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Systematic Review on the Role of Microfluidic Platforms in Advancing Scalable and Precise Microbial Bioprocessing

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ABSTRACT

Microbial bioprocessing is a key technology for the production of a wide range of biomolecules, including proteins, enzymes, antibiotics, and other bioactive compounds. In recent years, there has been an increasing interest in using microfluidic platforms for bioprocessing, due to the ability to precisely control and manipulate fluids at the microscale. Microfluidics offers a transformative platform for the manufacturing of biomolecules intended for clinical applications by addressing key technical challenges in scalability, precision, reproducibility, and the ability to study complex biological systems. In this review, various methods used to fabricate microfluidic platforms and the current state-of-the-art in the synthesis/production of biopharmaceuticals, polymers, bioactive compounds, and real-time monitoring in microscale bioprocesses are discussed. Additionally, the future trends and directions are highlighted. Overall, we envisage the utilization of microfluidic platforms to advance the field of microbial bioprocessing and applications in the biomedical field.

1 | Introduction

The synthesis of new biopharmaceutical compounds by recombinant organisms has been rapidly growing in recent years, leading to the development of an entirely new class of medications for a variety of conditions, which has been previously ineffective [1]. This growth has resulted in capacity constraints in the purification of manufacturing platforms, which necessitates eliciting less expensive and rapid ways to create and test new downstream processes [2]. In response to this need, researchers have turned to a promising technology so-called "microfluidic system," which advanced the development of new bioprocessing techniques. These systems include closed microfluidic channels, chambers, and components for a variety of small-scale laboratory methods and analyses [1, 3].

Microfluidic bioprocessing involves the use of microfluidic platforms for the cultivation of microorganisms and the production of biomolecules through fermentation, which offer several advantages over traditional fermentation systems, such as the ability to precisely control and manipulate small volumes of fluid [4], minimize material and energy consumption, enhance mass and heat transfer, integration of sensors and other analytical technologies, precise sampling processes, and the ability to perform

Abbreviations: 3D, three-dimensional; AMPs, antimicrobial peptides; DO, dissolved oxygen; FDM, fused deposition modeling; ISFET, ion-sensitive field-effect transistor; LAPS, light addressable potentiometric sensor; MEMS, microelectrochemical systems; PDMS, polydimethylsiloxane; PHAs, polyhydroxyalkanoates; PMMA, polymethylmethacrylate.

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parallel processing and high-throughput screening [2, 5–7]. These features make microfluidic bioprocessing an attractive tool for the production of high-value biomolecules, such as enzymes, proteins, polymers, and polysaccharides [1, 8], that have been utilized in a variety of biomedical applications, including drug delivery [9, 10], tissue engineering [11], and wound healing [12].

Furthermore, these systems have the potential to be applied in a wide range of fields, including biotechnology, bioimaging, electronics, energy, textiles, and gene delivery, by attempting to overcome the difficulties or challenges in traditional analyses [13, 14]. Microfluidic platforms provide diverse cell cultures and biomimetic in vivo like environments [15], which helped the field to evolve, leading to the introduction of organ-on-a-chip (OoC) systems. Notably, OoCs play crucial roles in understanding human physiology [16, 17] and disease [18, 19], accelerating drug discovery processes.

This review aims to provide an overview of the current state-of-the-art in microbial bioprocessing in microscale for biomedical applications, focusing on recent developments and key trends in the field including the use of different microorganisms and the diversity of biomolecules produced. Furthermore, this review provides the readers with an overview of the fabrication methods of microfluidic platforms embedded with sensors for real-time monitoring. As far as our literature search could ascertain, this is among the first reports combining the key aspects of the most current advancements in microfluidic bioprocessing for biomedical applications.

2 | Fabrication of Microfluidic Platforms for Bioprocessing

The properties of materials and methods used in the fabrication of microfluidic platforms affect the cost, performance, and function of these platforms [20, 21]. Surface properties, biocompatibility, durability, electrical and thermal conductivity, simplicity of fabrication, and capacity to fulfill reaction-specific temperature and pressure requirements should all be taken into account when choosing the material for the fabrication of microfluidic platforms. Glass, silicone, polymers, metals, ceramics, and papers are the most often utilized materials [5, 22]. Silicone is the first material utilized in the manufacturing of microfluidic devices. However, due to their superior properties such as mechanical flexibility, thermal stability, and biocompatibility, polymeric materials are used more frequently [23]. The polydimethylsiloxane (PDMS) polymer is mostly used due to its optical transparency, biocompatibility, and ease of manufacture. Additionally, PDMS regulates the cell's physicochemical environment through adjusting flow conditions. However, the molecular absorption of PDMS may influence the cellular response [24–26]. Another common polymer is polymethylmethacrylate (PMMA). PMMA is an amorphous thermoplastic that does not absorb small molecules and is more solvent compatible than PDMS. Hydrogels are used because they are biocompatible, allow diffusion, have low cytotoxicity, and support cell adhesion due to their properties similar to the extracellular matrix. Besides these, glass is a type of amorphous solid silicon material and is used in the fabrication of microfluidic devices because of its properties such as being chemically inert, transparent, and insulating. Aluminum, copper, and iron are used due to their cost, simplicity, and the ability to withstand high heat and pressure [27, 28]. The fabrication method should comply with the characteristics of the material and the cost should also be taken into consideration.

Microfluidic platforms for bioprocessing are typically fabricated using a combination of microfabrication techniques, including photolithography [29], microelectromechanical systems (MEMS) [30], and laser micromachining [31]. One common method for fabricating microfluidic platforms is through the use of photolithography, which involves the use of light-sensitive materials and masks to define patterns on a substrate (Figure 1A). These patterns are then transferred to the substrate through a series of chemical etching and deposition steps [32–34], which allows the creation of precise and complex microfluidic channels and chambers on the substrate [35].

Another method for fabricating microfluidic platforms is through MEMS [30], which involves the use of microfabrication techniques to create mechanical and electromechanical devices at the microscale (Figure 1B) [41]. Soft lithography (Figure 1C) involves the use of elastomeric stamps or molds to transfer patterns onto a substrate, which can be utilized to form microfluidic channels and chambers with a wide range of shapes and sizes [42]. Laser micromachining (Figure 1D) utilizes a focused laser beam to remove the excess material from a substrate, allowing the creation of precise and complex microfluidic structures [43]. Additionally, three-dimensional (3D) printing (Figure 1E) focuses on the use of additive manufacturing technique to fabricate (3D) structures with complex and customized geometries [34, 42, 44]. A wide range of methods can be used to fabricate microfluidic platforms for bioprocessing, each with advantages and limitations (Table 1).

Parameters such as surface roughness, aspect ratio, and typical working size of the platform that can be obtained vary depending on the fabrication method. Therefore, desired features and capabilities for a specific application can be achieved by using a combination of these methods [41].

Apart from fabrication, sterilization methods play a crucial role in ensuring the safety and integrity of microfluidic devices. While short-wavelength UV light has been widely used for sterilizing polydimethylsiloxane (PDMS) microfluidic devices, its effectiveness on Fused Deposition Modeling (FDM) polymers is limited. Unlike PDMS, FDM polymers are not as transparent to germicidal UV-C light, making UV sterilization less suitable for these polymers. Some of the FDM polymers such as Nylon PA 12 offer compatibility with autoclave sterilization [46]. However, majority of the FDM polymers and poly (methyl methacrylate) (PMMA) are incompatible with autoclave sterilization due to low glass transition temperatures, which fall below the standard autoclave temperature of 121°C. To address the sterilization needs of these kind of materials, alternative techniques have been explored, including sterilization with ethylene oxide, hydrogen peroxide, immersion in ethanol, and gamma irradiation [47].

3 | Real-Time Monitoring of Microfluidic Platforms for Bioprocessing

Understanding the bioprocesses in-depth to facilitate their successful and quick development at an early stage is a vital step for

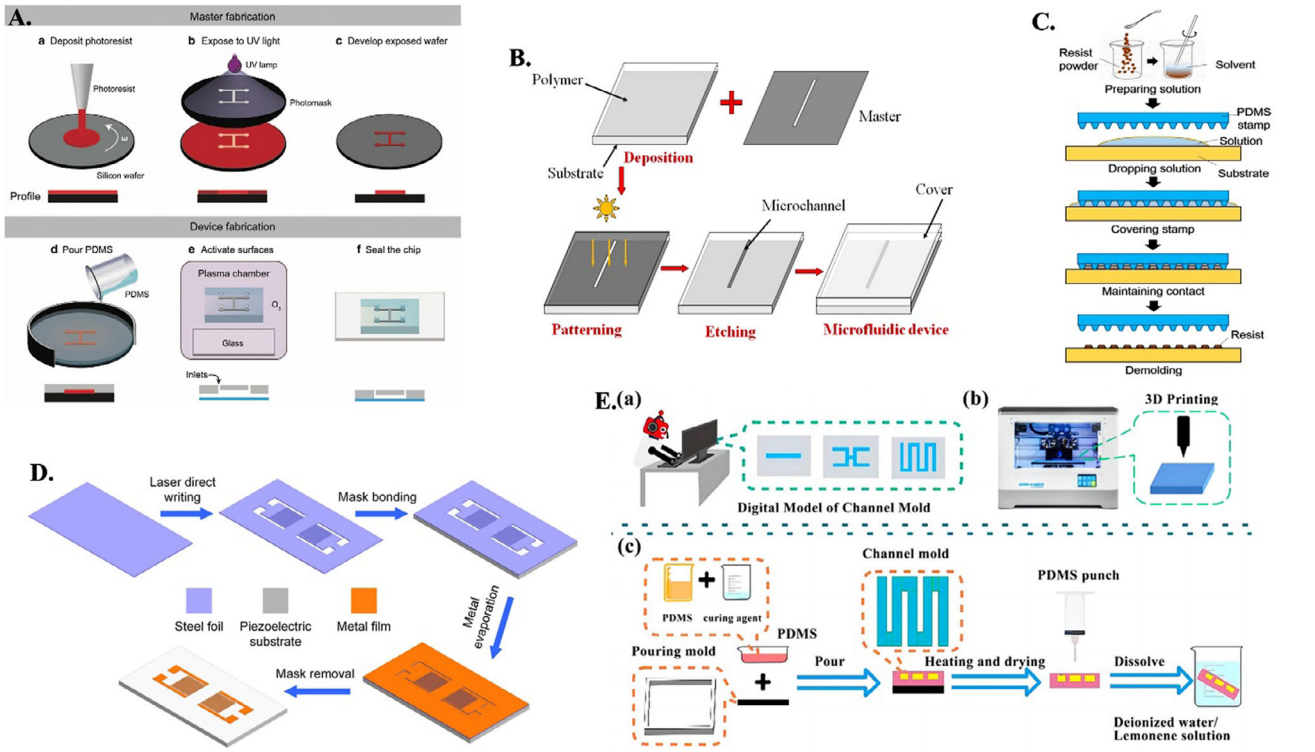

FIGURE 1 | Fabrication methods in microfluidic platforms for bioprocessing. (A) Photolithography involves the use of light to transfer a pattern onto a photosensitive material, which is then developed to create the desired structure by Aleklett et al., open access publication under CC-BY license [36]. (B) Microelectrochemical systems (MEMS) involve patterning a photosensitive material using light to create features on a substrate by Zhang et al., open access publication under CC-BY license [37]. (C) Soft lithography involves using a soft, elastomeric mold to transfer a pattern onto a substrate material. The mold is typically made by micro-molding in capillaries. Reproduced under the terms of the Creative Commons Attribution 4.0 International License [38]. (D) Laser micromachining involves using a focused laser beam to etch patterns into a substrate material by Wang and Qian, open access publication under CC-BY license [39]. (E) 3D printing involves the use of a 3D printer to fabricate a microfluidic platform by depositing layers of material (such as polymer or hydrogel) on top of each other by Wu et al., open access publication under CC-BY license [40].

the bioprocessing industries. This needs to be realized with the least amount of work and expense to gather a large amount of data that is pertinent to the production scale. Although it is possible to collect massive amounts of data regarding critical process parameters such as pH, temperature, cell density, and dissolved oxygen at the production scale, the accompanying costs of process development may be unaffordable [48]. Microfluidic platforms can be used as a scaled-down model to gain an understanding of microbial or mammalian cells function at the molecular or genetic level and allow continuous measurement and control of key process parameters by integration of sensors. For a variety of bioprocesses, microfluidic systems must demonstrate their ability to faithfully mimic large-scale growing conditions as well as the physiological metabolism and production potential of the microorganism. By lowering the amount of scaling-up and scaling-down iteration loops, this could result in a shorter time needed for strain selection and medium optimization.

Process analytical technologies (PAT) such as electrochemical and optical sensors are routinely used for real-time monitoring of critical process parameters and product quality attributes during bioprocessing [49, 50]. With real-time measurement, PAT tools provide quick process control to integrate desired product qualities into the finished product. A deeper understanding of the product and process, along with the intricate relationships between materials, process variables, environmental factors, and their impact on product quality, is crucial considering the focus on incorporating quality into the product [51]. Indeed, the miniaturization of microfluidic platforms with integrated sensors offers the potential to improve the precision and speed of bioprocess control [52, 53]. There has been a growing body of research in recent years on the use of microfluidic platforms for real-time monitoring of bioprocesses (Table 2).

Preetam et al. examined the emergence of microfluidics for next-generation biomedical devices and highlighted the potential of these platforms for real-time monitoring of fermentation processes [34]. Sensors can be manufactured directly as part of the microfluidic design or integrated as part of the overall device and detailed reviews of optical chemical sensors for temperature, pH, dissolved oxygen (DO), and carbon dioxide have been previously presented [54, 59].

3.1 | Optical Sensors

Optical sensors based on fluorescence or scattering are well characterized and widely used in macroscale and microscale applications such as chemical biology, industrial biotechnology, or microbial bioprocesses. Optical sensors are non-invasive,

TABLE 1 | A summary of various techniques used for the fabrication of microfluidic platforms for bioprocessing.

Technique	Materials	Advantages	Disadvantages	Limitations	Refs
Photolithography	Photoresist, silicon	High resolution (< 100 μm), mass production	Complex process, high cost, require cleanroom	Surface must be flat, not suitable for 3D	[34]
Microelectromechanical systems (MEMS)	Silicon, glass	Highly integrated, high resolution (< 1 μm), low power consumption	Complex process, high cost, small scale	Limited by the properties of the material	[30]
Soft lithography	Polymers	Low cost, simple process, biocompatible	Low resolution (> 100 μm), not suitable for high temperature	Surface must be smooth and featureless	[42]
Laser micromachining	Various	High precision, can be used on most materials	High cost, require skilled operator	Limited by the absorption of the material	[45]
3D printing	Various	Versatile, rapid prototyping	Low resolution (> 100 μm), limited materials	Materials properties can be different than bulk materials	[42, 44]

TABLE 2 | Techniques and sensors used in real-time monitoring of bioprocessing at microscale.

Technique	Advantages	Disadvantages	Limitations	Refs
Optical sensors (e.g., spectrophotometry)	Non-invasive, highly sensitive, can provide real-time data	Requires calibration, can be affected by interference from other components in the system, photobleaching of dye	Limited by the absorption/emission properties of the target analyte, an expensive read-out system	[54, 55]
Electrochemical sensors	High sensitivity, easy to integrate in miniaturized format, and low read-out cost	Can be affected by interference from other ionic species in the system, requires frequent calibration	Limited by the electroactivity of the target analyte	[55]
Microfabricated sensors	High resolution, small size easy to integrate with microfluidic platforms	Can be affected by fouling and requires specialized fabrication techniques	Limited by the mechanical or electronic properties of the sensor material	[56]
Biomarker-based biosensors	High specificity can provide information on cellular metabolism	Requires purified biomarkers, which can be affected by interference from other biomolecules in the system	Limited by the availability and stability of the biomarkers	[57, 58]

highly sensitive, low cost, and can be easily miniaturized [60], which makes them an excellent choice for single-use bioprocessing microfluidic devices. Generally, materials used for sensor fabrication should not affect the growth, productivity, and viability of microbial culture. Additionally, incorporated sensors are required to be sterilized without compromising sensor performance and can work consistently in various medium conditions [61]. Cell biomass is an indicator for determining the effect of culture conditions on growth rate, consumption or production of metabolite, and specific productivity of proteins during microbial bioprocessing. Optical density-based sensors for monitoring microbial biomass can involve light from a light-emitting diode (LED) being guided through the microfluidic bioreactor via optical fibers and measured at a photodetector. Zainal Alam et al. developed a low-cost and small-footprint optical density sensor consisting of a microcontroller and wireless data transfer using a smartphone to monitor *Saccharomyces cerevisiae* in a PMMA microfluidic chip in the range of 0.25–15 g/L [62]. Soares et al. developed an optical fiber-based quasi-elastic light scattering sensor for monitoring cellular growth kinetic parameters for *S. cerevisiae* during perfusion cultivation. However, this method is not suitable for larger diameter cells such as mammalian cells due to the saturation of signal at even low concentrations and can be susceptible to misalignment, which can cause loss of transmitted light [63]. Kitahara et al. designed a microfluidic device, which was connected to Jar microbial fermenter with the acquisition of high-definition images of yeast cells for monitoring morphological changes during ethanol production

[64]. Separately, machine learning techniques have been used for the analysis of real-time monitoring data for optimization of process conditions and prediction of deviations [64, 65]. Real-time monitoring in microfluidic platforms has been shown to be effective in optimizing fermentation processes and improving product yield and quality [66].

Oxygen and pH are critical process parameters of any aerobic microbial fermentation since they can influence cell growth and its behavior, yield, and viability. Within a microfluidic bioreactor, optical chemical sensors based on fluorescence quenching or luminescence are typically used to measure dissolved oxygen. Generally, an optical oxygen sensor consists of a chemical dye mainly based on ruthenium or metalloporphyrin fixed with a polymer matrix, and the sensitivity of the sensor is determined by the permeability of polymer matrix and fluorescent properties (lifetime or intensity) of the dye. Monitoring pH of microbial fermentations is as valuable as oxygen since it can affect enzyme activity, productivity, and cell growth. Generally, pH is measured by optical sensors based on the absorbance or fluorescence of pH-sensitive dye that is immobilized as a thin layer on polymer [67]. Hanson et al. compared the average difference in pH readings between the optical and electrochemical pH sensors in two cell media was only 0.04 pH units [68]. Melnikov et al. compared an optical oxygen sensor based on a porphyrin metal complex and an amperometric Clark electrode within a microfluidic device for yeast culture and showed better results with the optical method [55]. Funfak et al. used 8-hydroxypyrene-1,3,6-trisulfonic acid trisodium salt (HTPS) dye-doped polymer microparticles for optical pH monitoring during cell cultivation by using LED-based miniaturized flow through fluorometer [69]. A miniaturized pH holographic sensor with 3 nL working volume was presented by Chan et al. to measure the pH during microbial growth of *Lactobacillus casei* (*L. casei*) in a glass-PDMS microfluidic chip (Figure 2A) [70]. The sensor measures a pH change via a blue shift in a resultant holographic replay wavelength and shows comparable results to a standard pH meter. Zhang et al. described a PMMA-PDMS-based microfluidic device with 150 µL working volume with integrated optical sensors for pH, DO, and optical density (OD) for microbial fermentation of *Escherichia coli* and *S. cerevisae* by comparing results with traditional cell culture devices in terms of OD, pH, and DO [71]. A PC-PDMS-based 1 mL microfluidic bioreactor was developed for perfusion cultivation of recombinant human growth hormone (rhGH)- and recombinant interferon alfa-2b (IFNα-2b)-expressing strains of *Pichia pastoris* consisting of eight fluid inputs which provide media to the 40 µL reservoirs on the chip integrated with optical sensors (pH, DO, and OD) and valve architecture (Figure 2B) [72].

Uniform mixing was realized by deflection of the PDMS membrane, while real-time temperature measurement and control were achieved using an integrated circuit temperature sensor. Perfusion was achieved using a polyether sulfone (PES) membrane (1 cm diameter 0.2 µM) that was integrated into one of the three growth chambers to collect perfusate [77]. The platform was further used to produce a single dose of two biologics of recombinant human growth hormone (rHGH) and interferon-α2b in less than 24 h from a single genetically engineered yeast strain [72]. As such, Totaro et al. have developed a multifunctional system containing 12 individual reactor elements incorporated with optical sensors for OD and dissolved oxygen with 15 µL working volume for the cultivation of lactic acid-producing *S. cerevisiae* strains under batch and perfusion mode (Figure 2C). The device consisted of one inlet and one outlet for medium supply and removal, along with one separate channel for cell inoculation. The microfluidic bioreactor showed 4- to 6-fold higher production of lactic acid compared to the shake flask within 3 h during perfusion cultivation [73]. Parekh et al. have developed a 3D printed microbioreactor with integrated sensors for pH, DO, and optical density for batch and continuous cultivation of *E. coli* and *P. pastoris* during optimization of protein production (Figure 2D). The platform has used a 3D printed manifold for media switching and a pressurized fluid driving system for media delivery with a minimum flow rate of 0.7 µL/min. They have shown higher protein production for *P. pastoris* with pure methanol as a feed compared to methanol-sorbitol and methanol-glucose mixed feed [74]. Wang et al. developed a microfluidic Raman sensor using single-ring negative-curvature hollow-core fiber to quantitatively detect glucose and ethanol with LOD (0.32 g/L) for glucose during the fermentation of *Saccharomyces boulardii*. High throughput perfusion-based optofluidic device is developed to screen microbial strains for small molecule productions by using Berkeley lights Beacon system, which shows correlation with lab scale fed-batch bioreactors [78]. The system produced a 50%–70% saving in time by handling up to 5×10^3 mutants in less than 8 days. Currently, the system relies on fluorescence detection, which restricts the choice of analytes, however, for wider applications, there is a need for a universal detection method [79]. A novel capillary wave microbioreactor of 7 µL working volume was integrated with an optical sensor for the detection of pH, DO, biomass, and glucose (Figure 2E). The device with 4 mm diameter and 1 mm depth was fabricated from glass by using femtosecond laser direct writing and mounted on a black 3D printed polylactic acid block. The oxygen sensor was modified to measure glucose by measuring the uptake of oxygen by glucose oxidase in the presence of glucose. The device was able to measure 15 mM concentration of glucose over 8 h of cultivation of *E. coli* [75]. Hasan et al. have developed a PMMA-based microfluidic chip immobilized with glucose binding protein (GBP) on NI-NTA agarose beads which was connected with a bioreactor after micro-dialysis device for automated fluorescence measurement of glucose (Figure 2F). They were able to measure up to 260 mM glucose concentration with an accuracy of 81.78% with an RSD of 1.83% [76].

3.2 | Electrochemical Sensors

Electrochemical sensors generally depend on the interaction between receptors and analytes producing a signal change in terms of current, voltage, impedance, or conductance. Electrochemical biosensors can be specific and sensitive toward the analyte with a fast response time and have the advantage over optical methods in terms of being dependent on the optical setup, such as path length or optical properties of sensor materials. Moreover, using standard microfabrication approaches they are more amenable to miniaturization than optical systems and arguably less costly read-out systems [80]. Amperometric sensors can be used to measure oxygen concentration by electrochemical reduction of oxygen, in a similar manner to that of a Clarke oxygen electrode, and have the advantage of the ease of integration within microbioreactors [81, 82]. An ion-sensitive

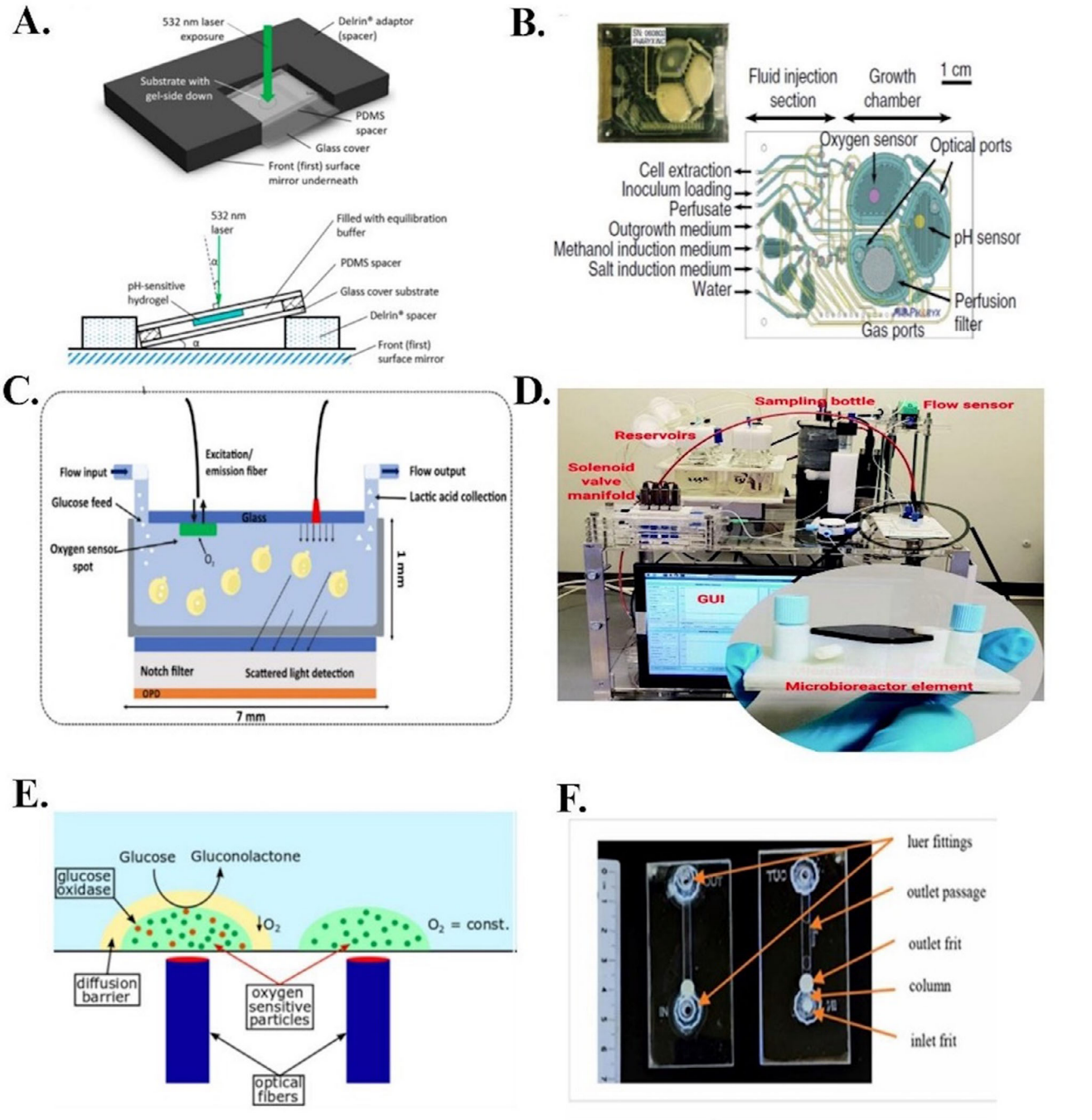

FIGURE 2 | (A) A PDMS-glass microfluidic chip containing pH-sensitive hydrogel to monitor pH during growth of *Lactobacillus casei* Shirota. Reproduced under the terms of the Creative Commons Attribution 4.0 International License [70]. (B) A PC-PDMS-based 1 mL perfusion microfluidic bioreactor integrated with optical sensors for monitoring of pH, DO, and OD by Perez-Pinera et al., open access publication under CC-BY license [72]. (C) Multifunctional microfluidic reactor array equipped with OD and DO sensors described in Totaro et al., open access publication under CC-BY license [73]. (D) Schematic of 1 mL inkjet 3D printed polymer microbioreactor integrated with optical sensors for DO, OD, and pH monitoring by Parekh et al., open access publication under CC-BY license [74]. (E) A working mechanism of glucose measurement using oxygen optical sensor and glucose oxidase by Viebrock et al., open access publication under CC-BY license [75]. (F) A PMMA microfluidic chip for glucose monitoring using glucose binding protein. Reproduced under the terms of the Creative Commons Attribution 4.0 International License [76].

field-effect transistor (ISFET) based pH sensor can work over a range between pH 2 and 12 with less than a second of response time and precision of about 0.01 pH units. Additionally, the ISFET sensor is stable over a wide temperature range from −45 to 120°C. ISFET sensors have higher sensitivity with rapid response and better reproducibility performance but suffer from signal drift and the requirement of a reference electrode which will increase the cost of a microfluidic fermenter [81, 83]. Welch et al. produced an extended gate ISFET sensor along with a pseudo reference electrode to monitor and control pH inside the microfluidic

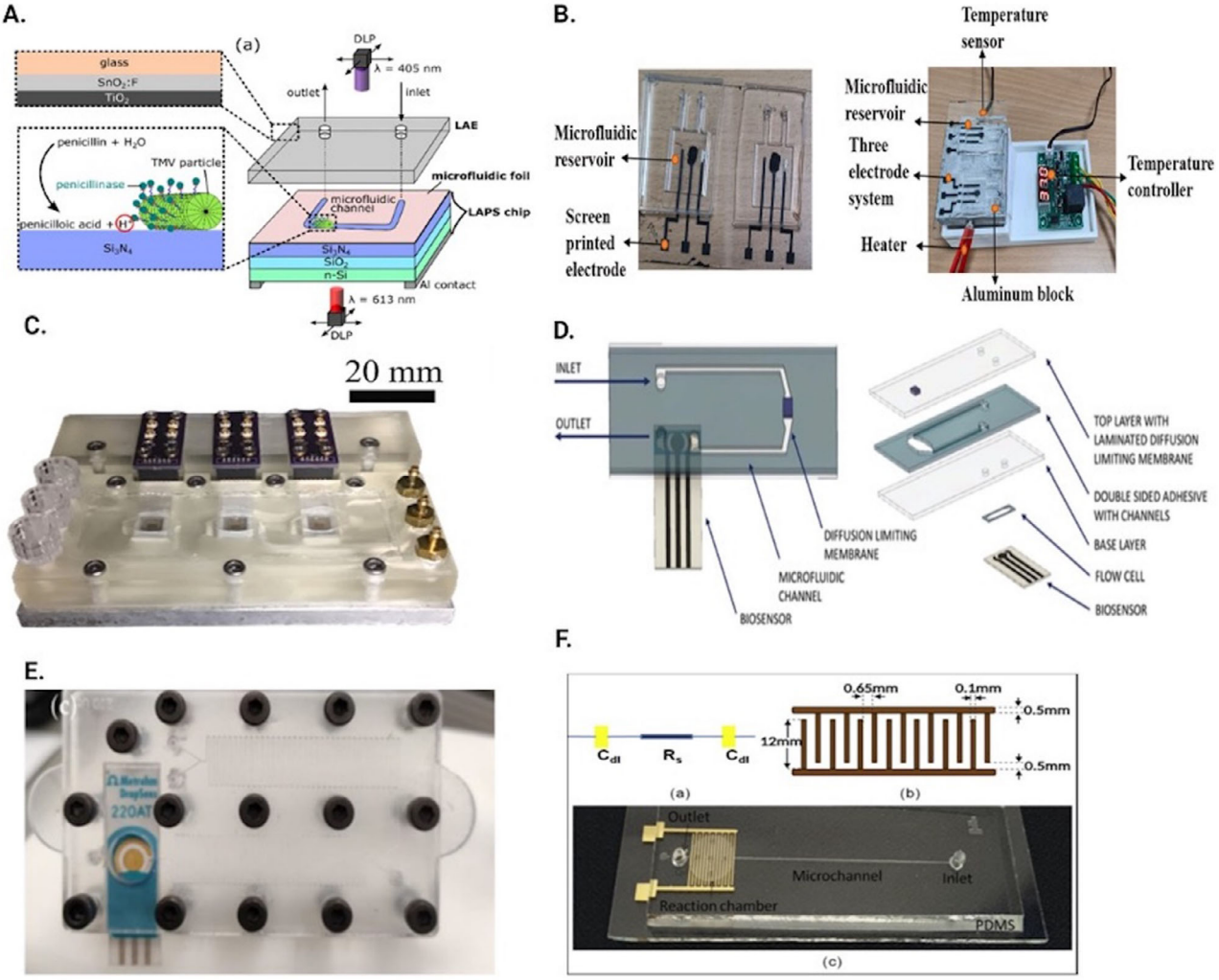

FIGURE 3 | (A) Schematic of LAPS sensor for measurement of local pH gradients inside the microfluidic chip by Welden et al., open access publication under CC-BY license [85]. (B) microfluidic device with screen-printed electrodes and aluminum thermal block with temperature controller for bacterial growth and measurement by Fande et al. Reproduced under the terms of the Creative Commons Attribution 4.0 International License [86]. (C) 3D printed microfluidic device with interdigitated electrodes for biofilm growth monitoring by McGlennen et al., open access publication under CC-BY license [87]. (D) Schematic view of biosensor microfluidic chip by Panjan et al., open access publication under CC-BY license [58]. (E) 3D printed microfluidic chip containing passive micromixers and Dropsens sensor for glucose monitoring by Podunavac et al., open access publication under CC-BY license [88]. (F) Glass-PDMS-based microfluidic device with gold interdigitated electrodes coated with aptamer for detection Ranibizumab by Bhardwaj et al. Reproduced under the terms of the Creative Commons Attribution 4.0 International License [57].

device with 90 nL working volume within 20 s of step change and 0.14 pH increments. A key issue is the reproducibility of the response of different ISFET sensors within microfluidic bioreactors [84]. A light addressable potentiometric sensor (LAPS) was presented to detect local pH gradients inside the microfluidic channel using a light addressable electrode (LAE) (Figure 3A). Here, microfluidic foil was sandwiched between LAE and LAPS where penicillinase enzyme was immobilized inside the channel using plant viral particles. The local pH changes were generated by enzymatic cleavage of penicillin which was measured by photocurrent voltage with LAPS [85].

Impedance is an attractive method for online cell viability monitoring in microbioreactors since it is specific to viable cells and can be easily incorporated as part of a microbioreactor. Commercial macro biomass sensors measure cell concentration in β dispersion in a range between 0.3 and 10 MHz [84]. Goh and Ram described a microfluidic device with an interdigitated electrode with 254 μM separation and width to measure viable Chinese hamster ovary cell density with R^2 of 0.75 [89]. Fande et al. have presented a microfluidic device with three electrode system which can be classified as Ag/AgCl ink as the reference electrode, graphene mesoporous carbon as the working electrode, and carbon ink as the counter electrode (Figure 3B). The device was fabricated using a glass substrate and PDMS with a working volume of 476 μL. The device yielded a linear range for monitoring the growth of *E. coli* from 0.336×10^{12} to 40×10^{12} CFU/mL, a quantification limit of 1.05 CFU/mL, and a detection limit of 0.35 CFU/mL [86]. Indeed, carbon-based materials have shown great potential as nanosensors to detect a wide diversity of molecules, expanding their application in the medical field [90]. Lei et al. have shown that impedance can be

used for monitoring of cell proliferation and chemosensitivity within a perfusion 3D cell culture microfluidic chip with a syringe pump for medium feed over a 5-day period [91]. The electrochemical sensor array for the measurement of biomass, DO, pH, and temperature was integrated into the 100 μL microbioreactor for aerobic batch cultivations of *Candida utilis* [92]. McGlennen et al. have developed 3D printed microfluidic chip to monitor the growth of *Pseudomonas aeruginosa* biofilm by using a pair of 50 gold interdigitated electrodes with spacing and width of 10 and 15 μM, respectively (Figure 3C) [87]. The electrodes were further modified with poly (4-styrenesulfonic acid) doped with pyrrole coatings to improve the stability of impedance measurements under abiotic and biofilm growth conditions. Panjan et al. have developed 3D printed microbioreactor integrated with OD sensor and glucose oxidase-based electrochemical biosensor for real-time monitoring of growth and glucose consumption during *S. cerevisae* cultivation with sensitivity to more than 20 g/L (Figure 3D) [58]. Similarly, a 3D printed microfluidic chip with passive micromixers integrated with glucose oxidase-based electrochemical sensor for the detection of glucose in cell culture medium in the range of 0.1–100 mg/mL (Figure 3E) [88]. Fernandes et al. developed a multi-function microfluidic platform with integrated sensors for the real-time monitoring of fermentation processes to meet the demand for high-throughput, quick, and cost-effective screening in bioprocesses [6]. Monitoring of protein concentration during fermentation is typically carried out by tedious and expensive offline analytical methods such as high-pressure liquid chromatography (HPLC). The absence of inline or online analytical tools increases the cost of the process as well as lowering the yield. To ease some of these issues, microfluidic chips have been developed based on electrochemical detection for protein quantitation that can be integrated into bench scale or micro scale fermenters. Microfluidic-based approaches are appealing alternatives to these existing methods, as all fluid handling steps can be automated [57, 93]. Bhardwaj et al. developed a PDMS/glass-based microfluidic chip with aptamer as a receptor and interdigitated gold electrodes for non-Faradic impedimetric detection of Ranibizumab, the LOD and linear range were found to be respectively 25 and 25–100 nM (Figure 3F) [57]. This microfluidic chip was also used to detect Lucentis in the fermenter with respective limit of detection and linear range of detection of 8.5 and 8.5–100 nM along with correlation with an HPLC-based method. The microfluidic chip device provides label-free, low-cost, and rapid analysis as it does not require pre-processing steps [94]. However, this methodology requires the generation of specific aptamers for each analyte using the SELEX method. Microfluidic platforms integrated with electrochemical sensors are also used in the diagnosis of diseases. For example, in a study by Viter et al., antibodies against SARS-CoV-2 virus proteins were detected in blood serum with a microfluidic platform integrated with an optical system. A ZnO tetrapod-based electrochemical biosensor, which showed significant sensitivity to target molecules, was used and flow was performed through the microfluidic system. Anti-SARS-CoV-2 antibodies were observed in real time with photoluminescence measurements. The response time of the biosensor was shortened by reducing the volume and flow rate of the microfluidic platform. Thus, a disease detection system was developed for the determination of anti-SARS-CoV-2 antibodies [95].

Over the past few decades, optical and electrochemical sensors have made great progress, and their variety of applications is growing including biotechnology and cell culture. The opto-electrochemical read-out enables integration into the generally polymer- and glass-based microfluidic devices for real-time monitoring of pertinent cell culture, and bioprocess variables have been effectively shown. A mass transfer step is very critical to achieve a consistent response since the indicator dye and the sample are in different stages which results in a delayed pH reaction. As a result, optical pH sensors often react more slowly than glass electrodes [96]. Nonetheless, the materials used for optical sensing must be long-lasting and responsive without drift. Another issue is that pH luminescent sensor spots dye is unreliable within the months due to gradual degradation by oxidative quenching effect. For the monitoring cell, this characteristic reflects difficulties with luminous or fluorescent dyes in general [97]. Additional restrictions include photobleaching, leaching, and interference from ambient light. This limitation can be overcome by the creation of new luminous or fluorescent dyes or by structurally altering them for long-term stability. The choice of sensor formats and integration also depends on device geometries, materials, and bonding/assembly techniques [98]. Electrochemical sensors for bioprocessing are made using a variety of microfabrication patterning techniques that allow for the control of the surface area-to-volume ratio, increasing the active surface area and producing a sensing surface with high sensitivity. It is challenging to increase reproducibility and stability for long-term sensor operation due to the absence of chemical stability and biological adsorption on the sensing electrode surface. However, there is a possibility that the continuous charging of the electrode would increase corrosion on the metallic substrate, which could prevent the signal from being detected. There is a need to develop anti-fouling methods or surface modification techniques to stop cells, proteins, amino acids, and nutrients in the culture medium [97] from adhering to the detecting surface. The development of more straightforward and portable devices is one of the enhancements required for microfluidic applications. Therefore, all the parts required for effective analyte monitoring must be compact, miniaturized, and robust to achieve real lab on a chip applications such as cell and tissue-based microsystems, process optimization, and high throughput screening [99].

4 | Bioprocessing on a Micro-Scale for Biomedical Applications

Microfluidic devices are used in the synthesis of nanomaterials [24, 100] and various substances such as polymers, polysaccharides [101, 102], antimicrobial [103, 104], and antiviral compounds [105, 106]. One of the main advantages of using microfluidic platforms for microbial fermentation is the possibility to precisely control the process conditions such as flow rate, temperature, pH, and nutrient availability on the growth and metabolism of microorganisms [28, 48, 64]. In that respect, computational modeling is invaluable to understand the mass transfer dynamics and the relations between the flow rate, nutrient availability, and hydrodynamic dispersion coefficients, to increase the yields of the end products [107]. Different polymers are synthesized in microfluidic devices offering benefits for the formulation of effective drug and genetic material carriers (Figure 4A) [108, 109]. For

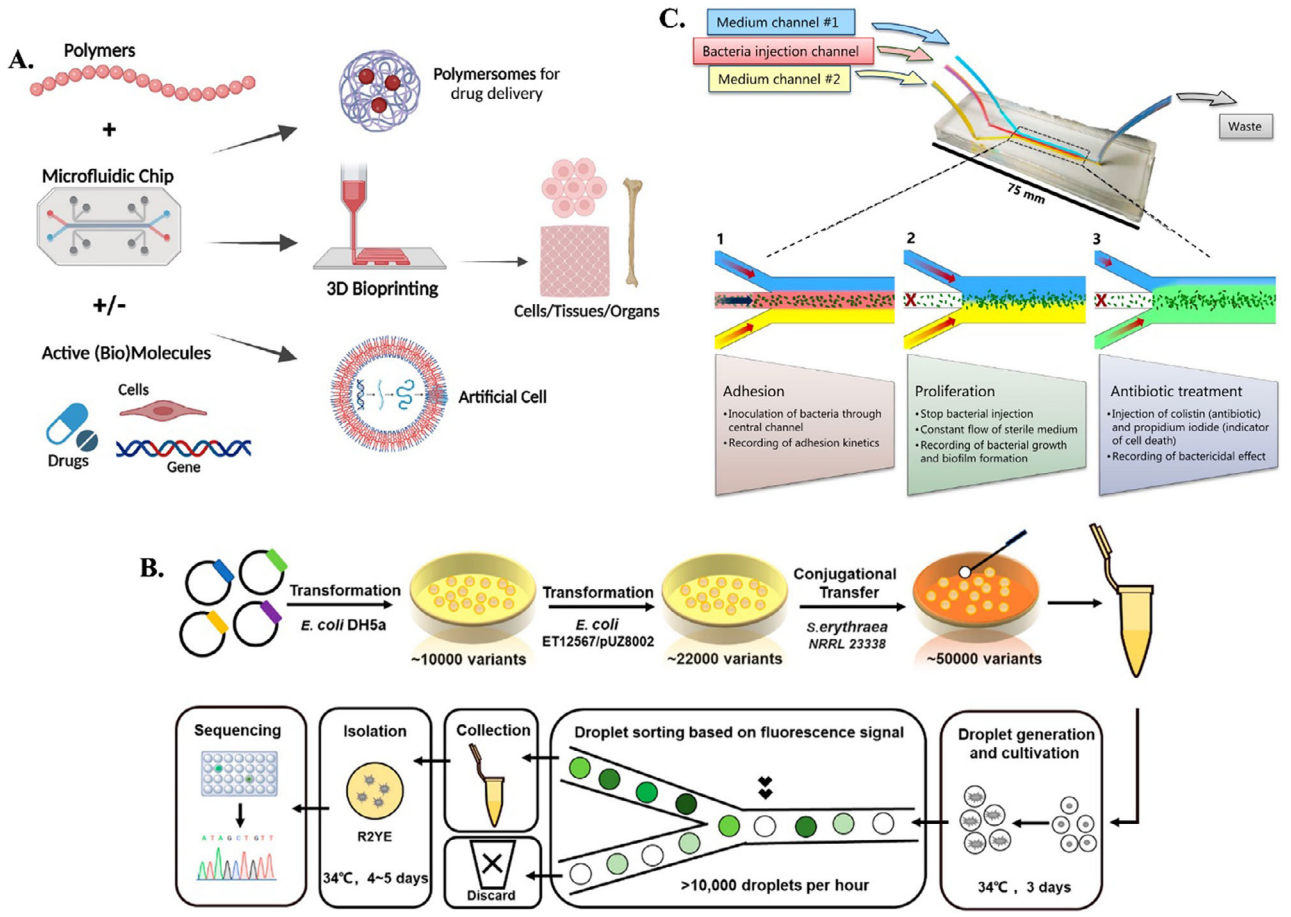

FIGURE 4 | Depiction of the production of (A) polymers by Damiati et al., open access publication under CC-BY license [108], (B) antimicrobials by Yun et al., open access publication under CC-BY license [104], and (C) antivirals by Straub et al., open access publication under CC- BY license [112] in the microfluidic platform used in biomedical applications.

example, polyhydroxyalkanoates (PHAs) are natural bioplastic polymers that decompose completely. PHAs synthesized from *Pseudomonas fluoroscenes*, *E. coli*, *Aeromonas hydrophila* 4AK4, *Ralstonia eutropha* B5785, and *Lactococcus lactis* can be used as drug carriers and medical devices due to their high biocompatibility, non-toxicity, and lack of immunogenicity. PHAs produced from *A. hydrophila* 4AK4 can also be utilized as scaffolds in tissue engineering owing to their high mechanical strength [101, 110]. Giduthuri et al. used a PDMS microfluidic platform for the production of PHAs from gram-negative bacterium *Cupriavidus necator*, which is known to synthesize up to 90% of its dry-weight PHAs under extreme conditions. In this platform, high-quality platinum wires were placed perpendicular to the microwells and used as electrodes for dielectrophoresis. Resultantly, the cytoplasmic conductivity of bacteria and the production efficiency of PHAs were reported to increase due to the uptake of rare earth elements by dielectrophoresis [102]. In another study by Menegatti et al., malic acid, which is a biodegradable and bioabsorbable water-soluble polymer, used as a drug carrier in biomedical applications was produced. For this purpose, *S. cerevisiae* cells were used and fumaric acid hydration and L-malic acid production were carried out in the microbioreactor, which consisted of 2-layer PMMA with Y-Y-shaped microchannels, and the cells were immobilized in the hydrogel layers at the bottom and top. The highest fumaric acid concentration was reached when the liquid height of the microbioreactor was 200 μm and the hydrogel thickness was 400 μm [111].

The production of antimicrobial compounds such as antibiotics and antimicrobial peptides is carried out in microfluidic platforms through microbial fermentation (Figure 4B). The capacity of several species to produce antibiotics, including *Penicillium, Streptomyces*, and *Bacillus* has been consistently investigated [113]. A study by Wink et al. demonstrated the use of a microfluidic platform, which included simultaneous epifluorescence microscopy and electrospray ionization mass spectrometry (ESI-MS) for the analysis of streptomycin produced in droplets by a gram-positive filamentous prokaryote *Streptomyces griseus*. The microfluidic platform was produced with the photolithography technique, and the encapsulation of S. *griseus* spores was carried out in 200 pL volume sections on the platform. After incubation to grow spores in the droplets, the droplets were analyzed by fluorescence and ESI-MS. Even at the picogram level, the secondary metabolites were reported to be easily identified [103]. In another similar study, Mahler et al. integrated a mass spectrometry unit based on direct electrospray ionization and injection of single droplets into the PDMS microfluidic system, which enables the determination of streptomycin produced by *Actinomycetales* strains at the single droplet level even in the presence of dense biomass. This droplet-based microfluidic platform stands out as a

promising alternative to elicit new antimicrobial compounds by high-throughput screening at the single-cell level [114]. Another study by Yun et al., using a droplet-microfluidic-based platform, demonstrated increased production of a large-spectrum bacterial infection antibiotic called erythromycin by the filamentous actinomycete *Saccharopolyspora erythraea* NRRL 23338, with metabolic engineering and a well-characterized promoter panel. Erythromycin level was determined by fluorescence-activated droplet sorting integrated into the platform. The wild strain of *S. erythraea* exhibiting low erythromycin productivity and gene expression has been reported to increase with this platform [104]. Microbial fermentation of antimicrobial peptides (AMP) using microfluidic platforms is another niche for biomedical applications. Since AMPs are bioactive small proteins that are protective against gram-positive, gram-negative bacteria, fungi, parasites, and viruses, they are regarded as the next generation of antibiotics [115, 116]. Microorganisms can produce AMPs, and some well-known examples are nisin and gramicidin from *L. lactis*, *Bacillus subtilis*, and *Bacillus brevis* [117]. Nuti et al. produced water-in-oil double emulsion droplets in the microfluidic system, which has inlets for the inner aqueous phase, the oil phase, and the outer aqueous phase. When the produced emulsion was combined with pneumolysin, a β-barrel cholesterol-dependent cytolysin from *Streptococcus pneumoniae*, and injected into another microfluidic device, long-term observation in a hydrodynamic capture array was carried out. In this platform, the cells were labeled with superparamagnetic beads for individual cell capturing and easily attracted to the wells when the microfluidic device was placed on a magnet. The developed system provides a new perspective for the discovery and development of membrane-active antimicrobials [118].

Antiviral compounds that inhibit the growth or reproduction of viruses are also produced in microfluidic platforms by microbial fermentation. Bacteria secrete extracellular polysaccharides (antiviral compounds), which exhibit biological properties, including anti-inflammatory, anti-cancer, anti-microbial, antioxidant, and immunomodulatory, and have an inhibitory effect on a wide variety of viruses, including DNA and RNA viruses (Figure 4C). Typically, the inhibitory effect is associated with the viral adsorption and/or replication phases in host cells. Fungal polysaccharides exhibit antiviral activity in a way like bacteria. Animal, human, and plant viruses can be inhibited by fungus polysaccharides such as glycan, chitin, mannan, or lentinan [119, 120]. For example, in the study of Raekiansyah et al. the effects of Brefeldin A antiviral compound, produced by strain *Penicillium* sp. FKI-7127, on dengue, Zika, and Japanese encephalitis viruses were investigated [106]. In another study by Biliavska et al., the antiviral activity of exopolysaccharides produced by lactic acid bacteria of the genera *Pediococcus*, *Leuconostoc*, and *Lactobacillus* against human adenovirus type 5 was investigated [105]. In addition to microbial bioprocessing for the production of various biomolecules, microfluidic platforms are utilized to study the interactions between microorganisms and the environment at the microscale, providing insights into the mechanisms underlying microbial growth and metabolism. For instance, in a study, a microfluidic platform was designed to enable in-depth control over uniform laminar flow conditions while bacterial adhesion and biofilm formation of *E. coli* was monitored in real-time. The effect of medium composition on biofilm formation by the bacteria was successfully investigated [112], which might accelerate the development of new antimicrobial biomaterials. We anticipate an increase in the number of studies focusing on the microbial production of polymers, polysaccharides, and antimicrobial and antiviral components in microfluidic devices as iterations are much more rapid for the ultimate aim of optimization.

5 | Future Trends and Conclusion

Microfluidic technology has been increasingly used for different biotechnology applications in recent years. These microfluidic platforms are portable, disposable, reproducible, have low material requirements, scalable, and allow high-throughput applications. These benefits make them perfect for a wide range of biomedical applications, such as tissue engineering, extracellular vesicle production, pharmaceutical synthesis, drug delivery, screening, diagnostics, wearable biosensors, organ-on-a-chips, and microreactors for in situ production of multiple substances. Classical microfluidic systems are relatively simple systems consisting of closed microchannels. However, with technological advances, they can be used with biosensors, optical filters, and electronic circuits to improve microfluidic pumping and mixing within microchannels.

Microbial fermentation in microfluidic platforms has emerged as a promising approach for the production of a wide range of biomolecules for biomedical applications. Currently, efforts should be devoted to the integration of downstream processing to the existing microfluidic platforms, coupled with robust sensing and monitoring techniques [121]. In the future, the use of microfluidic platforms for microbial fermentation is expected to play a significant role in the development of personalized and precision medicine. By combining the know-how and expertise in microfluidic platforms with genetic engineering and synthetic biology tools, it will be possible to design and engineer microorganisms that can produce customized biomolecules and bioproducts on demand [122]. This will enable the production of personalized vaccines, drugs, and diagnostic agents that are tailored to the specific needs of individual patients [32]. The use of microfluidic platforms for the production of antimicrobial peptides, small molecules [123], natural products, and biopesticides is also expected to increase in the future, as these biomolecules are often difficult to obtain through traditional chemical synthesis methods due to their complex structures [124]. However, more in-depth studies are required to understand the diffusive dynamics of substrates utilized for production [125]. Additionally, the cultivation of microorganisms in extreme environments, such as space or deep sea, is also being explored as a means to facilitate the discovery of new biomolecules and understand the limits of microbial life [11]. Microfluidic platforms that are capable of simulating these challenging conditions offer indispensable possibilities.

In conclusion, microfluidic platforms have the potential to completely transform biomedical bioprocessing by providing more accurate and efficient systems that are automated, scalable, and miniaturized. The future of microbial fermentation on microfluidic platforms is expected to be driven by the increasing demand for sustainable and cost-effective production of biomolecules, the continued development of new microfluidic technologies and materials, and the integration of microfluidic platforms

with other technologies such as bioprinting, nanotechnology, and machine learning. These advances are expected to fully exploit the potential of microbial bioprocessing at the microscale. As microfluidics field continues to mature and become more integrated with AI, synthetic biology, and automation, the field will likely become indispensable in the next generation of biotechnological innovations. Overcoming current challenges requires interdisciplinary collaboration between microbiologists, engineers, data scientists, and process developers. Continued interdisciplinary collaboration between engineering, biology, and clinical sciences will be key to translating these innovations from bench to bedside.

Author Contributions

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Conflicts of Interest

The authors declare no conflicts of interest.

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