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# Application of three-dimensional printing in bacterial detection

**Tingting Cui[1,2], Ziye Xu[1,2], and Ting Cao[1,2]***

[1]Department of Laboratory Medicine, the First Affiliated Hospital, Zhejiang University School of Medicine, Hangzhou, Zhejiang, China.

[2]Key Laboratory of Clinical *In Vitro* Diagnostic Techniques of Zhejiang Province, Hangzhou, Zhejiang, China.

## Abstract

Diseases caused by bacterial infections, especially pathogen variations and drug-resistant bacteria, are a serious threat to human health globally. Accurate and rapid identification of bacterial pathogens is essential for early diagnosis of infections and timely treatment of disease. At present, the routine diagnostic methods for identifying bacterial pathogens in the clinic are bacterial culture, immunological detection, genetic analysis, etc. These methods are fundamental for bacterial detection and clinical diagnosis of pathogen infections in daily life. Notably, three-dimensional (3D) (bio)printing technology provides more options for bacterial detection due to its personalized design and manufacturing. Compared with traditional methods, 3D printing provides a more convenient, rapid, and accurate bacterial detection platform, thus meeting the urgent needs of clinical and scientific research. Here, we have summarized the research progress in 3D printing technology for bacterial detection, including bacterial detection sensors, microfluidic chips, bioprinting microarray technology, artificial intelligence, and spectroscopy, providing a scientific reference and addressing gaps in 3D printing-assistance bacterial detection. Additionally, technical advantages, challenges, and future development trends are also discussed in related healthcare fields.

***Keywords:*** Three-dimensional (3D) printing; Bacterial detection; Bacterial infection

***Corresponding author:**
Ting Cao
(tingcao@zju.edu.cn)

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## 1. Introduction

Bacterial detection, particularly early diagnosis of bacterial infection, is a global concern,[1] made more urgent by the increase in pathogen variations and drug-resistant bacteria.[2] Conventional bacterial detection methods in clinics consist of culture-based detection, immunological identification, and molecular biology technology, but still require further improvements in detection rate, sensitivity, specificity, and cost.[3] In recent years, three-dimensional (3D) (bio)printing technology has introduced new approaches to microorganism research,[4,5] as it is currently used in surgical and medical devices, drug delivery, and tissue engineering,[6,7] with the advantages of rapid prototyping, easy complex geometry fabrication, customization of heterogeneous component assembly, and cost-efficiency.[8] However, a comprehensive summary of (3D) printing in bacterial detection is lacking, which is crucial for advancing both 3D printing and clinical

bacterial diagnostics. Thus, this review aims to provide a comprehensive overview of 3D printing applications in bacterial detection. Concurrently, technical advantages, challenges, and future development trends are also discussed in related healthcare fields.

## 2. Current status of bacterial detection

Bacterial pathogens cause a variety of human diseases.[9] Given the increasing bacterial infection rates, simple, rapid, and low-cost detection strategies are urgently needed.[10] A series of technical methods are currently employed for bacterial detection in clinics, including traditional microbiological detection methods, immunological detection, and nucleic acid-based detection techniques.[11-15] Likewise, other analysis methods with 3D printing technology are being developed to enhance clinical bacterial detection, each with its intrinsic advantages and disadvantages.

Traditional microbial detection platforms based on morphology include smear microscopy and culture identification. Smear microscopy is one of the most basic methods for bacterial detection. It was widely used in the fields of medicine and biology soon after microscopy technology matured. By preparing and staining bacterial smears, the morphology, arrangement, and staining properties of bacteria can be observed under the microscope for preliminary identification and classification.[16] Bacterial culture is a classic method for bacterial detection. With the development of microbiology, researchers optimized media formulations and conditions to support the growth of various bacterial species.[17] By selecting appropriate media and environmental conditions, bacteria can proliferate *in vitro*; they can then be identified based on colony morphology and biochemical reactions. This method has high accuracy and can provide detailed biological information about bacteria, but it is time-consuming, usually taking days to weeks. Additionally, culturing fastidious bacteria and the complex operation also pose significant challenges that need to be addressed.[18]

Immunological detection is a detection method based on the specific recognition between antigen and antibody; its emergence and development are closely related to

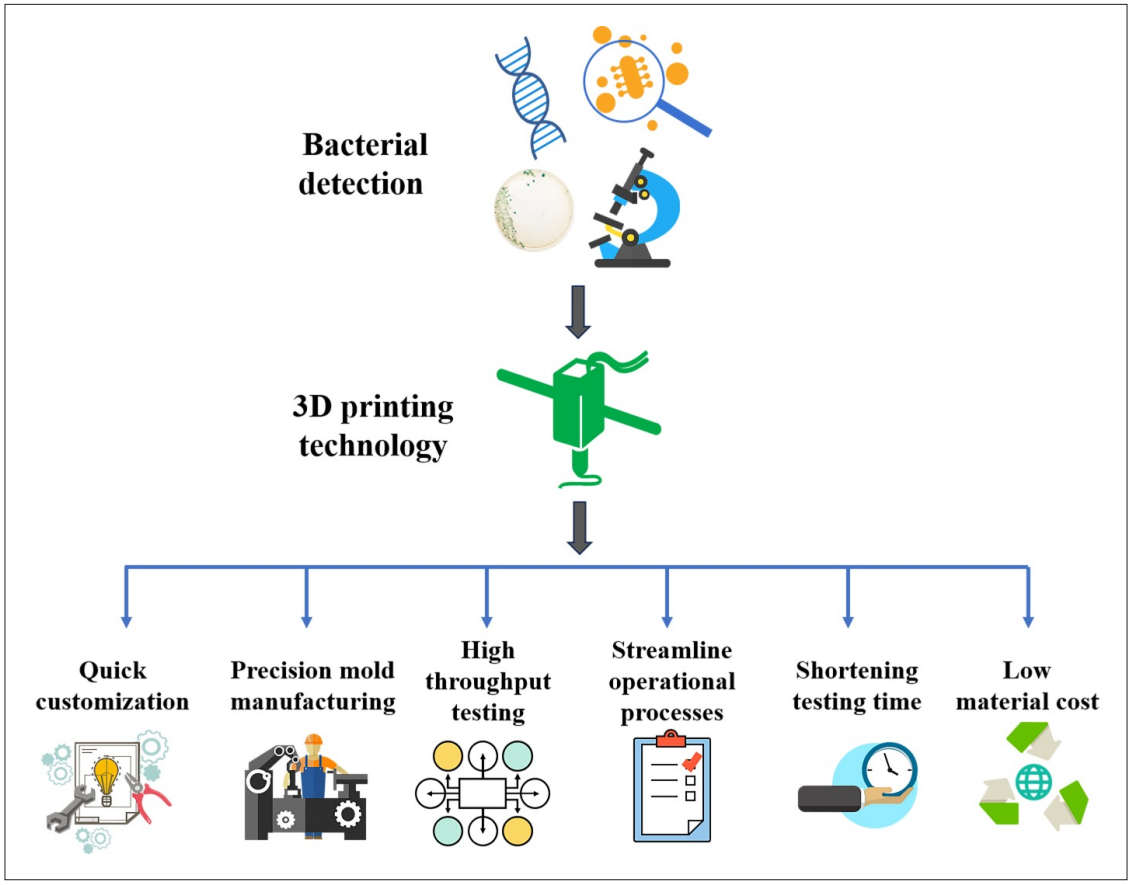


**Figure 1.** The advantages of 3D printing technology in bacterial detection.

advances in immunological research. Immunological detection targets pathogen proteins or metabolic substances, such as glycoproteins and polysaccharides,[19] to identify bacterial types mainly through specific antigens or antibodies during bacterial infections. This includes lateral flow devices, immunofluorescence, and enzyme-linked immune sorbent assay, among others.[20] This method has high sensitivity and good specificity; however, the detection results are affected by the quality of the antibody, with limited ability to detect new pathogens.

Molecular biological detection is a bacterial detection method that has emerged with the rapid development of molecular biology technology in recent decades. It uses unique gene sequences in bacteria for detection and is highly sensitive and specific. By extracting bacterial nucleic acid (DNA or RNA) from samples and using polymerase chain reaction (PCR) to amplify the target gene region, the rapid and accurate identification of bacteria can be achieved.[21] This method has high sensitivity and specificity, can be completed within a short time, and is widely used in the diagnosis and monitoring of infectious diseases. However, it is expensive, requires professional equipment and technicians, and may yield false positive/negative results.[22] Notably, isothermal nucleic acid amplification technology, an *in vitro* amplification method that uses enzymes with different functions and specific primers to rapidly amplify nucleic acids at constant temperature,[23] has been utilized for bacterial detection in recent years. This approach eliminates the thermal cycling step and simplifies the amplification process, providing a user-friendly operation condition.[24] Currently, the commonly used isothermal amplification methods include loop-mediated isothermal amplification (LAMP), nucleic acid sequence-based amplification (NASBA), recombinase polymerase amplification (RPA), rolling circle amplification (RCA), and hybridization chain reaction (HCR),[25] featuring advantages of simplicity, rapidity, and efficiency. However, aerosol contamination in the amplification process leads to false positive results; likewise, challenges in primer design and limitations in enzyme storage hinder its widespread practical application.[26] Capillary electrophoresis (CE) is a powerful tool for the identification and separation of charged compounds.[27] It is sometimes used in combination with various nucleic acid detection strategies to minimize the possibility of false/negative results. It has the advantages of high efficiency, small sample volume, and simple sample preparation, but along with shortcomings of time-consuming sample processing and high equipment cost.[28]

Next-generation sequencing (NGS), a representative of gene sequencing, has the advantages of high throughput and accuracy, displaying great potential for bacterial detection. By sequencing the whole genome or specific regions of bacteria (e.g., 16s rRNA region), accurate identification, typing, and drug resistance gene detection of bacteria can be achieved, particularly in complex samples.[29-31] However, it requires high data processing power for bioinformatics analysis and is relatively costly compared to other nucleic acid amplification methods, such as PCR.[32]

Gene chip technology uses nucleic acid hybridization to detect bacterial genes in samples. A large number of DNA or RNA fragments are hybridized on the surface of a solid carrier via base pairing; the hybridization signal is then detected using scanning equipment to obtain genetic information on the bacteria and achieve qualitative and quantitative detection.[33] Gene chips have high sensitivity and specificity and high-throughput capabilities, but there are challenges in terms of diagnostic speed, standardization, cost, and contamination risk.[34]

With advances in science and technology, chromatography and spectroscopy techniques based on bacterial proteins not only improve the accuracy, sensitivity, and speed of detection but also reveal a broader range of chemical and physical characteristics of bacteria. The primary principle of chromatographic detection of bacteria is using the distribution difference between the stationary phase and the mobile phase of bacteria or their metabolites through the chromatographic column and then analyzing separated components with the detector (i.e., usually mass spectrometry or other technical means) for qualitative analysis of separated constituents.[35,36] Chromatography has high separation efficiency, high sensitivity, and fast analysis speed for detecting bacteria, but it is challenged by complex sample pretreatment, high equipment cost, and technical requirements in operation. Spectrometry is used to qualitatively and quantitatively analyze bacteria by measuring the spectral signal produced by the interaction of bacteria or bacterial metabolites with light.[37,38] Its advantages—high sensitivity, high selectivity, non-destructiveness, rapidity, and efficiency—have demonstrated broad application prospects in bacterial detection. However, its disadvantages and limitations, such as high equipment cost, stringent sample requirements, and strong technical dependence, cannot be ignored.[39]

At present, the main challenges of bacterial detection are complex sample processing, long detection cycles, and the need for further improvements in sensitivity and specificity. Although traditional methods, such as Gram staining and PCR amplification, meet the needs of detection to a certain extent, they have limitations in dealing with rapidly mutating pathogens and improving detection throughput. A detailed comparison between various detection approaches is summarized in Table 1.

**Table 1. Characteristics of different bacterial detection methods**

| Detection strategy | Method | Target pathogen | Principle | Detection accuracy | Detection speed | Advantages | Limitations | Ref. |
|---|---|---|---|---|---|---|---|---|
| Conventional | Culture-based identification | Bacteria, fungi, and viruses | According to bacterial shape, size, and staining; requires a nutrient medium | High accuracy | Slow (days to weeks) | Cost-effective simple | Low sensitivity; laborious testing | [96] |
| | Immunological detection | Bacteria, fungi, and viruses | Exploits affinity between microbial antigen and mono/polyclonal antibody | Frequent false positives | Fast (few hours) | High specificity; processes many samples simultaneously | Dependence on antigen-antibody interaction | [20] |
| | PCR | Bacteria, fungi, and viruses | Uses unique gene sequences in microorganisms for detection | Frequent false positives/negatives | Fast (few hours) | High sensitivity and specificity | Expensive; requires equipment and technicians | [22,97] |
| | Isothermal nucleic acid amplification | Bacteria, fungi, and viruses | Uses unique gene sequences in microorganisms for detection | Accuracy higher than PCR | Fast (few hours) | Amplification at constant temperature; high sensitivity | Aerosol contamination causes false positives; challenging primer design and enzyme storage | [25,26,96] |
| | Capillary electrophoresis | Bacteria, fungi, and viruses | Intracellular metabolites, extracellular metabolites, or cellular components of bacteria are determined | Reduced false positives/negatives | Fast (minutes to hours) | High efficiency; small sample volume; simple sample preparation | Sample processing is time-consuming; high equipment cost | [27,28] |
| | Next-generation sequencing | Bacteria, fungi, and viruses | Sequencing of whole genome or specific regions, such as the 16s rRNA region | High accuracy | - | High throughput and accuracy; suitable for all microorganisms | Relatively high cost; require strained personnel to operate | [20,29] |
| | Gene chip technology | Bacteria, fungi, and viruses | Uses nucleic acid hybridization to detect bacterial genes in samples | Frequent false positives in multiple testing | Fast | High specificity and sensitivity; detects multiple pathogens simultaneously; easy to operate | Expensive equipment; requires a high level of operational skill | [33] |
| | Chromatography | Bacteria | Analyze bacterial metabolites or components; often combined with other techniques (e.g., mass spectrometry) | - | Fast | High separation efficiency; high sensitivity | Complex sample pretreatment; high equipment costs; requires technical expertise | [35,36] |

*(Continued...)*

**Table 1. Continue...**

| Detection strategy | Method | Target pathogen | Principle | Detection accuracy | Detection speed | Advantages | Limitations | Ref. |
|---|---|---|---|---|---|---|---|---|
| Conventional | Spectroscopy | Bacteria | Spectral signals from bacteria interaction or metabolites | - | Fast | High sensitivity and selectivity; non-destructive; efficient | High equipment cost, sample requirements, and technical dependence | [37,39] |
| 3D (bio) printing | Biological sensors | Bacteria | Establishment of the reactor; sample treatment and signal recognition | - | Fast | Simple operating device; improved applicability; low cost; high sensitivity and specificity; easy operation; fast response | 3D-printed materials cannot fully satisfy the requirement (stability, sensitivity, durability etc.) of biosensors; different printing parameters for different scenarios limits large-scale production of biosensors | [81,83,84,85] |
| | Microfluidic chips | Bacteria | 3D printing of microfluidic chips for signal recognition and transduction in bacterial detection | - | Fast | Enhanced detection limit; can culture and detect multiple pathogens simultaneously | Limited materials for 3D-printed microfluidic chips; 3D printing technology lacks uniform standards and specifications for chip production | [78,87,89] |
| | Bioprinting microarray technology | Bacteria | Biological probes (antibodies, DNA) can be precisely fixed on tiny matrices to form high-density microarray structures specific to bacterial targets | - | Fast | Easy to operate; rapid detection; potential for practical clinical application; enhanced bacterial capture efficiency | 3D-printed microarray technology is not well-established; only applicable for specific bacteria or pathogens; limited detection range | [90,91] |
| | Combination of AI and spectral technology | Bacteria | 3D bioprinting combined with AI and spectral analysis technology for bacterial detection | ≥99% | Fast | High throughput testing; non-culture detection; non-destructive testing | High technical complexity; complex data processing; limited testing range; lack of uniform standards | [95] |

Abbreviations: AI: Artificial intelligence; PCR: Polymerase chain reaction.


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Therefore, exploring efficient and accurate bacterial detection technologies remains a key research focus.

## 3. Overview of 3D (bio)printing technology

3D printing technology is a bottom-up manufacturing method that builds 3D objects by stacking materials layer by layer.[40] The key principle of 3D printing is slicing and stacking. Through slicing a 3D model into many thin layers, specific printing equipment, such as a 3D printer, prints out powdered metal, plastic, or other materials layer by layer and stacks the layers to form a complete 3D entity.[41,42] This operation endows 3D printing with the ability of complex geometry fabrication that is usually difficult to achieve with traditional manufacturing techniques.[43] With advancements in material science, computer technology, and precision manufacturing, 3D printing has been widely used in medical, aerospace, automotive, construction, food, and other fields.[44-47] Notably, 3D printing significantly accelerates medical development with the printing of medical devices, implants, and prostheses.[48-50] In addition, it can also be applied in tissue engineering and regenerative medicine with the bioprinting of cells, tissues, organs, and microorganisms, which is also known as 3D bioprinting.[51,52] There are three main types of 3D bioprinting, namely extrusion-based, jetting-based, and vat photopolymerization-based bioprinting.

Extrusion bioprinting is a technique for fabricating 3D structures through continuous extrusion of bioink.[53] It has strong compatibility and can print various types of biomaterials, including high-viscosity bioinks (e.g., cell membranes) and hydrogels. Typically, extrusion bioprinting has a low cost, but its low resolution limits its application in the fabrication of complex structures. Moreover, it is prone to nozzle blockage, and shear stress generated during the extrusion process may damage the cells and reduce cell viability.[54]

Jetting-based bioprinting is a technology that accurately controls the spatial arrangement of bioinks by contactless, on-demand injection of droplets at specific locations. It mainly includes inkjet bioprinting, laser-assisted bioprinting, acoustic bioprinting, microvalve-based bioprinting, and electrohydrodynamic jet bioprinting.[55] Samples prepared by inkjet bioprinting have high resolution and cell viability. However, this technology cannot print biomaterials with high viscosity and high cell concentration.[56] The benefit of laser-assisted bioprinting is that it can use printable bioinks of different viscosities with high printing resolution. Since shear stress is avoided, high cell viability can be achieved, but the cost is relatively high.[57] Acoustic bioprinting supports multi-scale bioprinting, enabling precise printing of everything from larger cell aggregates to individual cells in cross-scale 3D. This nozzle-free bioprinting system avoids clogging problems and also reduces cell damage, but its system is relatively complex and expensive.[58] A typical microvalve-based bioprinting system consists of a three-axis mobile robot platform and multiple electromechanical microvalve print head arrays. Each microvalve print head is connected to a separate gas regulator; biomaterials and cells can be simultaneously ejected from different print heads. It precisely controls the deposition of multiple types of cells and biomaterials with high cell viability, high throughput, and moderate printing resolution. High printing pressure and small nozzle diameter lead to reduced cell viability.[59] Electrohydrodynamic jet bioprinting uses an electric field to pull a cell-loaded droplet from the tip of a nozzle and facilitates high-resolution printing, but there are challenges such as limited selection of printable inks, disordered fiber arrangement due to charge accumulation within the printed fibers, and fabrication of smaller 3D structures.[55]

Vat photopolymerization-based bioprinting is a technology that uses photosensitive resins to fabricate 3D structures layer-by-layer using light-curing technology, featuring stereolithography (SLA), digital light processing (DLP), continuous liquid interface production (CLIP), computed axial lithography (CAL), two-photon polymerization (TPP), or other light-curing technologies.[60] Vat photopolymerization-based bioprinting provides high-resolution-printed structures and good connectivity between the printed layers, with the ability to create complex structures. The disadvantage of this approach is exposure to UV light, which leads to cell damage and high cost.[61] This method can improve the printability and cell viability of bioink by photorheological measurements and bioink wavelength synchronization.[62]

In the process of bioprinting, cell damage may be due to various causes, such as shear stress, thermal stress, and radiation stress.[63] Due to the intrinsic characteristics of each bioprinting technique, the factor affecting cell viability varies for different bioprinting methods. The effect of extrusion bioprinting on cell viability mainly lies in shear stress and material crosslinking. Shear stress can negatively affect cell viability; nozzle diameter and bioink viscosity can affect the range of shear stress values.[64] The precursor hydrogel or polymer solution for bioprinting must be crosslinked to form a stable 3D structure with good shape fidelity. The type and concentration of the crosslinking agent, as well as the duration of crosslinking, may affect cellactivity.[65] Cell viability in jetting-based bioprinting is mainly affected by shear stress, thermal stress, droplet impact velocity, and droplet volume. During the printing process, the limited nozzle size requires low viscosity and low concentration of bioink to reduce shear

stress and protect cells from severe damage.[51] Cell damage in vat photopolymerization-based bioprinting results from the radiation stress of the light source and the cytotoxicity of the photoinitiator.[63] Although the cell damage caused by the ultraviolet laser is not significant for cell printing, it is lethal to bacteria. Under UV light, microbial DNA absorbs the radiation, leading to thymine base dimerization, which inactivates the bacteria.[66]

A key component of 3D bioprinting is bioink, which embeds cells or microorganisms in biocompatible and bioactive materials (e.g., collagen, hyaluronic acid, alginate, and other natural polymers).[67,68] By precisely controlling the printing process, 3D bioprinting can construct 3D biological structures with biological activity and specific functions, implying great potential in tissue engineering, drug screening, and disease modeling.[69-72] In addition, bioprinting also can be used in various fields beyond human tissues, such as the manufacture of cultured meat, the development of more sustainable bioinks, the integration of machine learning (ML) into the 3D bioprinting process, bioelectronics, and space bioprinting.[73] Bacterial detection is one such example.

In the process of pathogen detection, 3D printing mainly serves in the construction of functional devices for microorganism capture, isolation, culture, and detection, thereby improving the efficiency and accuracy of bacterial detection.[74,75] Benefiting from its intrinsic superiority, 3D printing allows researchers to design microbial detection devices with specific shapes, structures, and functions according to actual needs. 3D printing technology allows for rapid customization according to different industries and specific needs to accommodate different types of pathogen detection. Lee et al.[76] used SLA to create 3D spiral microchannels around a cylindrical chamber, which can detect *Escherichia coli* (*E. coli*) in real food matrices quickly and easily. Pahlow et al.[77] used inkjet printing to prepare a cartridge system for convenient and cost-effective isolation of pathogenic bacteria from sputum samples. Besides, microfluidic chips fabricated by 3D printing can achieve high-throughput detection. Feng et al.[78] used 3D printing to develop a novel chip with an aptamer-based nano-interferometer capable of identifying four different foodborne pathogens (*L. monocytogenes, E. coli, S. typhimurium*, and *S. aureus*) with high sensitivity, i.e., with a limit-of-detection of 10 colony-forming units (CFU)/mL. Moreover, 3D printing technology simplifies the operation process of bacterial detection and shortens the detection time. Malhotra et al.[79] developed a cost-effective and portable 3D-printed electrochemical biosensor that facilitated the rapid detection of certain *E. coli* species in 15 min using 500 µL samples and cost only $2.50 per test. The advantages of 3D printing in bacterial detection are presented in Figure 1. These devices are capable of providing a larger culture area, more efficient chemical exchange, and more sensitive signal reports, thus enabling rapid and accurate detection of bacteria. In addition, 3D printing supports the hybrid printing of multiple materials, which provides more possibilities for bacterial detection. For instance, researchers can print appropriate biomaterials into complex 3D structures to study the growth characteristics and reaction mechanisms of bacteria under different conditions.[80] Compared with traditional bacterial detection methods, 3D printing can provide a more convenient, rapid, and accurate bacterial detection platform, thus meeting the urgent needs of clinical and scientific research.

## 4. Research and application of 3D (bio) printing in bacterial detection

### 4.1. Biological sensors for bacterial detection

3D printing enables the fabrication of miniaturized and integrated sensors capable of detecting the presence, type, and number of bacteria, making bacterial detection more portable and rapid. In particular, the combination of 3D printing and nanotechnology for sensor construction enables the rapid identification and detection of specific pathogens, which is favorable for early clinical diagnosis and has significant potential for real-world applications.

The most common use of 3D printing in bacterial detection biosensors is the development of reactors. For example, Xu et al.[81] constructed a 3D-printed integrated handheld biosensor that can replace PCR instruments for nucleic acid amplification, simplifying the operating device and improving its applicability in practice. Likewise, it integrates recombinase polymerase amplification-clustered, regularly interspaced short palindromic repeats/Cas12a protein (RPA-CRISPR/Cas12a) and lateral chromatography strip analysis for visualization and rapid analysis. As displayed in Figure 2A, it can be used to detect *V. parahaemolyticus* easily and intuitively in the field. Angelopoulou et al.[82] used fused deposition modeling to fabricate an electrochemical immunosensor for rapid and ultrasensitive detection of *S. typhimurium* in poultry samples. The sensor uses cadmium/selenium-zinc salt quantum dots (QDs) as a marker and consists of three electrodes made of carbon black (CB)-polylactide filament and a scaffold (printed from the polylactide filament).The sensor used biotinylated anti-*S. typhimurium* antibodies and subsequently streptavidin labeling of Cd/Se ZnS QDs to determine *S. typhimurium* in fresh chicken meat by sandwich immunoassay and quantify bacteria by measuring Cd(II) released after the acidic dissolution of

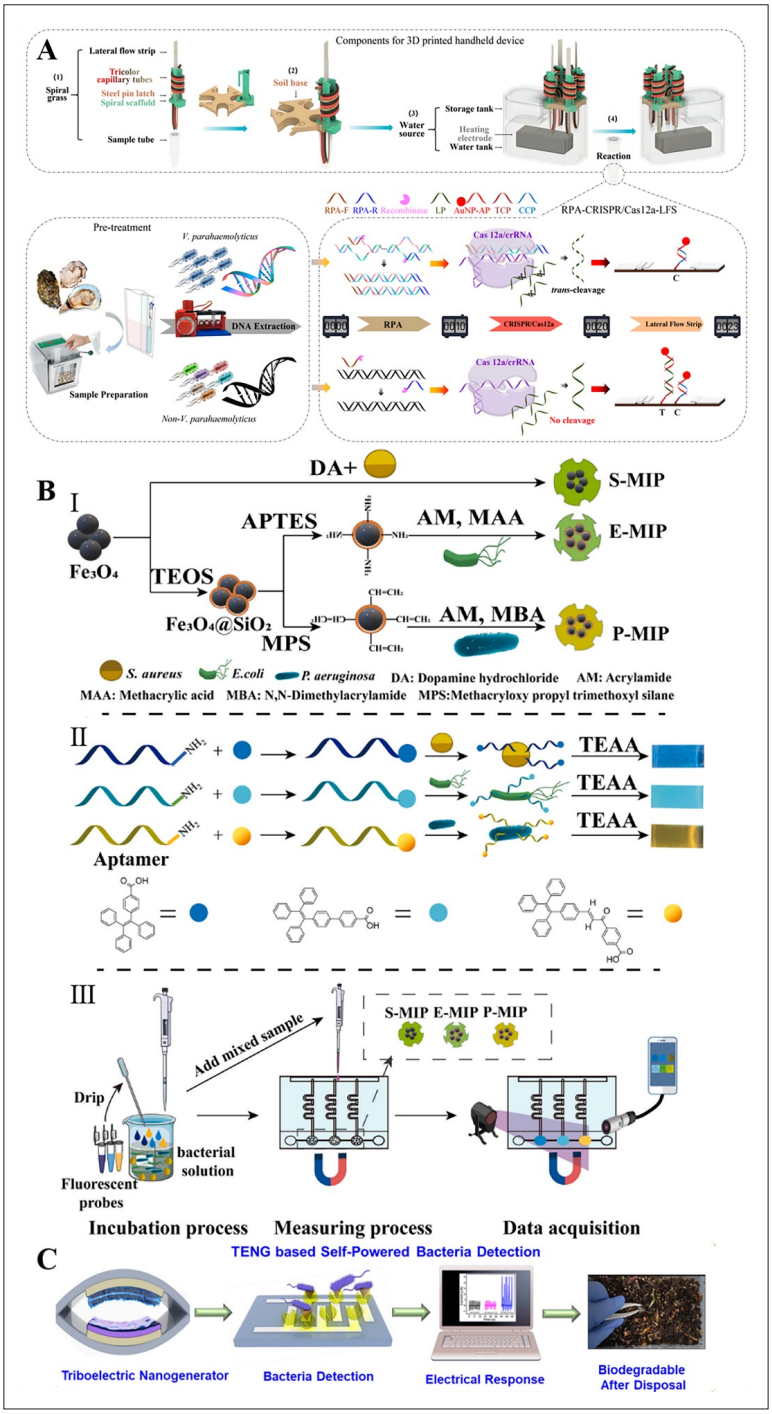


**Figure 2.** Biological sensors for bacterial detection. (A) Schematic diagram of *V. parahaemolyticus* detection with the integrated handheld biosensor. Adapted from ref.[81] (B) Illustration of the preparation of sensor device: (B, I) Synthesis of three magnetic molecularly imprinted materials; (B, II) Synthesis of fluorescent probes; (B, III) Schematic diagram of multiple detection of pathogenic bacteria. Adapted with permission from ref.[83] Copyright © 2023, Elsevier. (C) Schematic diagram of triboelectric nanogenerator (TENG)-based self-powered bacteria detection. Adapted from ref.[85] Abbreviations: RPA: recombinase polymerase amplification; LP: ligation probe; AP: AuNP-probe; TCP: test line capture probe; CCP: control line capture probe; CRISPR: clustered regularly interspaced short palindromic repeats; CRISPR/Cas: CRISPR-associated protein (Cas) system; LFS: lateral flow strip; crRNA: CRISPR RNA; TEOS: ethyl orthosilicate; DA: dopamine hydrochloride; APTES: aminopropyltriethoxysilane; MPS: methacryloxypropyl trimethoxyl silane. AM: acrylamide; MAA: methacrylic acid; MBA: N, N'-methylene diacrylamide; MIP: molecularly imprinted polymer; S-MIP: *S. aureus* imprinted material; E-MIP: *E. coli* molecularly imprinted material; P-MIP: *P. aeruginosa* molecularly imprinted material; TEAA: triethylammonium acetate; TENG: triboelectric nanogenerator. Miniaturized and integrated sensors made by 3D printing technology can be more portable and rapid to detect bacteria.

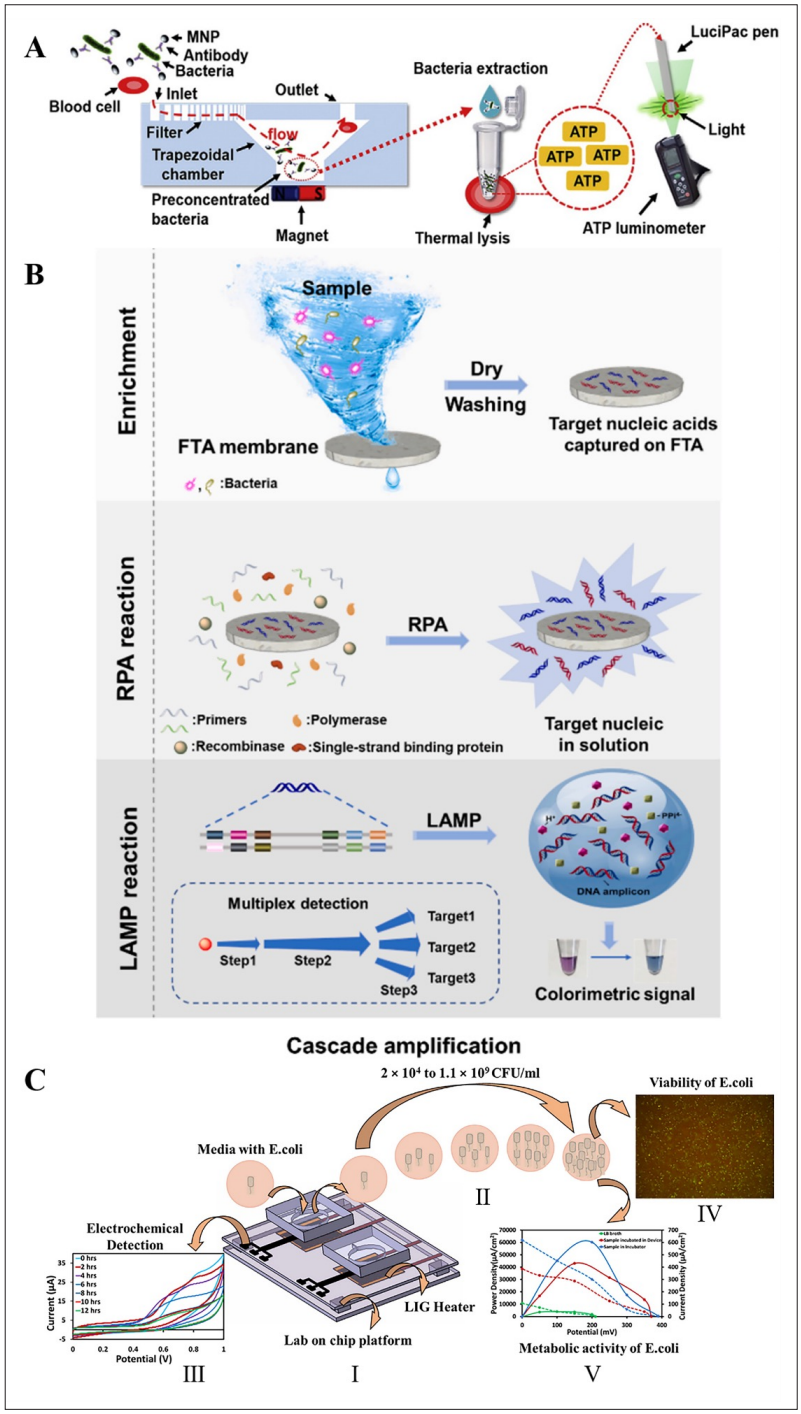


**Figure 3.** Microfluidic chips for bacterial detection. (A) Preconcentration of targeted bacterial cells in a 3D microfluidic magnetic preconcentrator (3DμFMP) with antibody-conjugated magnetic nanoparticles (Ab-MNPs). Adapted with permission from ref.[86] Copyright © 2016, Elsevier. (B) Design of flinders technology associates (FTA)-based multiplexed colorimetric assay for the detection of different pathogens. Adapted with permission from ref.[88] Copyright © 2021, Elsevier. (C) Schematic on the overview of the presented work: (C, I) microfluidics device integrated with screen-printed electrodes and laser-induced graphene (LIG) heater; (C, II) on-chip incubation of bacteria; (C, III) simultaneous electrochemical detection of bacteria; (C, IV) confirmation of viability through fluorescence imaging; (C, V) validation of metabolic activity by employing the cultured bacteria for a microbial fuel cell (graph represents the polarization curves). Adapted from ref.[89] Abbreviations: MNP: magnetic nanoparticle; ATP: adenosine triphosphate; FTA: flinders technology associates; RPA: recombinase polymerase amplification; LAMP: loop-mediated isothermal amplification; LIG: laser-induced graphene. 3D printing enables the multi-layer assembly of microfluidic chips in an automated manner, accelerating the development of complex sample processing for bacterial detection.

QDs by anodic stripping voltammetry (ASV). Yin et al.[83] developed a portable fluorescent sensor capable of rapid detection of various pathogenic bacteria (Figure 2B), using *E. coli, S. aureus*, and *P. aeruginosa* as target bacteria.. The transparent photosensitive resin was selected as the 3D printing material to prepare the microchannel device. The magnetic core-shell molecular imprinted polymer, using $Fe_3O_4$ and $Fe_3O_4$@$SiO_2$ as the matrix material, was fixed in the detection area by a magnetic field. It is then combined with a smartphone and an external lens to form a fluorescent sensor. This designed 3D model was used to enable the specific adsorption and detection of bacteria by magnetic core-shell molecular-imprinted polymers and fluorescent probes. Bacterial detection procedures, including incubation, enrichment, isolation, washing, and detection, were performed in the 3D model. The sensor has the advantages of low cost, high sensitivity, good specificity, easy operation, and fast response.

In addition, 3D printing technology also plays a role in sample treatment and signal recognition for bacterial biosensors. Zhang et al.[84] designed a quantitative sensing system based on 3D-printed Ag ion-selective electrodes for rapid and sensitive detection of bacteria in biological fluids that can be used for diagnosis such as urinary tract infections. This bacterial detector consists of three parts: a 3D-printed Ag-selective electrode (signal detection and transduction), a potentiometer (sensing and data processing), and a 3D-printed filter (sample preparation). Due to charge interactions, some of the extracellular Ag can bind directly to the bacterial cell wall; therefore, by detecting the loss of Ag added to the bacterial solution, the number of bacteria can be detected. Figure 2C features a biodegradable sensor system integrated with a triboelectric nanogenerator (TENG) for the detection of common pathogenic bacteria (*E. coli*) in the environment.[85] In this system, *E. coli* is detected by a simple carbohydrate-protein interaction mechanism using D-mannose functionalized 3D-printed polylactic acid (PLA) and brushed Agelectrodes. The adsorption capacity of D-mannose is usually altered by changing the concentration of *E. coli*, resulting in changes in electrical resistance. Therefore, the proposed biosensor can detect bacterial concentration by monitoring the output current.

### 4.2. Microfluidic chips

3D printing technology can accurately fabricate microfluidic chips with complex microchannel structures for bacterial detection, thereby simplifying the experimental process and improving detection efficiency. Microfluidic chips usually refer to miniaturized devices that engrave 10–100 μm microchannels in the chip, which can control the pattern of channels and the flow of fluids to achieve rapid and accurate detection of microorganisms. The tiny size concentrates chemicals in the chip, thus improving detection efficiency. The multilayer assembly of microfluidic chips, which can be realized with the assistance of automated 3D printing, accelerates the development of complex sample treatment for bacterial detection.

Park et al.[86] developed a bacterial reactor for sample processing displays by utilizing 3D printing to fabricate a 3D microfluidic magnetic preconcentrator (3DμFMP) with millimeter-scale features (Figure 3A); the all-in-one plastic device could selectively concentrate 100 mL of *E. coli* 700-fold in 1 h. Additionally, the device can withstand high temperatures and extreme pH values, and it can be recycled via chemical sterilization. Kim et al.[87] also reported a 3D-printed microfluidic platform that enabled bacterial preconcentration and purification of genomic DNA to improve the detection limit for molecular diagnostics of target pathogens in blood samples.

Besides sample processing, 3D-printed microfluidic chips can also be used for signal recognition and transduction in bacterial detection. Feng et al.[78] designed a microfluidic chip to detect multiple pathogens simultaneously using light. Their microfluidic chips are fabricated using 3D printing technology and can be modified for specific pathogens. The chip is divided into four sections, each dedicated to the detection of a specific pathogen. If the specific pathogen is present in the sample, it binds to the assay surface, the interaction of which transduces into an optical signal. The chip features an aptamer-based nano-interferometer that allows researchers to rapidly detect a variety of common bacteria at very low concentrations, such as *E. coli, S. typhimurium, L. monocytogenes,* and *S. aureus.* It has high sensitivity and specificity, with a detection limit of 10 CFU/mL, providing a cost-effective platform for distinguishing and screening different foodborne pathogens. Yin et al.[88] fabricated an integrated microfluidic chip using a clear methacrylate-based resin through 3D printing technology to rapidly detect SARS-CoV-2 and human intestinal pathogens in wastewater. It integrates nucleic acid extraction, two-stage isothermal amplification, and colorimetric detection on a chip (Figure 3B). The colorimetric signal during nucleic acid amplification is recorded in real time and analyzed by a programmed smartphone without the need for sophisticated equipment. Srikanth et al.[89] developed a simple, cost-effective, and miniaturized lab-on-a-chip platform to simultaneously culture and detect bacteria (Figure 3C). The temperature required for bacterial culture was provided by an optimized laser-induced graphene heater. A microfluidic chamber was integrated into a screen-printed electrode for electrochemical detection

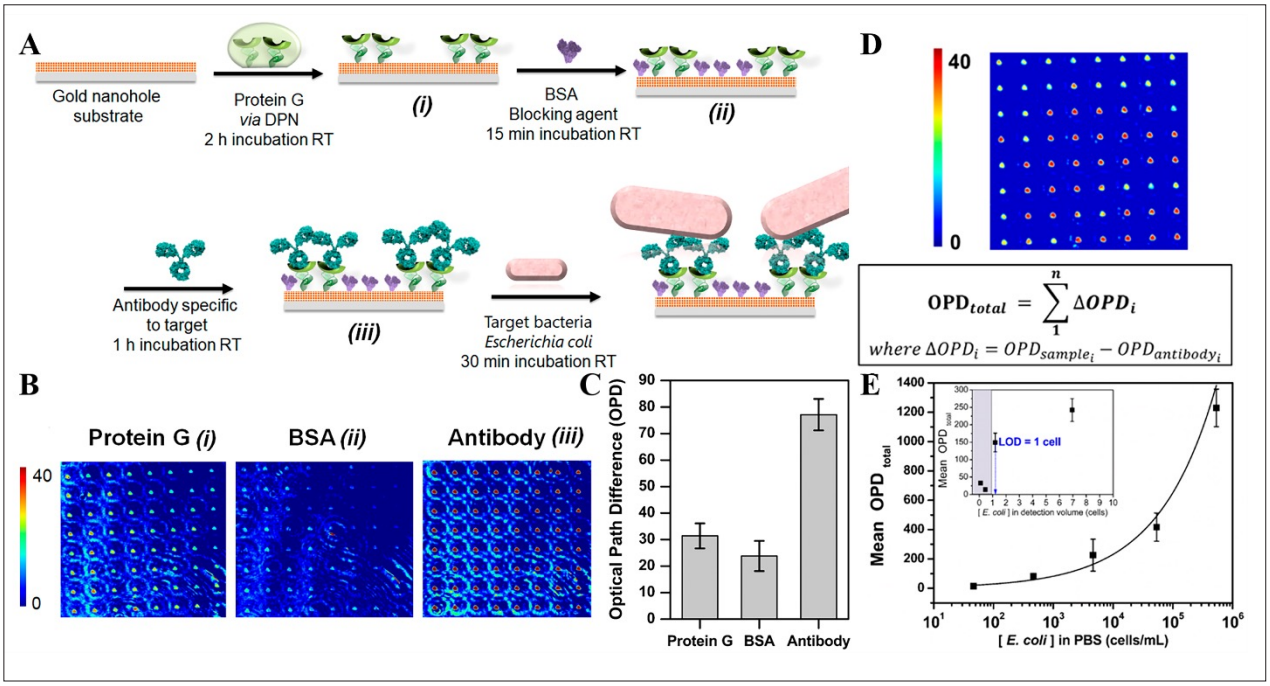


**Figure 4.** Bioprinting microarray technology for bacterial detection. (A) Biofunctionalization strategy (scheme not to scale). (B) Representative interferometric images for protein G spotting (B, i), BSA blocking (B, ii), and antibody immobilization, (B, iii) with the false color image indicative of the optical path difference (OPD) intensity. (C) Mean OPD signal (± standard deviation [SD]) obtained for inter-array (8 × 8, i.e., 64 microspots). (D) Interferometric image after *E. coli* incubation and the equation used to calculate the OPDtotal. (E) Calibration curve of E. coli in phosphate-buffered saline (PBS) without preconcentration (i.e., without bacteria enrichment) in a wide dynamic range of 10–106 cells/mL. (E, inset) Plot indicating the experimental limit of detection (LOD) in terms of cells in a constant detection volume of 150 µL. Adapted with permission from ref.[90] Copyright © 2019, American Chemical Society. 3D printing technology can precisely immobilize biological probes to form high-density microarray structures. These microarrays can specifically bind to target bacterial molecules to achieve rapid detection and identification of bacteria. Abbreviations: DPN: dip-pen nanolithography; RT: room temperature; BSA: bovine serum albumin; OPD: optical path difference; PBS: phosphate-buffered saline.

of bacteria. Through 3D packaging, a handheld device integrated with a portable potentiostat was realized, expanding practical applications.

### 4.3. Bioprinting microarray technology

By using 3D printing technology, biological probes (antibodies, DNA fragments, etc.) can be precisely fixed onto tiny matrices to form high-density microarray structures. These microarrays enable specific binding to target bacterial molecules to realize rapid detection and identification of bacteria.

Dey et al.[90] reported a portable point-of-care testing device based on lensless interference microscopy (LIM). Using high-performance nanoplasma technology and customized bioprinted microarrays, the device enables direct quantification of label-free bacteria (*E. coli*). The entire process from sample treatment to data processing was completed in only one operation within 40 min. As displayed in Figure 4A, protein G was first bioprinted onto a clean hydrophilic gold nanopore surface, using bovine serum albumin (BSA) as a blocking agent for non-specific absorption. The substrate was subsequently incubated with an *E. coli*-specific polyclonal antibody at a concentration of 500 µg/mL. After rinsing and drying the array, *E. coli* was incubated for quantification. Figure 4B and C indicates that the array generated after bioprinting protein G was visible, and an OPD value of about 30 was observed, confirming its binding to the substrate. Biomolecule height, surface density, and accumulated mass are reflected in the OPD signal. When BSA is anchored to the gold surface, the blocking step results in a decrease in the OPD signal, which effectively reduces the spot height, and finally a significant increase in the OPD value due to antibody attachment to protein G microspots. In this work, a high sensitivity of 1 bacterial cell was achieved by calculating the total OPD enhancement and testing the relationship between the concentration of *E. coli* in phosphate-buffered saline (PBS) and the average total OPD value (Figure 4D and E). The assay involves direct label-free quantification of *E. coli* without sample pretreatment, such as PCR, and/or the use of secondary antibodies or markers, providing quantification

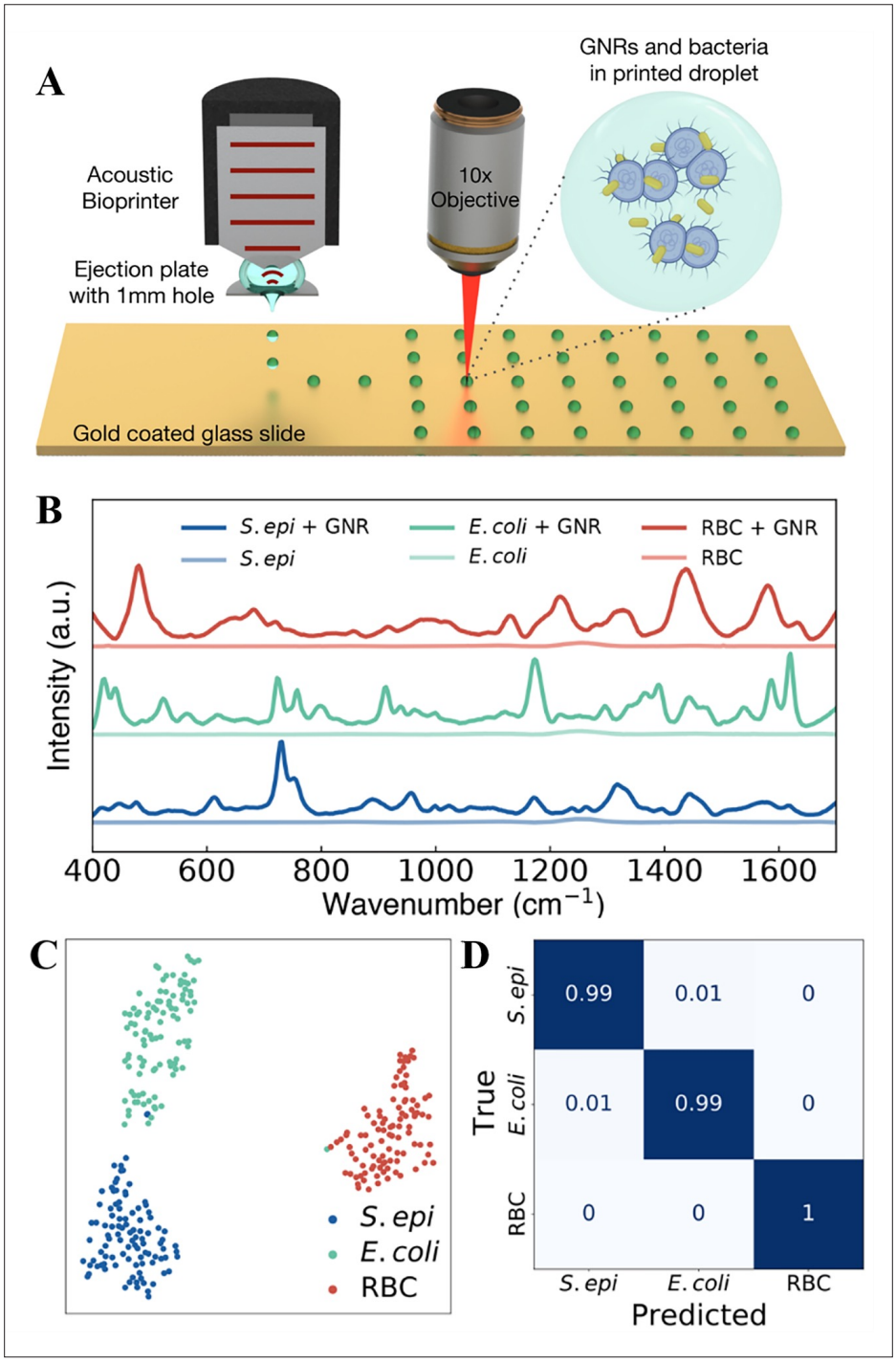


**Figure 5.** Combining AI and spectral technology for bacterial detection. (A) Schematic of acoustic printing platform and confocal Raman setup. (B) Mean surface-enhanced Raman spectroscopy (SERS) spectra of 100 measurements, each taken from single droplets printed from three cell lines (*S. epidermidis*, *E. coli*, and red blood cells [RBCs]) mixed with gold nanorods (GNRs). (C) A two-component t-distributed stochastic neighbor embedding (t-SNE) projection across all 300 Raman spectra acquired from droplets printed with GNRs. Data is plotted after performing a 24-component principal component analysis (PCA) for dimensionality reduction. (D) Normalized confusion matrix generated using a random forest classifier on the 300 spectra collected from single cell-line droplets of *S. epidermidis*, *E. coli*, and mouse RBCs mixed with GNRs. Adapted from ref.[95] Abbreviations: GNRs: gold nanorods; RBC: red blood cell; SERS: surface-enhanced Raman spectroscopy; t-SNE: t-distributed stochastic neighbor embedding; PCA: principal component analysis.

through a simple one-step process and demonstrating significant potential in practical applications.

Al-Taie et al.[91] used 3D printing to fabricate novel sampling matrices for the detection of respiratory pathogens in exhaled air. Fused deposition modeling was used to print polyvinyl alcohol (PVA) and create a multilayer matrix to trap bacteria and enhance bacterial capture efficiency. Quantitative assessment of bacterial recovery and detection of PVA sampling substrates was subsequently performed using quantitative PCR (qPCR), suggesting that the use of a PVA matrix could effectively recover mycobacteria. Compared with gelatin, PVA matrix is more environment-friendly due to its higher solubility in distilled water.

### 4.4. Combining artificial intelligence and spectral technology

The detection of bacteria by artificial intelligence (AI) is mainly based on ML, deep learning, and other algorithms. By training and learning with a large number of bacterial samples, the automatic identification and classification of bacteria can be realized based on extracted key information of bacteria.[92] For example, Nehal et al.[93] utilized a highly sensitive lab-on-a-chip with deep learning AI for the detection of bacteria in water. Cui et al.[94] developed an AI-assisted smartphone-based colorimetric biosensor for visual, rapid, and sensitive detection of pathogenic bacteria by measuring the hyaluronidase secreted by bacteria. The combination of 3D bioprinting technology with AI and spectral analysis technology can further improve the intelligent level of bacterial detection. AI algorithms can analyze complex biological signals and assist diagnostic decision-making, for which spectroscopic techniques, such as Raman spectroscopy and fluorescence spectroscopy, can provide bacterial molecular fingerprints and enhance the specificity and sensitivity of detection.

Safir et al.[95] developed an acoustic bioprinter, as shown in Figure 5A, which uses acoustic bioprinting technology in combination with Raman spectroscopy to achieve high-throughput identification of bacteria in blood. In this method, samples are digitized into single cells or a few cell droplets by acoustic bioprinting; each droplet is then rapidly detected using Raman spectroscopy, and the results are finally classified using ML to achieve the purpose of non-culture detection of pathogens in complex multicellular samples. As displayed in Figure 5B, the detection of *S. epidermidis*, *E. coli*, and mouse red blood cells indicated that almost no Raman signal was observed in droplets without gold nanorods (GNRs). Machine analysis was subsequently performed (Figure 5C); there were clear sample differences between each data set and cell type. Multiclass analysis of complex samples using a random forest classifier validated that the classification accuracy of all samples is ≥99% (Figure 5D).

In summation, 3D printing has great application potential in bacterial detection due to its outstanding intrinsic features. However, the limitations of the technique warrant further research in the field of 3D printing to advance its application in bacterial detection. When using 3D printing technology for bacterial detection, the materials involved should have a certain degree of biocompatibility and stability to ensure the activity and accuracy of bacteria in the detection process. In addition, the issue of equipment cost remains significant. Although the cost of 3D printing equipment is gradually decreasing, high-performance 3D printing equipment is still expensive. Compared with traditional biosensors, microfluidic chips, and other manufacturing technologies, 3D printing technology is still not sufficiently mature. The application of 3D printing technology to bacterial detection also requires certain professional knowledge and skills, including 3D modeling knowledge, material selection, printing parameter setting, and complex data processing ability.

## 5. Conclusion and future perspectives

3D printing technology, as an emerging manufacturing method in the biological field, has demonstrated great potential in life sciences and exhibited its unique advantages for bacterial detection. It can act as a sample treatment tool, signal recognition element, signal transporter, signal amplification pathway, and bioreaction carrier, all of which enable 3D printing technology to achieve more efficient, sensitive, and accurate bacterial detection. Personalized devices fabricated by 3D bioprinting can be customized according to specific detection needs, further accelerating bacterial detection in various scenarios.

However, several challenges remain. For example, optimizing printing parameters and material selection to improve the detection sensitivity and accuracy continues to be a long-standing challenge in bacterial detection. Additionally, the cost and time should also be considered, particularly the high cost of high-precision equipment and biomaterials, which limit large-scale production and widespread application of 3D printing. Moreover, the lack of standardized technical protocols and testing procedures can lead to discrepancies between laboratories or research institutions, affecting the accuracy and repeatability of results.

With the development of materials science, bioinformatics, AI, and other technologies, 3D printing technology has a broad application prospect in bacterial detection. Hence, we anticipate that 3D printing will adopt following enhancements for bacterial detection:

(i) Material innovation: More types of biomaterials and composites with higher performance will be developed to meet the specific needs of bacterial detection.

(ii) Combination of 3D printing and other advanced technologies: Advanced techniques (nanotechnology, gene editing technology, etc.) will be developed to obtain more efficient, accurate, automated, and integrated bacterial detection platforms.

(iii) Intelligent control and big data proceeding: The introduction of AI and ML can be further adapted to assist with bacterial detection.

(iv) Optimization: The standardization and normalization of 3D printing technology in the field of bacterial detection, along with establishing unified technical standards and operating procedures, would enhance the reliability and comparability of test results.

In conclusion, 3D printing technology is rapidly advancing in the field of bacterial detection. This review provides a comprehensive overview of 3D printing technology in bacterial detection, with the aim of offering a scientific reference and insight for bacteria-related studies. With advancements in technological development and interdisciplinary research, we believe that 3D printing technology will make significant contributions and breakthroughs in the field of bacterial detection in the future.

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## Conflict of interest

The authors declare no conflicts of interest.

## Author contributions

*Conceptualization:* All authors
*Writing – original draft:* Tingting Cui
*Writing – review & editing:* Ting Cao

## Ethics approval and consent to participate

Not applicable.

## Consent for publication

Not applicable.

## Availability of data

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