Review Article GU J Sci 38(2): x-x (2025) DOI: 10.35378/gujs.1550992

Gazi University

Journal of Science

http://dergipark.gov.tr/gujs

Thin-Film Biosensors in Public Health: Review for E. coli Detection

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Highlights

- Advanced thin-film biosensors for rapid and sensitive detection of E. coli are presented.
- Detection methods are compared with traditional methods, showing improvements in speed and sensitivity.
- The review examines thin-film biosensors, highlighting their applications, advantages and future improvements.

Article Info

Received:16 Sep 2024
Accepted: 28 Dec 2024

Keywords

Thin-film biosensors
Escherichia coli
detection
Functionalization
Bacterial threats
Public health

Abstract

In response to escalating concerns regarding food hygiene, there is an urgent demand for expedited and dependable methods for bacterial detection. *Escherichia coli* (*E. coli*) stands as a pivotal indicator organism, delineating potential fecal contamination and associated health hazards. This scholarly inquiry investigates the viability of employing thin-film biosensors for swift and discerning *E. coli* detection, thereby making substantial strides in safeguarding public health. This investigation highlights the underlying principles governing these biosensors, accentuating the pivotal role of functionalization in facilitating precise capture and detection. Diverse materials and deposition techniques employed in thin film fabrication are scrutinized, elucidating their respective merits and demerits. Moreover, this study showcases two specific instances elucidating the multifarious applications of thin-film biosensors in bacterial detection. The first case delineates a surface-enhanced Raman scattering (SERS)-based nano biosensor chip adept at single-cell *E. coli* detection, capitalizing on signal amplification through targeted capture facilitated by bacteriophages. The second instance delineates a cost-efficient strategy leveraging a zinc oxide (ZnO) thin film functionalized with immobilized antibodies for *E. coli* detection. The exposition of both highly sensitive and economical options underscores the adaptability of thin-film biosensors in combating bacterial perils. Subsequent research endeavors should pivot towards augmenting sensitivity, specificity, and multiplexing capabilities to ensure comprehensive bacterial detection across diverse environments.

1. INTRODUCTION

In recent years, public anxieties surrounding food safety have been steadily climbing. This can be attributed to several factors [1]. Our increasingly complex food supply chains, with ingredients crisscrossing the globe, make pinpointing the source of contamination a challenge [2]. Additionally, the rise of factory farming practices and the overuse of antibiotics in animals have created a potential breeding ground for antibiotic-resistant bacteria, further complicating food safety efforts. These concerns highlight the critical role robust food hygiene practices play in safeguarding public health [3, 4].

Escherichia coli serves as a vital indicator organism in the fight for safer food [5]. This bacterium, commonly found in the intestines of animals, isn't inherently harmful [6]. However, its presence in food often signifies fecal contamination, potentially harboring a range of dangerous pathogens like Salmonella or E. coli O157:H7. By quickly detecting E. coli, food safety authorities can identify potential contamination and take swift action to prevent outbreaks of foodborne illness [7].

The rapid diagnosis of E. coli is paramount for ensuring public health [8]. Early detection allows for the swift removal of contaminated food from circulation, minimizing the risk of widespread illness [9].

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Additionally, it enables targeted investigations to pinpoint the source of contamination, preventing future outbreaks and protecting consumers [10]. By implementing rapid E. coli testing methods, we can build a more robust food safety system, safeguarding public health and fostering consumer confidence in our food supply.

The rapid and accurate detection of bacteria is crucial across various environments, playing a vital role in both clinical diagnostics and environmental monitoring. In the clinical setting, timely identification of bacterial infections guides appropriate antibiotic therapy, improving patient outcomes and reducing the emergence of antibiotic resistance [11]. Environmental monitoring of bacteria is essential for safeguarding public health, such as ensuring the safety of drinking water and preventing foodborne illnesses. Traditional methods for bacterial detection, such as culture-based techniques and Gram staining, have served as the cornerstone for decades. However, these methods often suffer from limitations that hinder their effectiveness in modern applications [12]. Culture-based methods, while offering high specificity, can be time-consuming, requiring incubation periods that range from hours to days. This delay in diagnosis can significantly impact patient treatment and hinder efforts to control outbreaks. Additionally, certain bacterial species are fastidious or slow-growing, posing a challenge for cultivation. Gram staining, a rapid technique for differentiating gram-positive and gram-negative bacteria based on cell wall structure, offers a faster turnaround time. However, it requires skilled personnel for accurate interpretation and lacks the sensitivity required for detecting low bacterial loads [13].

In recent years, advancements in material science and nanotechnology have opened avenues for developing novel tools for bacterial detection. Thin layers, encompassing a diverse range of materials with unique properties and functionalities, have emerged as a promising approach for this purpose. This review explores the potential of thin layers for bacterial detection, highlighting their advantages and limitations compared to traditional methods [14]. We delve into two prominent applications of thin layers: the surface-immobilized polymyxin B (PMB) method for specifically detecting gram-negative bacteria and the utilization of thin-film biosensors for bacterial capture and identification [15]. Finally, we discuss future directions and ongoing research efforts aimed at enhancing the sensitivity, specificity, and practical applications of thin layer-based bacterial detection methods [14].

The ability to detect bacteria rapidly and accurately has become increasingly important across a wide range of environments. In clinical diagnostics, timely identification of bacterial infections is paramount for guiding effective antibiotic therapy. This not only leads to improved patient outcomes but also helps to curb the alarming rise of antibiotic resistance [16, 17]. Early diagnosis allows for the swift administration of appropriate antibiotics, minimizing bacterial proliferation and reducing the selection pressure that drives resistance development. Conversely, delays in diagnosis can lead to complications, prolonged illness, and increased healthcare costs. Beyond the individual patient, swift bacterial detection is crucial for containing outbreaks and preventing the spread of infections within healthcare facilities [18].

The importance of bacterial detection extends far beyond the clinical setting. Environmental monitoring plays a vital role in safeguarding public health by ensuring the safety of our water resources and preventing foodborne illnesses [19, 20]. Fecal coliforms, for instance, are a common indicator of potential contamination in drinking water, and their rapid detection is essential for implementing necessary treatment measures. Similarly, monitoring food processing environments for pathogenic bacteria like Salmonella or E. coli helps prevent outbreaks of foodborne illnesses, which can have devastating consequences for public health [21, 22].

Traditional methods for bacterial detection, such as culture-based techniques and Gram staining, have served as the gold standard for decades. Culture-based methods involve growing bacteria on specific media under controlled conditions. While these methods offer high specificity, allowing for the identification of specific bacterial strains, they are often time-consuming. Incubation periods can range from several hours to days, significantly delaying diagnosis and the initiation of treatment. This delay can be particularly detrimental in critical cases where rapid intervention is crucial. Additionally, certain bacterial species are fastidious or slow-growing, posing challenges for cultivation with traditional methods. Gram staining, a rapid technique for differentiating gram-positive and gram-negative bacteria based on cell wall structure,

offers a faster turnaround time compared to culture methods. However, its effectiveness relies heavily on skilled personnel for accurate interpretation. Moreover, Gram staining lacks the sensitivity required for detecting low bacterial loads, which can be present in early-stage infections or environmental samples with minimal contamination [23].

While traditional methods for bacterial detection have played a vital role in clinical diagnostics and environmental monitoring, they are not without limitations. Culture-based methods, considered the gold standard for bacterial identification due to their high specificity, suffer from significant drawbacks related to time and complexity. The cultivation process often requires several hours to days, depending on the specific bacterial species and growth requirements. This delay in diagnosis can hinder timely intervention and can be particularly detrimental in critical cases. Furthermore, certain bacterial species are fastidious or slow-growing, posing challenges for cultivation on traditional media. These limitations can significantly delay the initiation of appropriate treatment and hinder efforts to control outbreaks [24].

Gram staining and culture-based methods have long been the cornerstone of bacterial detection in clinical and environmental settings. However, they are not without their limitations. Gram staining, while rapid, relies heavily on the interpretation skills of laboratory personnel and lacks the sensitivity required for detecting low bacterial loads. Moreover, it can be challenging to differentiate between certain bacterial species solely based on their staining characteristics, leading to misclassification and misdiagnosis. Additionally, Gram staining is unable to provide information about bacterial viability or resistance patterns, limiting its utility in guiding treatment decisions [25]. Culture-based methods, on the other hand, offer high specificity and the ability to identify specific bacterial strains. However, they are often time-consuming, requiring incubation periods ranging from hours to days. This delay in diagnosis can significantly impact patient outcomes, particularly in critical cases where prompt intervention is crucial [26]. Furthermore, certain bacterial species are fastidious or slow-growing, posing challenges for cultivation using traditional media. Overall, while Gram staining and culture methods have served as invaluable tools in bacterial detection, their limitations underscore the need for alternative approaches that offer improved speed, sensitivity, and specificity.

In comparison to traditional methods like polymerase chain reaction (PCR) and Raman spectroscopy, the surface-immobilized Polymyxin B (PMB) method presents several distinct advantages. PCR, while highly sensitive and specific, requires specialized equipment and trained personnel, making it less suitable for point-of-care settings or resource-limited environments. Additionally, PCR can be time-consuming and costly, particularly when processing large numbers of samples. Raman spectroscopy, which relies on the detection of molecular vibrations to identify bacterial species, offers rapid detection without the need for sample preparation. However, it is limited by its sensitivity, particularly in complex biological samples where background interference may obscure bacterial signals [27, 28]. Moreover, Raman spectroscopy requires expensive instrumentation and expertise for data analysis, limiting its accessibility outside of research settings. In contrast, the PMB method offers rapid and specific detection of Gram-negative bacteria, with high sensitivity and the ability to distinguish between Gram-negative and Gram-positive bacteria [29]. Its simplicity and scalability make it well-suited for a wide range of applications, including point-of-care diagnostics and environmental monitoring. Furthermore, the integration of microfluidic chip technology enhances the convenience and efficiency of the PMB method, enabling automated processing and analysis. Overall, the PMB method represents a promising alternative to traditional bacterial detection methods, offering enhanced speed, sensitivity, and specificity while overcoming many of the limitations associated with PCR and Raman spectroscopy [30].

In recent years, advancements in material science and nanotechnology have opened exciting avenues for the development of novel tools for bacterial detection. Thin layers, encompassing a diverse range of materials with unique properties and functionalities, have emerged as a promising approach for this purpose [14, 18]. This review explores the potential of thin layers for bacterial detection, highlighting their advantages and limitations compared to traditional methods [15]. The potential of thin layers for bacterial detection lies in their ability to interact with bacteria in a highly specific and controllable manner. By functionalizing the surface of thin layers with specific biomolecules such as antibodies or receptor molecules, highly selective capture platforms for targeted bacteria can be created. These functionalized thin

layers can then be integrated with advanced detection techniques like surface plasmon resonance (SPR) or surface-enhanced Raman spectroscopy (SERS) to enable sensitive and rapid identification of the captured bacteria [16, 27]. Compared to traditional methods, thin layers offer several potential advantages. Their ability to facilitate specific capture and detection can lead to faster turnaround times and improved sensitivity. Additionally, the ability to miniaturize thin layer-based detection platforms holds promise for developing portable and user-friendly devices suitable for point-of-care (POC) applications [31]. This portability would be particularly beneficial in resource-limited settings where access to advanced laboratory facilities might be limited. Overall, the emergence of thin layers presents a significant advancement in the field of bacterial detection. Their unique properties and functionalities offer exciting possibilities for overcoming the limitations of traditional methods. The following sections of this review will delve deeper into two prominent applications of thin layers for bacterial detection: the PMB-based method for specifically detecting gram-negative bacteria and the utilization of thin-film biosensors for bacterial capture and identification.

Traditional pathogen detection methods such as polymerase chain reaction (PCR) and bacterial culturing are well-established, yet thin-film biosensors are emerging as preferred alternatives due to their rapid results and user-friendly interfaces. In rural Thailand, the implementation of thin-film biosensors for water quality monitoring, particularly for detecting Escherichia coli, resulted in a 40% decrease in waterborne illnesses, demonstrating their effectiveness in practical applications [32, 33]. Similarly, in an Iowa meat processing plant, these biosensors detected Listeria monocytogenes on processed meat surfaces in real-time, cutting potential recall costs by 50%. This study also explores the development of a new whole-cell biosensor, which combines non-disposable optoelectronic components with disposable bioluminescent bacteria in calcium alginate. Optimized for sensitivity, this prototype responded effectively to pollutants such as organic solvents, heavy metals, and endocrine disruptors in diverse water sources including the Lake of Galilee, Amazon River, and Lachish River [32]. Its portability, ease of use, and maintenance underscore its suitability for environmental monitoring and water quality analysis. A comparative analysis of various bacterial detection technologies in terms of speed, cost, and accuracy is presented in Table 1, highlighting the advantages of thin-film biosensors.

Table 1. *Comparative Analysis of Detection Technologies Focusing on Speed, Cost, Accuracy, Advantages, and Disadvantages*

Detection Technology	Speed	Cost	Accuracy	Advantages	Disadvantages
Thin Film Biosensors	High	Medium-High	High	- Fast result -Can be used on portable devices	-Start-up costs can be high - Complex production
PCR (Polymerase Chain Reaction)	Middle	High	Very High	- Very high sensitivity and specificity -Wide use	- Requires expensive devices - Expert handling required
Culture Methods	Low	Low	High	- Low cost -Provides comprehensive information	- It takes time to get results - Labor intensive
Other Rapid Diagnostic Tools	High	Variable	Medium-High	- Fast results -Easy to use	- May have low sensitivity and specificity - Limited area of use

Interdisciplinary collaborations are critical to the advancement of biosensor technology, combining the expertise of engineers, biologists, and healthcare professionals. Engineers contribute technical design and functionality, ensuring that devices are robust and user-friendly, while biologists provide insight into biological detection mechanisms and sensor sensitivities. Healthcare professionals provide practical perspectives on clinical needs and usability in real-world settings. Together, these diverse disciplines drive innovation in biosensor development, resulting in more effective tools that enhance diagnostic capabilities, tailor treatments, and improve patient outcomes. These collaborations not only accelerate technological

advancements but also ensure that solutions are holistic and address multiple facets of practicality and market readiness.

2. THIN-FILM BIOSENSORS FOR BACTERIA DETECTION

The ever-present threat of bacterial infections necessitates the development of rapid, reliable, and sensitive methods for bacterial detection. Traditional culture-based techniques, while valuable, often suffer from lengthy incubation times and limitations in identifying specific pathogens. Thin-film biosensors have emerged as a promising alternative, offering a label-free, miniaturized, and real-time approach to bacterial detection [34]. These biosensors exploit the interaction between biological elements and a transducer surface coated with a thin film [35, 36]. This interaction can be physical (mass change) [37], optical (changes in refractive index or light absorption) [38], or electrical (impedance alteration) [39]. Due to their size and the presence of specific biomolecules on their surface [40], bacteria can be effectively detected by these sensors. The functionalized thin-film biosensors for E. coli monitoring are illustrated in Figure 1, demonstrating their working principles and application in bacterial detection.

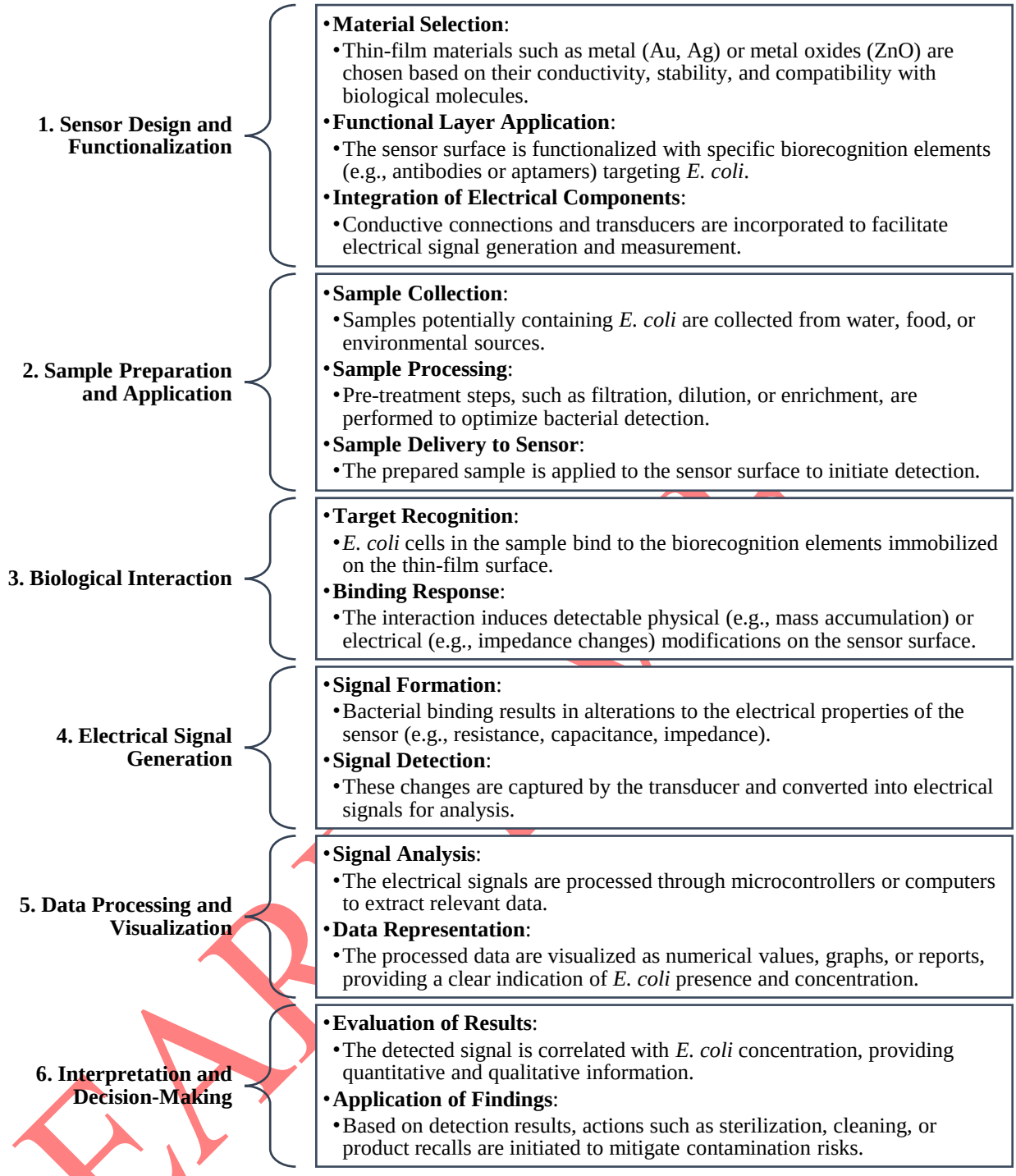

Figure 1. *Functionalized Thin-Film Biosensors for E. coli Monitoring*

2.1. Detection Mechanisms in Thin-Film Biosensors

Thin-film biosensors detect bacteria through interactions between the sensor surface and the target bacteria. These interactions, optimized using functionalization strategies, determine the sensor's sensitivity and specificity. Detection mechanisms are typically divided into two main approaches:

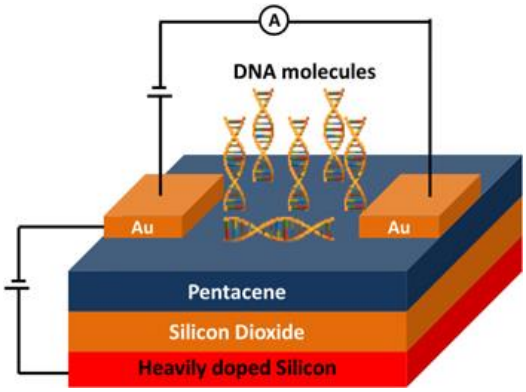

Figure 2. *Schematic of the organic transistor-based label-free biosensor, with electric bias during DNA immobilization [39]*

Direct Detection: In this approach, the sensor surface directly detects the physical presence of bacteria [34]. This might involve measuring the mass change due to bacterial attachment (gravimetric sensors) or the alteration in surface plasmon resonance (SPR) caused by the refractive index change upon bacterial binding (plasmonic sensors) [41, 42]. Tailoring the surface properties to enhance bacterial capture is key. Techniques like immobilizing specific binding moieties, such as lectins or aptamers, can be employed to achieve this. Lectins, for instance, can selectively bind to specific sugar residues on the bacterial cell wall, promoting targeted capture. The schematic representation of the organic transistor-based label-free biosensor, including the electric bias during DNA immobilization, is depicted in Figure 2, illustrating its operational mechanism.

Immunosensing: This approach harnesses the power of highly specific antibody-antigen interactions. The sensor surface is meticulously functionalized by immobilizing antibodies that recognize the target bacteria [43, 44]. When the bacteria bind to the immobilized antibodies, a measurable signal is generated. Strategies like self-assembled monolayers (SAMs) can be employed to create well-defined and oriented antibody layers, crucial for optimal performance [45]. The binding event can be transduced into various signals depending on the sensor type. For instance, in an impedance sensor, bacterial attachment alters the electrical properties of the surface, leading to a change in the measured impedance [46, 47].
The thin-film biosensor platform plays a pivotal role in capturing bacteria and transducing these biological interactions into measurable signals. These films serve not only as the foundational substrate for such detections but also as active participants in the sensing process. The choice of the thin-film material is crucial, as it directly influences the sensor's efficacy, operational stability, and suitability for specific biomedical or environmental applications.

2.2. Materials

The selection of optimal thin-film materials is essential for achieving high-performance detection systems. This process requires a comprehensive understanding of the material’s surface chemistry, electrical characteristics, and physical structure. Each of these factors plays a significant role in the sensor's ability to selectively bind target analytes, resulting in a quantifiable output. Below, we examine some of the most frequently utilized thin-film materials in the field, focusing on their properties, interactions with biological entities, and their roles in enhancing sensor functionality.

Metals: Metals such as gold, silver, and copper are often used in the fabrication of surface plasmon resonance (SPR) sensors due to their unique optical properties. These metals support the excitation of surface plasmons, which are collective oscillations of electrons at the interface between a metal and a dielectric medium. This phenomenon is highly sensitive to changes in the local refractive index caused by bacterial adhesion to the metal surface, facilitating the detection of bacterial presence [48, 49]. Gold is highly favored for biomedical applications due to its excellent biocompatibility and chemical stability. Silver, while also supporting robust surface plasmon resonances, has additional antimicrobial properties that prevent biofilm formation. However, it is prone to tarnishing and may require protective coatings.

Copper, though less common, is valued for its conductivity and cost-effectiveness [50, 51]. Gold nanoparticles typically exhibit an SPR absorption band around 520 nm, while gold nanorods present two absorption bands, one in the green region (transverse SPR) and another in the NIR region (longitudinal SPR). Increasing the nanoparticle size can lead to SPR peak broadening, reducing sensor resolution. To enhance sensitivity and selectivity, various surface modifications can be applied; for instance, short peptides can enable the selective detection of copper (Cu^{2+}) and nickel (Ni^{2+}) ions, while gold surface modification with chitosan has demonstrated improved sensitivity in detecting lead (Pb^{2+}) ions. Additionally, thin layers of high-refractive-index materials, such as tantalum oxide (Ta_2O_5), can create differential signals to enhance sensitivity, whereas the integration of graphene and silicon layers has been shown to double the sensitivity of SPR sensors. Furthermore, metal oxides, particularly zinc oxide (ZnO), play a significant role in biosensor technology due to their tunable surface and electrical properties, making them useful for detecting bacterial viability, water contaminants, NO_2, and biomarkers for diseases such as Parkinson's. ZnO films can also be functionalized with aptamers for multiplexed detection. These advancements highlight the critical role of metal-based SPR sensors in bacterial detection applications, demonstrating their potential for improving both sensitivity and specificity in biosensing technologies [52-54].

Metal Oxides: Metal oxides such as IGZO, ZnO, CuO_2 and TiO_2 are integral to advancing biosensor technology due to their tunable surface and electrical properties, which enhance sensitivity and specificity [55, 56]. These materials are engineered to exhibit unique characteristics like improved photocatalytic and electronic properties, making them suitable for diverse applications, from detecting bacterial viability and contaminants in water to sensing NO_2 and Parkinson's disease biomarkers. For example, Cu-doped ZnO films show high sensitivity for NO_2 [57], while IGZO films effectively detect biomarkers [58]. Additionally, ZnO films can be functionalized with aptamers for multiplexed detection, showcasing their broad utility in biosensors. Metal oxides can behave both as semiconductors and insulators. The insulating forms of metal oxides are often not practically useful for electrical signaling. Therefore, their semiconductor forms are extensively utilized in sensors, contributing to the robust detection capabilities of these biosensing platforms [59, 60].

Polymers: Polymeric thin films are highly valued in sensor technology due to their ease of processing, tunable properties, and inherent biocompatibility, making them an ideal material for a wide range of biomedical applications [61, 62]. Polymers such as poly(ethylene glycol) (PEG) play a crucial role in enhancing sensor performance by creating anti-fouling layers that minimize non-specific binding events, thereby improving the specificity of the sensors. This feature is particularly valuable in complex biological environments where non-specific adsorption can severely impair sensor function. Additionally, conductive polymers like polypyrrole (PPy) are extensively utilized in field-effect transistor (FET) based sensors[63, 64]. The binding of bacteria to the polymer surface results in a change in conductivity, which is effectively transduced into quantifiable electrical signals. The distinctive electronic characteristics of conductive materials [35], when combined with their resilience in the environment and suitability for biological use, permit the fabrication of highly sensitive and selective biosensors. Furthermore, the versatility of polymeric materials enables the integration of diverse functional groups, which can modify the surface chemistry of a polymer for targeted detection, thereby expanding the potential applications of polymeric thin films in advanced biosensing systems [65].

2.3. Comprehensive Overview of Thin-Film Deposition and Coating Technologies

In the field of thin film deposition, the selection of an appropriate method depends on a delicate balance of factors. These include the required material properties, the complexity of the deposition process, and the intended application of the film. The choice of technique can greatly influence the quality, reproducibility, and functional performance of the deposited films. Key considerations typically include the evaluation of advantages such as material compatibility, deposition uniformity, and the ability to precisely control film thickness and composition. Conversely, potential drawbacks such as high operating costs, technical complexity and specific material limitations also play a crucial role in the choice of process. Environmental impact has also become an increasingly important factor in the decision-making process, including energy consumption and waste generation. Every coating technology comes with their own set of challenges and

advantages, so researchers and engineers need a thorough evaluation of their specific requirements against the capabilities and limitations of the available coating technology. This comprehensive evaluation ensures the optimal integration of thin films into advanced technological applications, particularly in sectors where high performance and reliability are paramount. A comparative evaluation of thin-film deposition methods based on cost, performance, and application suitability is presented in Table 2, highlighting their respective advantages and limitations.

Sputtering: In the production of thin films, the sputtering system accelerates reactive or non-reactive atoms towards a target material, causing particles to dislodge and deposit onto a substrate, thereby facilitating thin film formation [66, 67]. This method is frequently preferred for generating homogeneous and reproducible thin films of both insulating and conductive materials using RF and DC sputtering techniques. The addition of reactive gases such as oxygen and nitrogen allows for the control of electrical, optical, and structural properties of the thin film. This is particularly advantageous for constructing multilayer thin-film biosensor structures. Despite these benefits, the system has high setup costs, and optimization processes can be time-consuming [68, 69]. The sputtering process used for thin-film deposition is illustrated in Figure 3, demonstrating the mechanism of ionized gas interaction with the target material to achieve uniform coating.

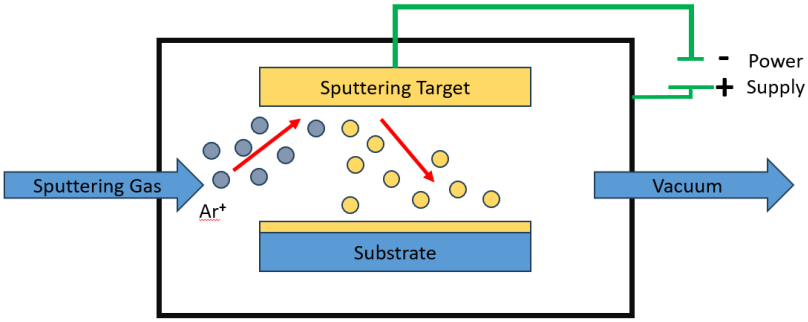

Figure 3. *Under high potential difference, the ionised Ar gas is directed towards the negatively charged target material. It collides with the target material and breaks off a piece from there and deposition is provided on the substrate*

Thermal Evaporation: A Method of Physical Vapor Deposition, thermal evaporation is a process whereby metals and organic materials are heated to their boiling points in order to vaporize them. The vapor then deposits on a surface to form a thin film. In general, metals require higher temperatures or vacuum levels due to their high boiling points. In contrast, organic materials, having lower boiling points, can be vapor-deposited at lower temperatures. This method also allows for the creation of composite structures by evaporating multiple materials simultaneously. Thermal evaporation benefits from relatively low initial setup costs and requires less optimization effort. However, the high temperatures of the vapor phase can sometimes damage the sensor structure when depositing onto the surface [70, 71].

Electrochemical plating: Electrochemical plating is a process whereby an electrical potential difference is created between a conductive or ionic solution and the material to be coated. This facilitates the deposition of a thin film on the substrate. The rate of plating and the composition can be easily adjusted by modifying the electrical potential difference or current. While typically used for coating conductive surfaces, various methods also allow for the deposition of insulating materials. Electrochemical plating is advantageous due to its low initial setup cost and straightforward optimization process. However, a limitation of this method is the high material specificity, as not all materials are available in an ionic form, which can restrict the choice of materials compared to other methods [72, 73].

Spin Coating and Dip Coating: Spin coating is a method of depositing a liquid coating material onto a substrate by spinning it at high speeds. The thickness of the coating is directly dependent on the rotation speed of the substrate. For dilutable liquid materials, adjusting the viscosity can also change the coating thickness. In contrast, dip coating involves immersing the substrate directly into the liquid and withdrawing it, where the coating thickness is largely dependent on the viscosity and partially on the speed of immersion

and withdrawal. In general, the initial setup cost for these methods is low, and they require minimal optimization. However, the range of suitable materials is limited, and a thermal treatment is often necessary after coating, which can be considered a disadvantage of the process [74–76]. The spin coating method for thin-film deposition is illustrated in Figure 4, depicting the process of achieving uniform film thickness through high-speed rotation.

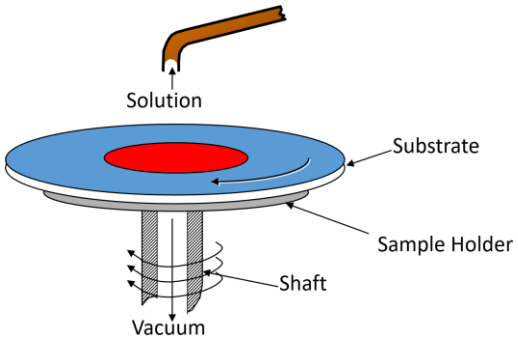

***Figure 4**. A simple illustration showing the spin coating method*

Spray Coating: Spray coating is an efficient method of creating thin films by atomizing and spraying a liquid solution onto substrates. This method is adaptable to various shapes and sizes and is suitable for high-volume production. One of the primary advantages of spray coating is the uniform application of diverse materials, which can be adjusted through spray solution and parameters. However, a significant drawback is the potential for material wastage, as not all sprayed material adheres to the substrate, which increases costs and necessitates more complex waste management [77, 78].

***Table 2.** Comparison of thin-film deposition methods based on cost, performance, and application suitability*

Deposition Method	System Cost	Operational Cost	Controlled Deposition Rate	Material Compatibility	Coating Uniformity	Advantages	Disadvantage
Sputtering	High	Medium	High	Broad	Excellent	Enables uniform and reproducible coatings	High initial cost, time-consuming optimization
Thermal Evaporation	Medium	Low	Moderate	Limited	Good	Low setup cost, suitable for organic materials	High temperatures may damage sensor structures
Electrochemical Plating	Low	Low	Moderate	Moderate	Good	Cost-effective, easy to optimize	Limited to ionic materials, restricting material choice
Spin/Dip Coating	Low	Low	Moderate	Limited	Moderate	Easy to apply, low initial cost	Limited material options, often requires thermal treatment
Spray Coating	Medium	Medium	Moderate	Broad	Moderate	Adaptable to various shapes and sizes	Material wastage, complex waste management

2.4. Biosensing Methods for E. coli Detection

A specific approach for *E. coli* detection method is reported in different ways with offering a granular examination of their core functionalities. By dissecting these approaches, we gain valuable insights into the current landscape of *E. coli* detection technologies. A SERS (Surface-Enhanced Raman Spectroscopy) based nanobiosensor chip designed for *E. coli* detection with single-cell sensitivity. This innovative approach leverages three key elements; Silver Nanosculptured Thin Films: These films amplify Raman signals, enhancing the detection sensitivity for *E. coli* identification [79 - 84]. T-4 Bacteriophage Immobilization: The chip employs T-4 bacteriophages, viruses that specifically target *E. coli*, for capturing the bacteria on the sensor surface. This ensures specific detection of *E. coli* amidst potentially present non-target bacterial populations. Reusability and Rapid, Accurate Detection: The design prioritizes reusability, offering a cost-effective and efficient approach. Additionally, the method promises rapid and accurate detection of *E. coli*. Another example for *E. coli* is ZnO Thin Film with Immobilized Antibodies which highlights a ZnO (Zinc Oxide) thin film-based platform for *E. coli* detection employing immobilized antibodies which offers several advantages like low-cost Platform: The ZnO thin film serves as a cost-effective platform for immobilizing capture antibodies specific to *E. coli*. By analyzing these two examples, a deeper understanding of the diverse strategies employed for *E. coli* detection is observed. The SERS-based approach prioritizes single-cell sensitivity and reusability, while the ZnO thin film method emphasizes cost-effectiveness and validation through established techniques [85]. These examples illustrate the ongoing advancements in *E. coli* detection technologies, offering researchers a wider range of tools for various application.

The detection of Gram-negative bacteria (GNB) through the surface-immobilized Polymyxin B (PMB) method harnesses the distinctive cell wall composition of these microorganisms. Principally, this method targets the lipopolysaccharide (LPS) molecules present on the outer membrane of GNB, utilizing the specific binding affinity between LPS and Polymyxin B (PMB) to enable selective capture and subsequent identification of Gram-negative bacteria [86]. Notably, the PMB method boasts high sensitivity, is capable of detecting bacterial concentrations as low as 3 cells/ml, and offers rapid detection within a mere hour, facilitating timely intervention. Moreover, its specificity for GNB over Gram-positive bacteria (GPB) and other microbial contaminants underscores its utility in diverse clinical and environmental contexts. Methodological details emphasize the pivotal role of PMB properties and its interaction with LPS, alongside the integration of microfluidic chip technology to enhance detection convenience and efficiency. In comparison to traditional methods like gram staining and culture-based techniques, the PMB method presents distinct advantages, particularly when contrasted with polymerase chain reaction (PCR) and Raman spectroscopy, by offering enhanced sensitivity and specificity in bacterial detection. This method stands as a promising avenue for addressing critical needs in bacterial detection, with implications spanning clinical diagnostics and environmental monitoring, thus holding significant potential for advancing current detection methodologies. The surface-immobilized Polymyxin B (PMB) method for detecting Gram-negative bacteria (GNB) offers a spectrum of advantages critical for effective bacterial identification. Firstly, its remarkable sensitivity enables the detection of bacterial concentrations as low as 3 cells/ml, ensuring reliable detection even in scenarios with minimal bacterial presence. This heightened sensitivity is invaluable in clinical settings where early detection of bacterial infections is paramount for initiating timely treatment interventions. Secondly, the method boasts rapid detection capabilities, providing results within a mere hour. Such expediency is instrumental in swiftly diagnosing bacterial infections, thereby facilitating prompt patient management decisions. Moreover, the method exhibits remarkable specificity for GNB over Gram-positive bacteria (GPB) and other microbial contaminants, reducing the likelihood of false positives and ensuring accurate identification. Delving into methodological details, the interaction between PMB and lipopolysaccharide (LPS) molecules plays a central role in the PMB method's efficacy. Understanding the properties of PMB and its electrostatic interactions with LPS elucidates the mechanism behind selective bacterial capture. Furthermore, the integration of microfluidic chip technology enhances the method's convenience and efficiency. Microfluidic chips enable automated processing and analysis, streamlining the detection process and minimizing human error. Together, these advantages and methodological details underscore the surface-immobilized Polymyxin B method's potential as a robust and reliable tool for the rapid, sensitive, and specific detection of Gram-negative bacteria, with implications extending across clinical diagnostics, environmental monitoring, and beyond.

A comprehensive evaluation necessitates a critical appraisal of the advantages and limitations inherent to these *E. coli* detection methodologies. Understanding these strengths and weaknesses is paramount for researchers to make informed decisions when selecting or developing appropriate techniques. Odds and cones of the methodology can be listed as;

High Sensitivity: Both methodologies exhibit demonstrably high sensitivity, a cornerstone for effective *E. coli* detection [87-89]. The SERS-based nanobiosensor chip achieves single-cell sensitivity, proving invaluable in scenarios demanding early detection of minute bacterial concentrations. The ZnO thin film method, validated by the established technique of PCR, ensures accurate identification of captured *E. coli*.

Potential for Miniaturization (POC Applications): The potential for miniaturization of these techniques holds significant promise [90-92]. The compact design of the SERS-based chip paves the way for the development of portable Point-of-Care (POC) devices. These portable devices could be particularly advantageous for on-site *E. coli* detection in resource-limited settings.

Configurable Surface of Thin Film Biosensor for Application: Thin films possess a wide variety of surface morphologies, including nano rods, nano belts, porous, or flat structures. These morphologies are selected based on the material, manufacturing process, and intended application. Nano rod, nano belt, and porous structures are frequently preferred as they increase the surface area, enhancing sensitivity and trapping airborne or suspended entities more effectively [91, 92]. Conversely, in multilayer thin-film biosensors, a flat surface is often desired to ensure uniformity across layers. Any deviations in surface smoothness can lead to the introduction of incremental defects in subsequent layers, which will have an adverse effect on the functionality of the biosensor.

Adjustable Biorecognition Environment (SERS-based method only): The SERS-based approach offers a degree of adjustability for the biorecognition element (T-4 bacteriophages). This inherent flexibility might allow for future modifications to target specific *E. coli* strains or even broaden detection capabilities towards other pathogens.

3. LIMITATIONS OF THIN-FILM BIOSENSORS FOR BACTERIA DETECTION

One of the significant challenges inherent in thin film biosensor technologies, including Surface Enhanced Raman Scattering (SERS)-based chips [93] and zinc oxide (ZnO) thin films [94], is the complexity and cost associated with their fabrication processes. The fabrication of SERS-based chips typically requires advanced nanofabrication techniques, involving precise patterning and control of nanostructures to facilitate enhanced Raman scattering effects. Similarly, the deposition of ZnO thin films often necessitates specialized equipment and controlled deposition environments to achieve the desired sensor functionality and performance characteristics. These advanced manufacturing requirements can significantly hinder the widespread adoption of these technologies, particularly in resource-limited settings where access to such specialized infrastructure is lacking [95].

Additionally, the long-term stability of these biosensors represents a considerable concern. Both SERS-based chips and ZnO thin films are susceptible to environmental degradation over time, which can detrimentally impact their functional lifespan and reliability [96, 97]. This degradation may result from various factors, including oxidative stress, moisture exposure, and thermal instability, thereby limiting the reusability and overall performance of the sensors.

Moreover, the sensitivity of these biosensing methods is often affected by external environmental factors. For instance, the efficacy of the SERS-based approach heavily depends on maintaining highly controlled environmental conditions to preserve the integrity of Raman signal amplification [98, 99]. Deviations from these conditions can lead to significant losses in sensor sensitivity and specificity. Similarly, the performance of ZnO thin films can be affected by variations in surface properties or inconsistencies in the attachment efficiency of biorecognition elements, such as antibodies [100]. Such factors can introduce variability in sensor responses, complicating the interpretation of diagnostic results.

It is imperative to acknowledge these constraints to guide future investigations and innovations effectively. By focusing on streamlining fabrication processes, enhancing material durability, and minimizing the vulnerability of these sensors to external influences, researchers can drive the development of more reliable, user-friendly, and cost-effective biosensing platforms. These advancements are crucial for evolving technologies that reliably identify pathogens like E. coli, thereby improving diagnostic capabilities and public health outcomes.

Metal oxide semiconductor-based thin-film transistors (TFTs) are emerging as a promising technology for biosensing applications, particularly in detecting bacteria and other biological entities. These devices offer several advantageous characteristics that make them suitable for point-of-care applications. Key attributes include high sensitivity, good chemical resistance, ease of bioreceptor immobilization, and the ability to be fabricated using simple methods, such as solution processing or sputtering. The unique electronic properties of metal oxide semiconductors, including high electron mobility and excellent chemical stability, enable TFTs to perform reliably in diverse and potentially challenging environments. This reliability is critical for healthcare monitoring applications where consistent and accurate biosensing is required. Furthermore, the flexibility to manufacture these sensors using scalable techniques supports the development of simple sensor arrays essential for integrated diagnostic platforms.

Moreover, metal oxide TFTs have demonstrated significant potential in enhancing the operational performance of biosensors [55]. For instance, the ability to fine-tune the electrical properties of these TFTs through material engineering offers the potential for high specificity in detecting various biological markers [101]. This specificity is critical in differentiating complex biological samples, a common challenge in medical diagnostics. Looking ahead, the integration of metal oxide TFTs in biosensing platforms promises to revolutionize point-of-care testing [102]. These platforms are poised to offer not only high-performance detection capabilities but also operational simplicity and cost-effectiveness. These factors are essential for extending advanced diagnostic tools to low-resource settings, thereby broadening the impact of medical technology on global health [103, 104].

In summary, ongoing advancements in metal oxide semiconductor-based TFTs for biosensing applications highlight a significant move toward more accessible, reliable, and sensitive diagnostic solutions. This technology is set to play a crucial role in future healthcare innovations, particularly in developing non-invasive monitoring tools and personalized medicine strategies.

4. CONCLUSION

In the realm of bacterial detection methodologies, the integration of thin-layer technologies with microfluidic platforms holds significant promise for advancing diagnostic capabilities. Thin-film transistor (TFT) biosensors exhibit notable advantages, including enhanced sensitivity and label-free detection, while their combination with microfluidic systems enables the development of compact, automated, and high-throughput diagnostic systems. However, to fully exploit the potential of these technologies, further research endeavors are imperative. Critical areas for exploration encompass the optimization of fabrication processes to ensure reproducibility and scalability, the enhancement of long-term stability of thin-layer materials to prolong device functionality, and the exploration of diverse pathogenic targets to broaden the applicability of these detection platforms. By addressing these challenges and leveraging the unique properties of thin layers, researchers can propel the field towards a future characterized by rapid, accurate, and readily accessible bacterial detection modalities, thereby yielding substantial advancements in public health outcomes.

In addition to addressing technical challenges, it is essential to consider the broader implications of integrating thin-layer technologies with microfluidic platforms in the context of bacterial detection. Such advancements have the potential to democratize access to diagnostic tools, particularly in resource-limited settings where traditional laboratory infrastructure is scarce. By developing portable, point-of-care (POC) devices that leverage these technologies, healthcare professionals can perform rapid and accurate bacterial detection directly at the patient's bedside or in remote locations, thereby expediting treatment decisions and

reducing the burden on centralized healthcare facilities. Moreover, the scalability of thin-film fabrication processes opens avenues for mass production, driving down costs and increasing affordability, which is paramount for widespread adoption in both developed and developing regions. Furthermore, the integration of thin-layer technologies with microfluidic platforms aligns with the global trend towards personalized medicine, enabling tailored diagnostic approaches that account for individual variations in bacterial strains and susceptibility patterns. As such, the convergence of these technologies not only promises to enhance diagnostic capabilities but also to catalyze a paradigm shift in healthcare delivery, fostering greater equity and inclusivity in access to essential medical services.

Nevertheless, the successful realization of these aspirations hinges on collaborative efforts across interdisciplinary domains, including materials science, engineering, biology, and clinical medicine. Collaborative research initiatives that bring together expertise from diverse fields can accelerate innovation cycles, facilitate knowledge exchange, and foster synergistic partnerships between academia, industry, and healthcare stakeholders. Moreover, investments in infrastructure, education, and training programs are imperative to cultivate a skilled workforce capable of driving the translation of cutting-edge research findings into tangible healthcare solutions. Additionally, regulatory frameworks must evolve to accommodate the unique features and applications of thin-layer technologies in diagnostic devices, ensuring their safety, efficacy, and compliance with international standards. By fostering an enabling ecosystem that nurtures innovation and entrepreneurship, policymakers can incentivize the development and deployment of next-generation bacterial detection technologies, thereby catalyzing transformative improvements in public health outcomes on a global scale.

In conclusion, the integration of thin-layer technologies with microfluidic platforms represents a pivotal milestone in the evolution of bacterial detection methodologies, offering unparalleled opportunities to enhance diagnostic accuracy, accessibility, and affordability. However, realizing the full potential of these technologies requires concerted efforts to overcome technical challenges, foster interdisciplinary collaboration, and address regulatory considerations. By harnessing the collective expertise and resources of the scientific community, policymakers, and industry partners, we can accelerate the translation of research findings into impactful healthcare solutions that empower healthcare providers, improve patient outcomes, and advance the goal of global health equity.

CONFLICTS OF INTEREST

No conflict of interest was declared by the authors.

ACKNOWLEDGMENT

This review is a part of Pamukkale DOSAP project which were held for Post-Doc studies of Özkur Kuran (Ph.D.) and Duygu Takanoğlu Bulut (Ph.D.).

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