfabrication techniques [82]. These materials are processed by standard photolithography methods. For this purpose, a thin light-resistant layer is placed on the surface of the wafer, and ultraviolet rays are used to transfer the patterns on the transparent mask [83].

Biocompatibility, reuse, optical transparency with less fluorescence background, compatibility with water and stable surface properties, such as wettability, surface absorption, and surface reactivity are among the characteristics of glass. This material also has a significant anodic binding capacity, which forms high resistance at high pressures. Today, glass is not the only material for the purpose; therefore, it requires manufacturing processes with expensive and time-consuming characteristics and is considered a very fragile material with inflexibility [84].

In microfluidic systems, the use of silica is limited due to its turbidity, which prevents optical inspection. Because glass has optical properties that can be easily adjusted, resistance to high pressure and chemical properties of the glass surface have made it an alternative for various biomedical applications [84].

Organic or polymeric materials

Organic polymers are more cost-effective compared to inorganic materials and are simply and quickly manufactured. If they have a series of desirable properties and some minerals. PDMS, polymethyl methacrylate, polystyrene, and polycarbonate are organic polymers used in microfluidic equipment.

Elastomer

The ability of elastomers to create a specific structure to change the molecular shape and combine them by external forces, such as tension or pressure is interesting. These shape changes are temporary and due to the plenty of cross-links in the structure, they can return to their previous shape without breaking or tearing [85]. This material can be deformed under weak stress and quickly return to its original state due to its low Young modulus and high elasticity. The most popular elastomer in the process of making microfluidic systems is PDMS. The emergence of soft lithography caused PDMS to make significant progress in the field of microfluidic systems fabrication [86].

PDMS is elastic and easily modified by oxygen plasma, and two layers of PDMS can be bonded together in structures such as closed channels, integrated valves, and connected chambers. It is also transparent for the visible light spectrum and provides the possibility of op-

tical reading. PDMS is non-toxic for most cells and is permeable for the transfer of oxygen and carbon dioxide, which can be useful for cell studies [87]. PDMS polymer has significant gas permeability and biocompatibility compared to other materials. Also, the construction of microfluidic systems using this type of polymer is done easily, which is done by mixing prepolymers, molding, and thermal curing of PDMS. This material is also suitable for making systems for biomedical applications with lower cost and acceptable optical clarity [85-88].

Molecularly, PDMS polymer has a structure consisting of Si-O, which leads to gas permeability and material transfer by having a porous matrix. With its high biocompatibility, this substance has had wide biological applications, such as cell culture, cell screening, and biochemical assays [89]. On the other hand, non-specific and uncontrollable absorption of molecules in the channel wall, formation of a gradient due to liquid evaporation, and internal flow limit the wider use of this type of polymer for biological purposes. Accordingly, other limitations of this material such as channel deformation and problems of resistance to pH changes have also been reported [90].

Thermosets

The most common use of thermosets is to resist negative light for the fabrication of microfluidic systems [58]. Negative light refers to light resistance, due to which the parts that are exposed to ultraviolet rays are connected, while the rest remains in solution and is washed [91].

In the manufacturing process with a thermostat, heat, and light radiation effects form strong cross-links. This reaction is irreversible and creates a strong structure without any change to the original structure like elastomers [92]. Thermosets will be optically transparent and structurally strong after baking. Also, they show high resistance with increasing temperature. Having these characteristics, thermostats have an important place in the design of optical sensors [93]. The possibility of three-dimensional construction of microfluidic systems using photopolymerization is also considered one of the important advantages of the thermostat. Another advantage of this composition is its high strength, which allows the fabrication of structures with high and free dimensions. The high cost of manufacturing with this type of material limits their applications in microfluidic systems.

Hydrogels

Another widely used material in various fields of biomedicine, sensors, and robotics is the soft materials of hydrogels, which are formed from highly porous three-dimensional nets of hydrophilic-based polymer chains and cause the diffusion of small molecules and biological particles. Biocompatibility, low cytotoxicity, biodegradability, controllable pore size, significant permeability, and suitable aqueous nature are useful properties of hydrogels. Also, these materials, like an extracellular matrix, show vital properties by imitating natural mechanical and structural templates of cell adhesion, proliferation and differentiation [94]. Hydrogel-based microfluidic systems have been developed today, which are very practical with their high biocompatibility for cell-containing fluids. These materials can also create channel forms through various approaches, such as 3D printing, photolithography and sacrificial mold methods [46].

Due to the inherent hydrophilic surfaces of Hydrogels, it does not require complex processing. Also, devices made of hydrogels have fewer mechanical defects compared to equipment made of conventional hard materials. These characteristics have made the microchannels of devices made of hydrogel tend to change shape under high-stress conditions, thereby finding more limited applications. Also, these materials show deficiencies in water absorption and swelling [95]. Therefore, hydrogels are rarely used as raw materials for making microfluidic systems. Maintaining integrity in the formation of fabricated devices is a challenge and the long-term use of such materials is limited [96]. Also, chemical modification of these materials is considered an effective method to increase the strength and mechanical stability of these materials. Using diacrylate Pluronic F127, Shen et al. succeeded in making a type of hydrogel with non-swelling properties for use in a microfluidic system. Such systems are specialized to maintain the mechanical strength and morphology of the channel when exposed to a solution environment [97, 98].

Paper

The invention of litmus paper in 1784 [99] is considered a turning point in the development of this type of material. This innovation made it possible to measure the acidity of various samples by using cellulose paper impregnated with pH-sensitive chromophores in addition to measuring pH in different places [100]. It took many years to form the basis for the development of the most widely used types of paper sensors such as lateral

flow measurement. Lateral flow assays were introduced in the 1970s and are used in modern medicine to identify a variety of pathogens and biomarkers. The blood sugar measuring device for diabetic patients invented by Clark and Lyon is considered one of the most famous POC medical diagnostic devices [100, 101]. Then, when the Whitesides group introduced microfluidic patterns on paper in 2007, which used paper modified with biomolecules to detect pathogens in microfluidic paper-based tests, a milestone in the development of these systems emerged [102]. In recent years, various materials have been evaluated for integration into microfluidic systems. Some of them were successful and some of them are still under development.

Microfluidic systems components

Based on their capabilities, microfluidic systems made it possible to use laboratory biochemical compounds in portable form. Generally, the chip-based platform is capable of sampling, extraction, filtration, separation, preconcentration, isolation, re-storage, and identification of biomarkers. Usually, microfluidic systems consist of micropumps, valves, micromixers, separators, and concentrators. Pumps and mixers are usual components of these systems for various microfluidic applications [103].

Pumping tools

For the development of diagnostic devices based on microfluidic flow which is the main cause of reactions in microfluidic systems, manipulating the fluid by external actuating force is not suitable. Because it is difficult for untrained personnel to adjust the connections between the pumps and the system. Therefore, many strategies have been reported for on-chip fluid manipulation. The sample and reagents are injected into the microfluidic system and flow through the channels of the system by the pumping mechanism. This mode is effective for reducing possible human error in handling materials and affects standardizing the reaction conditions and its repeatability [104, 105].

In a biosensor microfluidic system, the movement of samples and reagents requires creating a pressure to path flow the fluid from one direction to another. Peristaltic pumps are examples of positive pumps that have been proposed for use in microfluidics, although they all have an external power source or require repetitive motion to control the flow. Therefore, in microfluidic systems, fluid movement is formed by passive forces, such as gravity, capillarity, and pressure caused by the stimulation of chemical reactions and creating pressure. Capillary and wick properties have been widely used for fluid movement in POC diagnostic systems. For example, it is used in lateral flow assay (LFA) systems based on the wick phenomenon for the cost-effective movement of all kinds of samples for all kinds of measurements. In terms of design and application, micropumps have a high variety, which is grouped into two groups mechanical and non-mechanical pumps. Smaller examples of macro-sized pumps are mechanical micro-pumps, which consist of a micro-chamber, check valves, micro-channels, and an active diaphragm with the ability to move and transfer liquid [106].

Mechanical approach

Piezoelectric micropumps

In the late 1980s, the first piezoelectric-based actuated micropump was introduced. To activate the membrane in the pump part using an alternating electric field, a piezoelectric disk was used to stimulate the fluid. Peristaltic pumping through piezoelectric discs in three reaction chambers determined that liquids are transferable to carry out DNA amplification reactions. The built-in chip consists of reaction chambers at different temperatures in three cycles of PCR reaction formation, a peristaltic pump, and an integrated optical sensor. Due to the piezoelectric micropump, after 20 to 30 thermal cycles, high impact volume, strong driving force, and fast mechanical response are formed. This is while a challenge for the point diagnostic device is the integration of piezoelectric force-based fluid drive components [107].

Pneumatic micropumps

The design feature of peristaltic-based pneumatic micropumps is that they move with the help of air pressure and without electricity by creating pressure for fluids. The lack of need for electricity for such pumps eliminates the possibility of sparking by disconnecting or actively connecting the fluid flow in the channels using compressed air. The required sequential excitation control occurs through three external electromagnetic valves. Also, an S-shaped air channel in the fluid channel causes similar peristaltic pumping. At the same time, an external electromagnetic valve is required only for pumping, as the connected airflow causes sequential activation of the fluid channel. In these micropumps, the use of elastomeric materials, such as PDMS with low Young's modulus is common [108]. This diagnostic method is started automatically by connecting the antigens to the detection areas, followed by the addition of samples, washing buffer, secondary horseradish peroxi-

dase-conjugated antibody, development buffer and stop buffer in separate tanks sequentially with micropumps. Also, a pneumatic micropump is used to flow samples, examine cell separation, and collect cell nuclei through dielectrophoresis forces [109], while the performance of pneumatic micropumps is dependent on external air.

Centrifugal force

Centrifugal force based on microfluidic or compact disc (CD) is used to create fluid flow in a microfluidic system. The flow of centrifugal force causes the movement of fluids on the CD following the rotation speed of the CD and is controlled by the dedicated channels of microfluidic systems. This particular design enables simultaneous and identical CD-based measurements in parallel designs on the underlying CD and the screening of many multiple analytes simultaneously. This microsystem exists in two formats: The first type of automated biochips integrated with micropumps and microvalves for the pathogen's detection, and the second type of integrated automatic biosampling chips with peristaltic actuation has three sets of thin films with fluid pumping capability. A large commercial potential is predicted due to the extensive advances in CD technology and its advantages, such as precise rotation control and optical reading and simultaneous measurement of a large number of analytes in a short time and on a dedicated platform. Other types of these pumps are electromagnetic micropumps and electrostatic micropumps [110-112].

Non-mechanical approach

Flow-based on electroosmotic phenomenon

Electroosmotic flow is the movement of liquid induced by an applied potential in a porous material, capillary tube, membrane, microchannel, or any other liquid channel. Since the electroosmotic velocity is independent of channel size, the electroosmotic flow will have little effect as long as the electrical layer is much smaller than the specified length scale of the channel. Electroosmotic flow is important in small channels. Electroosmotic flow is an essential component in chemical separation techniques, especially capillary electrophoresis. Electroosmotic flow can occur in natural unfiltered water and buffer solutions [113-116].

Flow-based on dielectrophoretic forces

Dielectrophoresis is the induced motion of polarized particles in non-uniform fields and is an effective method for separating bioparticles, such as cells, viruses, proteins, and biological macromolecules. This induction separation is based on the movement of charged particles according to their charge, size and mass. The application of liquid flow based on the phenomenon of dielectrophoresis in the miniaturization of the LOC system has found a wide and new application [117].

Valve and mixer

The presence of special buildings inside the chip, such as channels, valves, mixers and pumps, gives the device the ability to enter one or more types of fluid into it and move along the channels. If needed, they can be stored in a part of the chip for a period. They are mixed and create a specific reaction, and finally, the main products and the resulting waste are transferred to the outside of the machine through the outlets [118]. Another way to control the fluid flow is to take the high surface-to-volume ratio in microfluidic systems and design passive capillary microvalves that take advantage of the geometry or wetting properties of the surface in microchannels. These passive capillary valves are preferably used to block and pass fluid flows to prevent the valve from interfering with biological fluids [119–128].

Molecular detection in microfluidic systems

Microfluidic instruments are used as a platform for biological and chemical analyses and as a tool at higher laboratory levels that require solutions or fluids for sample preparation or quantification. All these features are due to the micrometric scale of fluidic components of microfluidic systems, which increases the capability or efficiency of analyzer systems and provides more accuracy in quantitative biological measurements [129].

The application of microfluidic systems in the field of medicine can be divided into two categories:

1) The development of POC diagnostic tools, which is a basic solution for laboratory problems in the field of molecular diagnosis, to provide accessible technology to be used in providing health in resource-limited settings;

2) Microfluidic systems that are commonly used in research laboratories provide a creative solution to the problem of the impracticality of many tests or analyses that go back to basic studies of human biology [130].

Wells and channels embedded in microfluidic systems for fluid flow, which have dimensions of about 10 to 100 μm are the main characteristic of these systems. The volume of reagents in these systems is about nL and they

provide the ability to analyze and perform reactions in new conditions on the chip that do not have a similar sample in the laboratory scale. There are many practical benefits in the small reaction volume in chemical or biological analysis, but the fluid has physical and chemical properties on a nM scale. These properties can be used in microchannels or other components of microfluidic systems to control fluid flow [131].

One of the main advantages of conducting small-scale analyses and tests is the consumption of a small amount of sample, which can be seen in protein crystallization screening studies. One of the main challenges in protein crystallization is low efficiency during protein purification steps. Usage microfluidic systems with the chip-in-a-lab approach have been introduced as a tool in protein crystallization systems because it reduces the protein sample volume required for the test [132].

One of the other advantages of using a smaller reaction volume is increasing the concentration or density. Condensation and alignment in structures of microfluidic systems is another characteristic of these systems, which is widely used in the field of technology [133].

The high power of microfluidic systems can have many functional advantages in molecular diagnosis, such as optimizing or reducing the time of research studies and reducing consumption costs by increasing the number of reactions that are integrated simultaneously on a microchip and automatically. In other words, the scale of performing the reaction at the bench-top level can be an important principle and an obstacle to the analysis and review of the results and different analyses, and microfluidic systems provide the collection and access of inaccessible information [129-131].

When using on-site medical diagnostic equipment, ideal microfluidic systems should be designed and supplied as simple and small as possible. Microfluidic systems that can move and have a small size are certain self-sufficient devices that control the fluid movement in the system with the help of electromechanical devices. In addition, it comes with a suitable detector system for reading and determining the results of tests that can be easily analyzed [130].

During the review and analysis of test results, researchers use a series of external and large tools to detect the results after the test. The small design and format of microfluidic systems, which are made using biocompatible types, have made this technology compatible with many optical diagnostic methods, including microscopic photography and spectroscopy. A microfluidic system is not the end of research; however, these tools are viewed as a starting point for research in the field of diagnosis [129-133].

Infectious diseases are diseases caused by pathogenic microorganisms, such as bacteria, viruses and parasites with rapid transmission and infection among human or animal carriers through insemination or transmission through air or water. There are three strategies for managing infectious diseases as follows: 1) Controlling the source of infection; 2) Interrupting transmission routes; and 3) Protecting susceptible individuals. Among these strategies, the control of the source of infection is the most important and it is recommended that it requires early diagnosis, early isolation, and early treatment of related patients and requires the development of rapid, sensitive, and accurate diagnostic methods and kits [133].

Standard methods for the clinical diagnosis of infectious diseases are based on the culture of the pathogen and the identification or detection of antigens, antibodies, or nucleic acids specific to infectious pathogens. Compared with time-consuming conventional diagnostic methods, POC detection has several advantages, such as faster diagnostic speed, better sensitivity and specificity, lower cost, higher efficiency and on-site detection capability. To achieve this, the development of POC detection methods and related devices is key and should be prioritized. The rapid development of microfluidic technologies, microelectromechanical systems, nanotechnology, and materials science has led to the production of a series of portable, small, low-cost, and integrated devices for POC diagnostics of various infectious diseases [129-133].

Detection in microfluidic systems

LOC methods and microfluidic systems can have various applications in a wide range of sciences, such as drug tests and screenings, toxicology studies, medical laboratory analyses, assays related to determining physiological and metabolic status, identification of cells, biomarkers, biomolecules, immunoassays, genome studies, and DNA diagnostic tests [134]. Studies have shown that chip-scale analyses are better than bench-top tests due to speed, portability, sensitivity, and the possibility of integrating reactions. Examples that can be included as reagents in this field include proteins, cells, nucleic acids, and small molecules, which we will briefly describe [135].

Protein

Proteins are used in clinical blood samples, such as serum, plasma, saliva, urine, etc. for clinical diagnosis and disease status monitoring and evaluation. POC technologies are based on microsystems for protein detection, including immunoassay tests or enzyme assays. Laboratory diagnostic tests that are available today for POC diagnoses include the detection of various infections using specific antibodies, such as viral infections (influenza, retrovirus, and AIDS), bacterial infections (streptococcus, chlamydia, and treponema) and parasitic infections. In addition, these systems are widely used in diagnosing non-communicable diseases, such as cancer by identifying protein markers [35, 134].

Nucleic acid

Among the molecular diagnosis methods, diagnostic technologies based on nucleic acids play a critical role in the correct and specific diagnosis of the pathogen with an acceptable level of specificity and sensitivity compared to other diagnostic methods that have been proposed so far [35].

The reason for the importance of diagnostic technologies based on nucleic acids as an important tool among other molecular diagnosis methods includes the following items: 1) All living organisms use nucleic acids as a genetic factor and each strain of the organism in terms of sequence and quantity nucleic acids are different from others; 2) Nucleic acid sequences, regardless of whether they are single-stranded ribonucleic acid (RNA) or double-stranded DNA, consist of four basic bases that can be easily identified and manipulated; 3) Today's technologies in the field of molecular methods have made it possible to synthesize large amounts of nucleic acids in a short period of time with a reasonable economic cost; 4) Manipulation, purification and labeling of nucleic acids is relatively simple, and therefore the detection of nucleic acids has become a relatively common method in the identification of diseases; 5) Various developments in nucleic acid sequencing methods have developed and improved diagnosis technology according to clinical needs [134].

Miniature nucleic acid amplification systems are the key to POC molecular diagnostics. Diagnostic tests based on isothermal amplification such as loop-mediated isothermal amplification (lamp) and non-isothermal amplification, such as PCR have been transmitted to microfluidic systems and chips to improve and develop commonly used diagnostic tests [135].

The miniaturization of nucleic acid amplification reactions enables these techniques to analyze the results in less time and at a lower cost, reducing the risk of sample contamination and often increasing the efficiency of these reactions compared to macro systems [135].

Since the appearance of microfluidic systems, this technology has attracted a lot of attention by performing conventional molecular detection tests in a short time. Compared to the macroscale, the structure of microfluidic systems gives unique characteristics to diagnostic reactions. Including shorter time for sample analysis, faster mass and heat transfer, reduction of sample volume, and integration and automation of reactions [136].

The reaction mixture transferred to microfluidic systems, experiences interactions of several physical effects that lead to the creation of new physical properties in them. As a result, by optimizing the physical effects, we can expect the reaction to occur in conditions different from the macro environment. Also, the opportunity to perform such techniques that do not exist with these features on a macro scale becomes possible [137].

Nucleic acid amplification in microfluidic systems

Because obtaining a large number of nucleic acids is necessary for detection, it is often more useful to generate multiple copies of a target nucleic acid molecule by amplification methods instead of time-consuming culturing. Conventional techniques often involve multiple time-consuming steps including multiple steps of sample lysis, lysis purification to extract nucleic acids from cell components, nucleic acid amplification and target detection, which make them unsuitable for practical applications in the clinic. Also, nucleic acid-based detection requires expensive tools and reagents, trained technicians, and a long time due to complex analytical methods. Therefore, it is necessary to develop fast, low-cost, and sensitive methods to detect very low concentrations of nucleic acid-based biomarkers in samples [35].

Sample preparation

The sample preparation process is usually done with special equipment in well-equipped laboratories. Sample preparation from clinical samples can be combined in a miniaturized microfluidic system. A series of sample treatment methods such as lysis, DNA or RNA extraction, and purification to obtain high-quality nucleic acid [135-138].

On-chip cell lysis

Cell lysis is first performed to extract nucleic acid from the samples. Cell lysis methods take advantage of physical and chemical properties to destroy cell membranes by physical and chemical methods, such as special reagents, temperature, electric field and mechanical pressure. In the chemical lysis method, which is widely performed in laboratories, the cell membranes are dissolved using special reagents [134]. For example, a microfluidic chip by sample lysis has been designed to detect RNA of influenza A, H_3N_2 [135]. Thermal lysis of cells is done by placing them at a temperature of 80°C or more, thus their membranes are torn and their nucleic acid is released [136-138]. Another method of cell lysis is electrical, in which the transmembrane potential of the cell is set to its critical value and causes the cell membrane to break and release nucleic acids [139]. Mechanical cell lysis is used to break cell membranes by mechanical force methods [140]. The mechanical method is a relatively cheaper technique than the chemical and thermal method because special chemicals and reagents are not used and the extracted nucleic acids are safe from thermal damage. Accordingly, porous polymers have been used in the construction of microfluidic chips that provide mechanical shear force for cell lysis. The size of the pores of the polymer material is usually smaller than 1 µm, which creates a mechanical shear force with a certain flow rate [141].

On-chip nucleic acid extraction and purification

After cell lysis, there are nucleic acid extraction and purification steps, for their isolation nucleic acid replication inhibitors, such as proteins, polysaccharides, and lipid molecules are required. There are two common methods of using magnetic beads and paper [139-141].

Magnetic beads

Magnetic beads are formed by coating a core of magnetic material such as Fe_3O_4 with an active group to bind nucleic acids. Magnetic bead-based separation can be combined with elution of the nucleic acid with temperature, pH, or salt concentration. Magnetic nanoparticles were developed through a uniform distribution of Fe_3O_4 with an average particle diameter of about 300 nm covered by 20 nm functionalized silicon substrate for RNA trapping for rapid nucleic acid extraction from clinical samples [142, 143].

Nucleic acid amplification

Non-isothermal cycling amplification

PCR as the first innovative technique of nucleic acid amplification was invented by Kary Mullis and became a gold standard technique for clinical diagnostic [144]. A PCR consists of temperature. This process can theoretically increase the number of original DNA copies and now is miniaturized on microfluidic systems for point-of-care diagnostics real-time PCR [145, 146].

Isothermal amplification

Isothermal nucleic acid amplification methods are performed at one temperature, usually between 37°C and 65°C and due to this, the isothermal amplification system has made it simpler than PCR [147, 148]. The most popular isothermal DNA amplification systems are LAMP, recombinase polymerase amplification and helicase-dependent amplification [147, 148].

Detection of amplicons

Nucleic acid amplicons can be characterized by real-time or endpoint detection. Endpoint detection requires less instrumentation and provides easier-to-interpret outputs. Real-time methods integrate the amplification step with detection and have a wider dynamic range for quantitative analyte detection. Alternative methods for the detection of nucleic acid amplification, such as optical, mechanical, chemical, electrochemical, and nanomaterial-based detection have been developed [149, 150].

Fluorescence-based amplification detection is the most popular optical detection strategy in biosensors and has the advantage of sensitivity and selectivity developed in a low-cost integrated unit [149]. The fluorescent substance calcein, when added to the reaction mixture, combines with magnesium ions and prevents its fluorescent effect. By performing the reaction and releasing pyrophosphate ions, these ions with magnesium react and separate magnesium from calcein and produce magnesium pyrophosphate. As a result, free calcein will be able to radiate [149-153].

Another method is to use cationic polyethylene imine polymer with low molecular weight added to the LAMP mix, which causes precipitation of insoluble complex with DNA at the end of the reaction [152] .

The use of dyes, such as hydroxy naphthol blue and phenol red, which multiply positively by color change appears. The test based on these colors has a great advan-

tage compared to other tests; that is, the dye is added to the mix before the reaction starts, and there is no risk of contamination by adding the dye [153].

A completely integrated diagnostic system based on the LAMP reaction on paper with a LFA layer was used for the detection of the dengue fever virus. Choi et al. immobilized gold nanoparticles then antigen probe conjugated on a nitrocellulose membrane on the LFA layer to capture the target amplicons. After amplification, the nanoparticle-probe conjugate constructs bind to the LAMP products and generate a visible red signal that requires 15 min at room temperature [154].

Applications of microfluidic systems

Early and rapid diagnosis of diseases is critical because it can greatly enhance the recovery rate and prevent the spread of diseases. However, in many areas with inadequate medical facilities, timely diagnosis of diseases is challenging. Conventional medical diagnostic methods require high technology-based equipment and expert operators, which limits the utility of these tests. Along with the continuous development of technology, microfluidic systems have shown great potential to advance biomedical research that was previously unimaginable using conventional techniques. For point-of-care applications, these systems can quickly detect diseases at low cost. In this section, we will review the position and application of microfluidic systems in the diagnosis of human health, quality control of food products, and environmental assessments [155, 156].

Human health diagnosis

Infectious diseases are caused by pathogenic microorganisms and they can be transferred between people, thus threatening public health and potentially the economy. Suitable diagnostic tools are needed to provide sensitive, accurate, easy-to-use, and timely guidance to identify cases of the disease, and transmission disorders and prescribe appropriate treatment [155]. POC assays provide practical results close to the patient, thereby acting as a personal radar. In this section, we review the clinical needs of POC for several major pathogens including malaria parasites, human immunodeficiency viruses, human papillomavirus, *mycobacterium tuberculosis*, and SARS-CoV-2 [155, 156].

Compared to non-infectious diseases, infectious diseases can be transmitted exponentially among populations in a relatively short period. It is estimated that infectious diseases are the main cause of death and prevalence in developing countries and account for more than half of infant deaths [154]. Due to inadequate healthcare infrastructure and cost constraints, >95% of deaths from infectious diseases are due to a lack of proper diagnosis and treatment [155]. Many infectious diseases, including those mentioned above, are a great threat to human life and cause more than half of the deaths worldwide [156].

Malaria parasites

World malaria report 2020 states that there were 241 million cases of malaria in the world in 2020 and 627000 deaths. The incidence of malaria cases decreased from 81 in 2000 to 59 in 2015 and 56 in 2019, before rising again to 59 in 2020. In 2020, malaria deaths have increased by 12% compared to 2019. The increase in malaria cases was due to the disruption in service delivery during the COVID-19 pandemic [157]. Plasmodium parasites produce relatively species-specific proteins. This has been used as an advantage by a wide range of diagnostic devices via immune-chromatic-based assays known as rapid diagnostic tests for malaria. The functional principle of rapid diagnostic tests for malaria is based on the absorption of parasite-produced proteins by complexing them with antibodies that are designed in a line on a nitrocellulose strip so that a drop of blood is washed by several drops of buffer solution. The buffer solution contains a labeled antibody to produce a visually detectable reaction and provides a second line of control [158].

Human immunodeficiency virus

According to the announcement by the WHO, there is a requisite need for POC device detection and monitoring of HIV/AIDS in resource-limited settings. Early detection of HIV infection has prevented its unknowing transmission, which emphasizes the importance of early detection of HIV. POC devices for HIV detection should be accurate, cheap, straightforward, and simple to use for the detection of infection and quantification of $CD4^+$ T lymphocytes and HIV burden in resource-limited settings [159, 160]. For on-site application, Yang et al. designed a lateral flow strip for HIV assay in urine samples. A microfluidic immunoassay system was also developed. Based on the lateral flow strip with colloidal gold indicator, this system can perform sample loading, measurement, discharge, run, and detection in an integrated manner, which simplifies HIV testing. This system shows the result within a few seconds [161].

There are two main approaches to monitoring antiretroviral therapy effectiveness and HIV/AIDS progres-

sion: 1) The number of $CD4^+$ T lymphocytes, which is an indicator of the patient's immune system status after HIV infection; 2) Measurement of viral load as another indicator of virus amplification in blood plasma [162, 163]. Based on this, Shafiee et al. presented HIV detection technologies based on microfluidic systems based on $CD4^+$ T lymphocyte count, viral load measurement, and drug resistance assay [164].

Meanwhile, to find new ways for on-site infectious disease diagnosis and monitoring applications, Kim et al. used a new and effective method to accurately identify HIV viral particles in a patient whole blood without preprocessing. In this method, a sample of 10 µL of whole blood was taken from the finger prick and analyzed in a microfluidic system on which the anti-gp120 antibody was immobilized. In this system, by using two sets or two colors of quantum dots (Qdot525 and Qdot655 bound with streptavidin), viral particles are captured and identified by simultaneous labeling of envelope glycoprotein gp120 and Mannose-rich glycans [165].

Human papillomavirus

HPV viral DNA testing or Pap smear method followed by colposcopy and biopsy is currently the gold standard for cervical cancer screening in the world [166]. In a large randomized trial in India, providing one-step HPV screening based on DNA testing in women over 30 years of age significantly reduced the incidence of advanced cervical cancer and mortality by 50% [167]. However, these tests require expensive laboratory equipment and a reliable recall system, which makes them not applicable in resource-limited settings such as developing countries [168]. A total of 85% of the global burden of cervical cancer is related to developing countries [169]. Therefore, to improve the cervical cancer prevention status in developing countries simple, cost-effective, and in-situ methods for HPV with high sensitivity and high specificity are needed.

Karakaya et al. developed a paper-based microfluidic system with early detection of cervical cancer by measuring HPV 16 and HPV 18 identification. This extraction and purification system is developed based on paper, non-enzymatic amplification that is followed by electro analytical system to detect of HPV virus. In this study, a specific method of DNA modification with the aim of non-enzymatic amplification using magnetic beads and silver nanoparticles labeled with primers has been used. The system is capable of extracting more than 10-100 copies per mL of DNA in approximately 15 min with a single-step extraction process detected low concentrations in less than 10 min with high selectivity for HPV 16 and HPV 18 types from patient samples [170]. In addition, the nucleic acid sequence-based amplification (NASBA) method on a chip was developed by Gulliksen et al. In this way, it became possible to detect 0.1 mmol of human papillomavirus in a volume as small as 10 nL in glass-silicon microcapsules at 41°C [171].

M. tuberculosis

Liong et al. used the magnetic-based barcoding technology to *M. tuberculosis* DNA detection in 2.5 h. This research team described an integrated microfluidic system in which *M. tuberculosis* DNA is first exogenously extracted and then immobilized with complementary DNA on polymer beads. Then, the beads coupled with DNA probe conjugated magnetic nanoparticles, which paramagnetize the beads loaded with magnetic nanoparticles. Because beads loaded with paramagnetic nanoparticles can generate localized magnetic fields, beads loaded with different amounts of *M. tuberculosis* DNA produce different decay rates during nuclear magnetic resonance readings. This event provides the possibility of diagnosis [172].

A portable integrated microfluidic system capable of LAMP nucleic acid amplification was designed by Fang et al. to detect tuberculosis within 1 h. This chip, with sample-to-response capability, can perform DNA isolation, amplification, and reading results with the naked eye in single or multiple formats. This system was named iµLAMP and it was successfully used for the in-situ identification of pathogens [173].

Severe acute respiratory syndrome coronavirus 2

According to Natsuhara et al. a microfluidic system was introduced that is capable of sequentially dispensing samples into microchambers simultaneously, which provides a fast and easy response platform for the genetic diagnosis of several viral infectious diseases. In this study, the LAMP method was used to amplify and identify the specific target factor of DNA or RNA nucleic acid. Based on this, simultaneous detection of COVID-19 and other infectious diseases, such as seasonal influenza A and the 2009 pandemic influenza A, was detected within 30 min by color-based quantitative analysis with the naked eye [174].

Ganguli et al. presented a real-time LAMP reaction-based assay for the detection of the SARS-CoV-2 virus with comparable performance to validated assays using real-time PCR. This method has a detection limit of 50

RNA copies per µL of viral transport medium solution in 20 min and a detection limit of 5000 RNA copies per mL of artificial nasal mucus solution. Virus detection in less than 40 min using a cartridge and a smartphone-based reader demonstrates the applicability of this system for real-time testing [175].

Detecting antimicrobial drug resistance

The spread of new forms of drug-resistant microbes great importance a global concern, such as tuberculosis and COVID-19 [176, 177] and the current molecular techniques are complex, non-portable, and expensive and require facilities, trained personnel, and laboratories to perform are advanced. Therefore, the early diagnosis of such diseases is rapidly evolving and necessitates the need for cheap, fast, simple, portable, and accessible point-of-care diagnostics. The combination of microfluidic systems with propagation methods has been effective in realizing biosensor systems for antibiotic resistance detection. Lutz et al. developed a microfluidic system to *mec*A antibiotic resistance gene detection in *staphylococcus aureus* to detect less than one copy of this gene in less than 20 min [178].

Drug abuse and toxic compounds

The use of portable POC systems to identify drugs, such as cocaine is of great importance in different countries. Considering that a higher amount of cocaine concentration can be detected in saliva compared to intravenous injection [179], electrochemical sensors can be used by integrating into microfluidic systems. As an example, an aptasensor made of label-free nanocavity was developed for detecting cocaine with high sensitivity in human saliva and serum samples [180]. Also, a microfluidic chip equipped with an aptamer-based electrochemical sensor was made in blood serum with the aim of continuous and real-time monitoring of cocaine [181].

Whole-cell and virus detection

A sorting and phenotyping microfluidic-based platform was developed by Tay et al. for neutrophils via chemotaxis and the formation of neutrophil extracellular traps that could be used for low volumes of blood [182]. Thus, they directly purified on-chip neutrophils using cell migration biomimetic and affinity-based adsorption from blood. Then, the cells were exposed to preloaded chemotaxis and neutrophil extracellular trap stimulator. Furthermore, a microfluidic platform using integrated rod-shape gold nanoparticles was investigated for avian influenza virus detection it could detect viruses within 1.5 h and it provided a smaller limit of detection than the common method [183].

Microfluidic models for functional diagnostics in oncology

In the last decade, microfluidic systems became an important model for cancer investigation [184] and has shown the design of new methods of diagnosis, monitoring, treatment, and follow-up of the disease [185]. This emerging technology can detect cancer biomarkers more sensitively and accurately than classical platforms [186, 187]. Microfluidic systems have been used to monitor the migration of single cancer cells in the early stage of migration and collect kinetic information [188]. Microfluidic systems by simulating blood vessels have shown their effectiveness in examining tumor cells and other areas of cancer research. Despite progress in cancer treatment, tumor cell metastasis is responsible for more than 90% of cancer patients' deaths [189]. This technology can investigate behaviors, such as arrest and extravasation cells from the metastatic tumor site. Cancer modeling based on a microfluidic system or tumor-on-a-chip model has been introduced as an intermediate and complementary technology between animal models and 2D models [190–192].

The development and performance improvement of micro-RNA sensor systems has been the focus of researchers in recent years. Meanwhile, microfluidic technology has been combined with various measurement systems. As such, these systems have been used to achieve a high-performance atomic-level multiple micro-RNA assay platform [193]. Due to the disadvantages, such as the lack of separation and counting of cells in the monitoring of circulating tumor cells, microfluidic systems were developed for this purpose. A system that has multiple arrays and detection based on the size of tumor cells, a microfluidic platform for capturing prostate cancer cells was introduced [194].

Microfluidic devices for disease modeling

Microfluidic technologies have provided new opportunities, especially in the field of neuroscience, where the ability to organize cells to mimic brain structures in vitro has become possible. Microfluidic systems have provided the possibility of cell-scale simulation, regulation of function, and description of cell biology from a single cell to the organ-on-a-chip model. An organ-on-a-chip system on a chip is a device that simulates the physiological environment and function of human organs; it has been used to predict drug responses and effects on

organs. Accordingly, these systems have been widely used to mimic certain organs inside the body. The use of organ-on-a-chip technology in the pre-clinical stages of drug production has shown great potential. Drug development is a time-consuming, costly process with a low approval rate. Currently, animal models are not good predictors for studying how drugs react in humans [195]. Cytosensor microphysiometer was used as a foundation for the development of organs on a chip, to study the function and biochemical parameters of the cell [196], and also as a tool to study toxicology and pharmacology in biosensors [197].

Food safety control

The outbreak of food-related diseases has a significant effect on the economy. According to 2018 statistics, 1.5 million cases of foodborne illness in the US resulted in an economic burden of more than $17.6 billion [198]. It is necessary to develop advanced technologies in the process of preparing healthy food that meets the needs of the world population. Among these new technologies, there have been many researches related to microfluidic technology [198].

Among the applications of microfluidic systems in the food industry, its use has been to provide free chlorine with less chlorine consumption and less exposure time to reduce the population of *Escherichia coli* O157: H7 [199]. Also, Zhang et al. used microfluidic chips to extract strychnine as a bioactive compound from plant products [200]. Diagnosis of foodborne pathogens is based on the culture of bacteria on agar plates, which is a time-consuming method. However microfluidic systems allow cheap and efficient detection of small amounts of chemicals, antibiotics, pathogens, and toxins in food. Custom-designed microfluidic devices measure food safety indicators within minutes. Therefore, it is possible to control food safety from farm to table [198-200].

Pathogenic agents in food have been detected by measuring and quantifying the DNA of pathogenic cells [201]. In a study, a microfluidic system with an array of gold microelectrodes integrated with antibody-conjugated magnetic nanoparticles was developed to detect *E.coli* in beef. This system detected 1.2×10^3 cells of *E. coli* O157:H7 in beef samples at 35 min [202].

In another system, the three steps of DNA extraction, isothermal amplification, and *Salmonella* detection were integrated. All steps are fully automated within 30 min, while conventional methods take 3 to 5 days using many steps to complete the *Salmonella* detection test [203]. Also, systems were introduced for the detection of other food pathogens, such as *Listeria monocytogenes* [204] and norovirus [205]. Microfluidic devices have been used in the detection of toxic chemicals in food, such as contaminants, plant pesticides, and food additives. Zarghampour et al. detected biogenic amines as quality indicators of fishery products by combining electrochemical enzyme sensor arrays in microfluidic device [206].

Rapid identification of *Salmonella typhimurium* in food products is of great importance. For this purpose, Kim et al. designed a microfluidic nano-biosensor that is used for rapid detection of pathogenic *salmonella*. The use of quantum dots activated with anti-*salmonella* polyclonal antibodies was the basis of construction to identify this bacterium. Cells were isolated and concentrated using super paramagnetic particles and a microfluidic chip. In this invention, a portable fluorometer was designed to measure the fluorescence signal of quantum dot nanoparticles attached to *Salmonella*, and with this biosensor, the detection limit of 10^3 *Salmonella* colony-forming unit (CFU)/mL in food extract was achieved [207].

In another research, Zhu et al. invented a microfluidic system integrated with graphene oxide, which was based on fluorescent aptamers. The basis of their work was that graphene oxide exhibits reversible fluorescence quenching properties when it is absorbed or repelled by fluorescence. This fluorescence sensor can also be used to identify multiple pathogens; it can simultaneously detect *S. aureus* and *Salmonella enterica* with a detection limit of 0.11 CFU/mL, and therefore has a significant potential to widely detecting other bacterial and viral pathogens in food [208].

Since food contamination with heavy metals causes damage to nerve tissues and kidneys and is harmful to human health, in another study, graphene oxide attached with DNA and fluorescent material was developed as a fluorescent sensor in a μPAD. Found. Chemical pollutants, such as heavy metals Hg^{2+}, Ag^{+} and aminoglycoside antibiotic residues in food were detected simultaneously with this sensor; therefore, this low-cost and simple method showed an effective application in food safety control [209].

Considering that aflatoxins have serious toxic and carcinogenic effects on human health, their common diagnostic methods, such as immunoassay and high-performance liquid chromatography are costly and time-consuming. Therefore, Hu et al. developed a sensor that

operated on an integrated microfluidic chip for the detection of aflatoxin in corn samples based on a smectite-polyacrylamide nanocomposite and with fluorometric quantification capability with a detection limit of less than 100 parts per billion [210].

Challenge of microfluidic

One of the most important challenges of this technology can be mentioned in the design of the devices, the mass production of the device, and the cost of the tests. Therefore, microfluidic devices are produced from PDMS by a soft lithography method. This material has many positive features [71, 211]. Despite this, it is time-consuming to process [71]. It is also difficult to seal [212] and swells in the presence of non-polar organic solvents, which limits the chemicals with which it can be used [213]. As a result, it extracts hydrophobic molecules from the streams in contact with it and makes it difficult to maintain its surface [71, 213, 214]. Therefore, cost-effective materials and processes are an important challenge in the mass production of microfluidic devices. Using microfluidic devices is dependent on external equipment, such as a fluid flow pump or power supply. Microfluidic chips are not programmable and their design is practical. Progress in making microfluidic chips programmable has not been significant due to reliability, generalizability, and cost [215, 216]. Another important challenge is to integrate separate microfluidic chips with different and incompatible specifications into a single system [217, 218].

Conclusion

Today, the priority of POC diagnosis development is to build smart devices with mobile healthcare capabilities, which can revolutionize the level of personal healthcare monitoring, as the next generation of POC treatment. A wide range of smart-phone healthcare technologies are currently being developed, the most promising of them are smartphone-based POC technologies for readout of colorimetric or light emission based reactions, such as fluorescent, chemiluminescent, or electrochemical assay and lateral. The widespread availability of smartphones has provided an important opportunity for the integration of POC treatment. The storage and communication capabilities as well as the smartphone camera can be used for optical-based assay. Integrating POC treatment with these platforms and placing the data on a cloud-based server has made the concept of telemedicine accessible. Several smartphone-based systems and related applications have been commercialized to monitor and manage health parameters such as blood sugar, blood pressure, weight, pulse rate, electrocardiogram, and physical activity. The integration of POC treatment data into electronic health records and the use of artificial intelligence and data analysis for data mining and achieving rapid and accurate diagnosis have become more important. Some care devices provide the possibility of medical self-testing at home. In this regard, there are some investigatins about several easy-to-use diagnostic devices connected to the network. In this study, the integration of cloud systems and the analysis of results through online medical platforms have been investigated.

In addition, the technology of microfluidic systems is cheap, portable, and sensitive in diagnosis. Therefore, this technology can enable on-site medical diagnosis requirements. These devices equipped with diagnostic technologies such as real-time LAMP, surface plasmon resonance (SPR), etc. and integrated with a smartphone, have been successfully used in the detection of pathogens, examples of which were mentioned. Researchers are always looking for the development of small biosensors for POC diagnostics using simple diagnostic methods based on portable devices or microfluidic systems. In addition to high sensitivity and specificity, a suitable miniaturized biosensor should be capable of integration, automation, and multiplex detection for use in areas without trained personnel. Microfluidic technology will continue to be integrated with innovative applications by combining it with emerging sciences, such as artificial intelligence and metamaterials. Therefore, the microfluidic market will grow by 11.7% between 2019 and 2024 and is developing with various products in this field. The number of leading companies in this field exceeds 1000 companies worldwide in 2019. Since most of the protein biomarkers of the human body are present in blood, urine, or other fluids, they can be detected in these fluids. The preparation of urine and saliva is non-invasive and it is easy to obtain these samples. After these samples have been used for rapid POC detection methods. Saliva is a sample that has made non-invasive diagnosis of oral diseases and systemic diseases possible and therefore it is possible to replace blood. This sample has enzyme, electrolyte, protein compounds, nucleic acids, antibacterial compounds, and hormonal, cytokine, and antibody biomarkers. Saliva determines the state of health and diseases of the human body and the analysis of the physical state of the human body will show in medical research in the future. The economic value of these diagnostic products was about 23.6 billion dollars in 2016 and about 43.2 billion dollars in 2022 and it is expected to be 0.72 billion dollars for virus detection in 2027. COVID-19 and the flu virus reach all over the world. Improving access to POC devices through online platforms is a key

factor that will drive the growth of this market in the long term. Based on platform type and product type, LFA systems and blood glucose monitoring products accounted for the largest share of the global POC market in 2021, respectively.

Ethical Considerations

Compliance with ethical guidelines

There were no ethical considerations to be considered in this research.

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Authors contribution's

Conception, study design, data analysis and interpretation: Adele Rafati and Pooria Gill; Data collection and writing the original draft: Seyed Mahmoud Mahdi Zadeh Diva; Review editing and final approval: All authors.

Conflict of interest

The authors declared no conflict of interest.

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