Biotechnology Reports 45 (2025) e00872

Contents lists available at ScienceDirect

Biotechnology Reports

journal homepage: www.elsevier.com/locate/btre

Review

Bacteriophage-based biosensors technology: Materials, fabrications, efficiencies and shortcomings

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ARTICLE INFO

Keywords:
Phages
Biosensors
Diagnostics
Substrates
Immobilizations
Orientation
Detection

ABSTRACT

Ongoing research in biosensor technologies has led to advanced functional materials for healthcare diagnostics, and bacteriophages (phages), demonstrating exceptional utility due to their high specificity, accuracy, rapid, label-free, and wireless detection capabilities with minimal false-positive results. Phage-based-pathogen-detecting biosensors (PBPDBs) include surface plasmon resonance (SPR) biosensors, magnetoelastic (ME), electrochemical, and quartz crystal microbalance (QCM) biosensors. Commonly used substrates for PBPDBs are gold, silicon, glass, carbon-based materials, magnetic particles, and quantum dots. These substrates are chemically and physically modified to optimize phage orientation on sensor surfaces, enhancing bacterial capture. To address typical stability and issues encountered in traditional biosensor applications, phage particles and genetically modified phages are utilized to improve biosensor stability and increase detection efficacy while reducing assay time. Genetic modification in phages facilitated by CRISPR/Cas9 enables the tailoring of phages to target specific bacterial strains. This approach helps overcome the inherent specificity of phages and enables the detection of multiple pathogens in a single assay. Multiple pathogens can be detected through a single phage-based assay. This manuscript elucidates the fabrication methodologies and detection efficiencies of PBPDBs providing valuable insights into the development of practical, precise, and efficient biosensors for pathogen detection.

Summary: PBPDBs are emerging diagnostic tools for the detection of bacterial pathogens.

1. Introduction

Modern technological advances in material sciences, diagnostics analytics, and biotechnological approaches have enabled the enable the fabrication of efficient and precise phage-based-pathogen-detecting biosensors (PBPDBs) [1]. Phages are highly specific and selective towards their host bacterium, and they can discriminate between viable and dead bacteria cells. Phage-based detection bypasses the requirement for selective media, as phages rely on natural, selective binding to specific bacterial receptors for bacterial identification [2]. Integrating targeted phage recognition and releasing natural molecular markers from cells upon lysis constitutes an efficient approach for accurately detecting particular bacterial strains [3].

To enhance specificity, multi-phage biosensors utilize spatial separation and functionalization. Each phage type is immobilized on designated regions of the biosensor surface, which successfully minimizes cross-reactivity by allowing precise targeting against pathogens. Functional coatings further ensure selected phage-pathogen interactions. [4, 5]. Functional coatings further ensure selected phage-pathogen interactions. Distinct signal transduction methods, electrochemical impedance spectroscopy (EIS), magnetoelastic (ME), quartz crystal microbalance (QCM) biosensors, and surface plasmon response (SPR) generate unique signal patterns for each pathogen to avoid any interference [6,7]. Other recent studies by Quintela et al. and Handa et al. have identified that such simultaneous and accurate pathogen detection of pathogens *E. coli* and *Salmonella*, serves as evidence for multi-phage

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https://doi.org/10.1016/j.btre.2024.e00872
Received 20 June 2024; Received in revised form 11 November 2024; Accepted 19 December 2024
Available online 21 December 2024

biosensors in the complicated diagnostic arena [4,12].

Effective phage immobilization methods use various chemicals and cross-linking agents to bind phages efficiently to substrates such as gold, silicon, glass, carbon-based material, and magnetic particle [4]. However, there is a set of instability problems in PBPDS concerning phage degradation and problems with sensor performances when environmental conditions become unfavorable. The diverse studies done on PBPDS have achieved promising potential from phage particles or proteins for high heat stability and, hence, help address the most prevalent instability issues [8]. All this stability is crucial to the improvement of the reliability and robustness of phage-based biosensors toward their wider application in diverse settings. Engineered tail spike proteins (TSPs) of P22 phage are thermo-stabile, protease-resistant, and have better affinity, specificity, and binding properties [9].

Phage orientation is also critical for pathogen capture effectiveness. Phages can be ideally immobilized in a 'tail-up' [10] and 'head-down' orientation perpendicular to the surface, which orients the tail spike proteins outward [11]. This would improve the performance of biosensors since the tail spike proteins may be oriented for optimum recognition and binding to cell surface proteins of the target pathogen [12]. More importantly, appropriate orientation-let's say, especially 'tail-up'-radically increases the interaction possibilities of phage and bacterial cells, hence the effective capturing of the pathogen. A variety of strategies can be employed to achieve the preferred 'tail-up' orientation. Thus, selective anchoring of phages via their capsid heads by surface treatments or linker molecules leaves the tail regions free for direct exposure, with the head regions complex. Genetically engineered phages that display biotin on the head often employ the strong biotin-streptavidin interaction to promote homogeneous 'tail-up' attachment. Its strategic location brings top efficiency in the binding of the phage while it can still interact directly with bacterial cells; hence, it contributes toward the enhancement of biosensor sensitivity in pathogen detection.

The surface density of phages is another vital factor in the bacterial capturing affinity of the phages. While it remains very high at ideal density, approximately around 19 phages μm^{-2}, surpassing this level of density might cause steric hindrance, thus reducing capturing efficiency since phages may crowd one another, blocking the active binding sites [13]. While this is hindered by high densities, such as at 100 phages μm^{-2}, this can be counteracted by adequate phage orientation in a tail-up position by exposition of the binding sites, which retains high bacterial capturing affinity [14]. Also, genetically engineered phages displaying biotin on their attached through the streptavidin-biotin system show a surface density of 4.4 phages/m^2, representing a 15-fold increase in attachment efficiency[15]. This attachment strategy uses one of the largest affinities, namely the interaction of streptavidin-biotin, to effectively allow phages to attach steadily and soundly on the surface. The affinity of the streptavidin/biotin system is leveraged by genetic biotinylation that facilitates the attachment of phages on the surface [16].

Advances in genetically modified phages further enhance PBPDB functionality. For example, phages engineered to overexpress the alkaline phosphate (ALP) enzymes can reduce detection time, providing faster response and enhancing biosensor performance. The mechanism behind ALP overexpression accelerates detection and provides a clearer understanding of its impact on biosensor performance. Moreover, self-assembled monolayers (SAMs) are widely used as functional interlayers for phage immobilization covalently on sensor surfaces, enhancing stability, and increasing binding efficiency [17,18]. These are single layers of molecules that spontaneously organize a surface, altering its properties with high precision and stability. Various other studies demonstrated that the transformation of spheroid shapes became effective in increasing bacterial capture efficiency on sensors [19]. It has also been reported that the bacterial capture efficiency of phages in sensors increases when they transform into spheroids [20].

Phage-based detection biosensors would have very poor prospects for false positives since the phages infect only living, viable bacteria [3, 21]. In this respect, often phages are combined with other diagnostic detection methods such as quantitative PCR (qPCR) to provide further elimination of false positives. [22]. There is a pressing need to develop new diagnostic technologies using PBPDBs due to various important considerations regarding public health, food safety, and environmental monitoring. These can enable the quick detection of a pathogen in many cases, in a question of minutes-enabling timely intervention. This review aims to update information concerning technologies and strategies adopted in the fabrication of PBPDBs by showing their efficiency and precision in pathogen detection capabilities.

2. Fabrication of PBPDBs

2.1. The phages as an advanced functional material in PBPDBs

Phage-based bacterial detection is an innovative method that has shown promising results in pathogen detection, inspiring biosensor technologies to use phages as biorecognition elements in pathogen-detecting biosensor platforms [28]. Symmetrical phages exhibit higher bacterial capture efficiency than asymmetrical phages [29]. The detection efficiency of PBPDBs significantly varies based on the phage types, phage particles, genetically modified phages, and immobilization strategies. In PBPDBs, numerous phages are employed, each selected for its specific ability to target and infect a particular bacterial host. The choice of phage depends on the pathogen of interest for detection. The most used phages in PBPDBs are lytic and non-lytic, as chosen for specific applications in biosensors. The lytic T-series phages (T2, T4, T7) can lyse bacteria efficiently, hence useful in rapid detection [29,16,35], while the non-lytic M13 phages [36], such as M13KO7 [34], allow for continuous infection, which is important for phage display (Table 1). Lambda and P22 [13] integrate into the host genomes, allowing for longer periods of detection; however, single-stranded PhiX174 [32] and MS2 phages are simple, and their cycles of infectivity are very fast, thus ideal for high-throughput screening purposes. Pathogen-specific phages include B1-7064 and D29 [33], BP14 [37], and NRGP56 [42], each targeting very specific pathogens, while broad-host-range phages include *Tbilisi* [43] and E2 [38,39,40], robust at detecting antibiotic-resistant strains. The phages can thus be grouped into categories according to their types. Genetically modified phages also remain a central focus of the fabrication of PBPDBs, which enhances biosensors' specificity, sensitivity, and versatility, enabling the detection of a wide range of bacterial pathogens with high accuracy [44]. Genetically modified PBPDBs offer effective recognition and capture of their targets. They can be efficiently used on biosensor surfaces, i.e., phages with biotinylated capsids can be immobilized on streptavidin-coated surfaces [45]. The fusing of glutathione S transferase (GST) with RBP named Gp48 of *Campylobacter* phage NCTC 12673 showed increased bacterial detection on SPR biosensor reflected by a low detection limit of 10^2 colony-forming units (CFU/mL) [46]. Genetic modifications of phages can increase the expression of alkaline phosphatase enzymes released while infecting the bacteria, thus increasing the detection sensitivity [47]. The lost activity or function of phage can be restored using genetic engineering [45]. The endorhamnosidase activity of tail spike protein has been reported to be restored using genetic engineering, showing good capture efficiency for *Salmonella* cells (10^3 CFU/mL) on a gold surface. However, the coated surface did not detect *Escherichia coli*, which was used as a control pathogen [9]. The specific reporter genes can be inserted into phages whose expression upon infection of a target cell allows the detection of its product [45].

The use of phage particles, including tail proteins, tail fibers, and receptor binding proteins (RBPs), in biosensors has immense diagnostic potential as the detection technique is truly highly selective, sensitive, and rapid for detecting bacterial pathogens (Fig. 1) [3]. In 1960, Watson pioneered using phage coat proteins for the detection of bacteria by employing fluorescently labeled antibodies [48]. These phage proteins

Table 1
Phage-based biosensors: substrates, techniques, efficiency, and limitations.

Contents	Characteristics/observations	References
Substrates used in the fabrication of PBPDBs	Gold	[1]
	Silicon and glass	[2]
	Carbon-based materials	[3]
	Magnetic particles	[4]
	Quantum dots	[5]
	Nanofibers, and nanoparticles	[6]
	All the substrates are positively charged to allow the phage to bind on its surface through its capsid part.	[42]
	Substrates with positive charges enhance phage attachment	[16,53]
	The chemical functionalization of the substrates increases the phage depositing density	[53,14]
	A single phage-based assay, conjugated with QDs of varying sizes can detect multiple pathogens simultaneously	[51].
Phage-based biosensors	SPR biosensors, QCM, magnetoelastic (ME) biosensors, and electrochemical types such as amperometric and EIS biosensors, often incorporate PBPDBs	[7,8]
	The lytic T-series phages (T2, T4, T7) can lyse bacteria efficiently, hence useful in rapid detection	[29,16, 35]
	Phages like Lambda and P22 integrate into the host genomes, allowing for longer periods of detection and PhiX174.	[13,32]
	Life cycle of MS2 phages are very fast, thus ideal for high-throughput screening purposes	[13,32]
	Phages like B1-7064 and D29, BP14, and NRGP56, target specific	[33,37, 42]
	pathogens, while broad-host-range phages include *Tbilisi* and E2 robust at detecting antibiotic-resistant strains	[38,39, 40]
	Genetic modifications of phages can increase the expression of phages enzymes released that increase the detection sensitivity	[47].
	The specific reporter genes can be inserted into phages whose expression upon infection of a target cell allows the detection of its product	[45]
	The use of phage particles, including tail proteins, tail fibers, and RBPs in biosensors has immense diagnostic potential	[3]
Principle of phage attachment and immobilization	Phages are immobilized through their functional groups of amino acid side chains.	[9]
	Tailed phages are immobilized through electrophoretic and electrostatic interaction.	[10]
	Phages physisorption does not require any chemical modifications such as biotinylation or crosslinking	[11–13].
	Physical adsorbed phage based sensor has response time as fast as within 3 minutes was able to detect cells as low as 100 cells/millilitre.	[14].
	Phages density is higher when immobilized by chemical methods	[15]
	DTSP self-assembled monolayer mediated attached T4 phage on SPR surface can be regenerated with 20 mM NaOH for repeated use	[16]
	Applying surface activation techniques, such as cysteamine + glutaraldehyde, DTSP, and electric field orientation enhance sensitivity	[11]
	Genetically modified phages well immobilize and have high detection efficiency	[17].
Phage orientation on biosensor	The desired phage orientation can be achieved by applying a positive voltage	[18]
	The external electric field induces polarization, allowing the phages' "head-down and tail-up"	[14,25]
	With head-down and tail-up improve the performance of biosensors	[12]
The detection efficiency of PBPDSs	Phage based SPR sensors detect pathogens within the LOD range of 104 to 107 CFU/mL.	[21,22].
	Phage based SPR sensors can detect pathogen within 15 min	[16]
	The multifunctionality of phage-based biosensors can also be extended to the detection of more than one pathogen in a single platform.	[23].
	Multiplexed detection possible with one phage based biosensor assay	[24,5]
	The LOD of phage-based ME biosensors is higher than Q-PCR.	[25].
	The decrease in physical dimensions of phage-based-magnetoelastic sensors increases their sensitivity	[26].
Advantages of phage based biosensor	The sensitivity of phages toward target bacteria reduce the chances for false-positive results	[27]
	Phage cocktails may employed to capture a wider range of target bacteria in the event of encountering phage-resistant bacteria	[28].
	The phage-based sensor showed promising advantages such as facile operation, low cost, selectivity, and high sensitivity	[64].
	Phages are highly specific and selective towards their host bacterium, and they can discriminate between viable and dead bacteria cells.	[2].
	Phage-based detection bypasses the requirement for selective media, as phages rely on natural, selective binding proteins.	[2].
	Phage-based detection biosensors would have very poor prospects for false positives since the phages infect only living, viable bacteria.	[3,21]
	Phages can be combined with other diagnostic detection methods such as qPCR to provide further elimination of false positives.	[22].
Shortcomings and limitations in PBPDSs and possible solutions	Some phages even degrade the host receptors enzymatically to break the signal variability from the biosensor platform	[30]
	High densities are often actually found to cause agglomeration of immobilized phages, in which the over-immobilization of phage leads to the satirical hindrance effect	[32].
	The genetic instability of phages can undergo multiple passaging that may introduce, over time, mutations that can alter the host range, hence the specificity and/or long-term reliability of phage biosensors	[29].

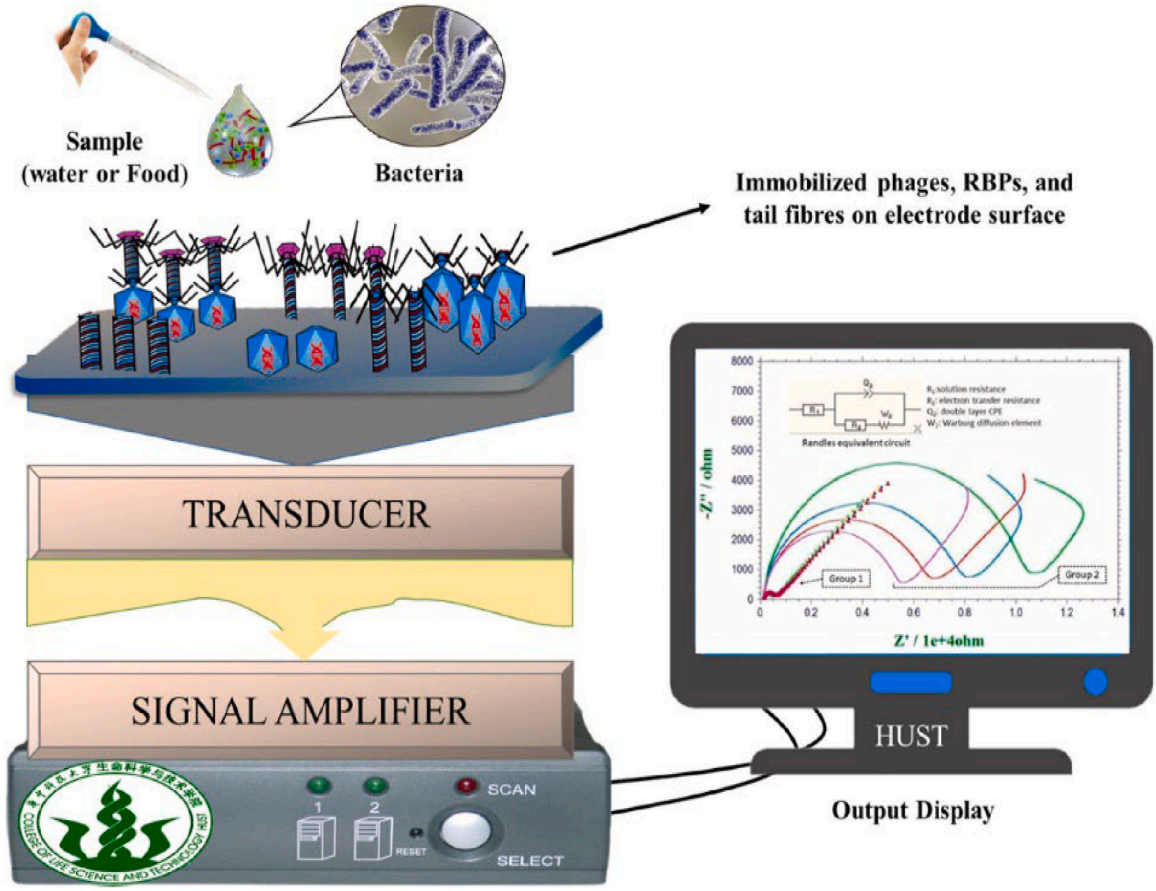

Fig. 1. Schematic representation of typical PBPDBs showing whole phages and phages RBPs immobilized on the electrode surface, transducer, amplification of signals proportional to analyte detection, and output display system. Copyrights 2021, Elsevier [28].

bind specifically to the surface of host bacteria, making them ideal sensing elements for PBPDBs [8]. The C-terminal part of the 1132 amino acid tail protein J confers phage lambda host specificity to its host bacterium, *E. coli* K-12, by utilizing LamB as a receptor [49]. This specificity of binding underlines the full potential of the phage tail proteins in the development of biosensors as very selective sensing elements. Several researchers have explored this special feature of the phage tail proteins for the development of various unique biosensing systems.

For instance, Shin et al. prepared a new SPR biosensor based on a fragment of phage lambda tail protein, which proved the potential of tail proteins in the selective detection of pathogens [50]. In this direction, a wild-type Det7 tail protein, namely Det7T, has been used as a sensing element in an SPR biosensor where a ~2130 base pair product from PCR amplification enhances the detection sensitivity due to specific bacterial recognition.

In one such example, Singh et al. bioengineered a biosensing platform targeting *Salmonella* Typhimurium by using P22 phage tail spike proteins, a category of proteins possessing high thermostability, resistance against proteases, and good affinity and specificity towards the target bacteria. These properties attributed to TSPs make them highly efficient probes for pathogen capture and detection. [9]. Unlike other lytic proteins, P22 TSPs do not induce a lytic cycle when bound to the bacterial host. Alternatively, these proteins utilize endorhamnosidase enzymes to selectively recognize and cleave serotypes A, B, and D1 of *Salmonella*. Interestingly, the activity of this enzyme can reduce bacterial capture on detectors due to the potential lowered propensity of the cleaved bacterial cells to stay bound. This selective enzymatic cleavage served as the basis for the design of the biosensor since it would support the differentiation of target bacteria and reduce the occurrence of false positives on the sensor surface.

3. The substrates used in the fabrication of PBPDBs

Numerous substrates are used to fabricate PBPDBs, which serve as bases for immobilizing phages and allow them to interact with target analytes. The performance of PBPDBs depends on the type of substrate regarding sensitivity, specificity, and stability [6]. Common substrates used in the fabrication of PBPDBs include gold [16], silicon and glass [12], carbon-based materials [4], magnetic particles [5], quantum dots [51], nanofibers, and nanoparticles [52]. All these positively charged materials allow the phage to bind on its surface through its capsid part. [42]. This is important for stable immobilization, representing the coulombic attraction between the members of the capsid and the surface. Different studies have already addressed that materials with positive charges increase phage attachment by creating more electrostatic binding sites on the substrate surface [16,53]. The chemical functionalization of these substrates increases the phage depositing density, resulting in a higher capacity for bacterial detection than pristine substrates [53,14]. Quantum dots (QDs)-coated phages can detect pathogens even if they are present in low concentrations, with high quantum yield and brightness resulting in intense fluorescence emission even when excited with low-intensity light [51]. A single phage-based assay, conjugated with QDs of varying sizes with distinct emission wavelengths, can detect multiple pathogens simultaneously [51].

3.1. The phage-based biosensors

Commonly used biosensors, including SPR biosensors, QCM, magnetoelastic (ME) biosensors, and electrochemical types such as

amperometric and EIS biosensors, often incorporate PBPDBs [6,7]. EIS is used to characterize the stepwise assembly of bacterial biosensors, as it is a non-destructive and effective method for monitoring surface features, allowing for the understanding of chemical transformations and interfacial properties of the modified electrode [23]. The SPR is a spectroscopic method that uses plasmon electromagnetic waves to detect pathogens quantitatively and label-free [24]. The electrochemical sensors use phage as transducers and attach to the sensor surface through electric current. In phage immobilization through electrochemical sensors, the successful orientation of phage depends on the Debye length (L_D) between the sample solution and the sensor's surface [25]. The L_D refers to the distance over which electrostatic interactions between charged particles, in this case, the phage and the sensor surface, decay. Proper orientation is essential because it may directly influence the ability of the phage to recognize and bind the target bacteria, which are so crucial for the sensitivity and specificity of the sensor. In turn, at non-optimized L_D, phages could attach rather randomly or inadequately to poorly optimize the sensor for pathogenic detection. The ability to control the L_D in the sample solution directly improves the performance of the biosensor by its effects on exposing the active binding sites in the phage and aligning them properly for target biomolecule interaction. Phage-based ME biosensors are cost-effective, rapid, feasible, and LOD-competitive [26]; they detect pathogens wirelessly, in real-time, and in-situ, and have excellent detection limits, low cost, and possibility for field testing. Phages are immobilized on magnetoelastic resonators that act as a central component of ME biosensors; the changes in the resonant frequency are measured to detect the pathogens. A QCM biosensor, based on phage technology, quantifies analyte mass by using phages immobilized on the sensor's surface, which is crafted from quartz crystal [27].

3.2. *Gold substrates in PBPDBs*

Gold substrates are preferably utilized in electrochemical biosensors due to excellent electrical conductivity and the ability to form stable self-assembled monolayers. Van der Waals and hydrophobic bonding also immobilize the phages on the gold surface through their amine and thiol groups [54]. Such chemical bonds have high stability [16]. Gold capacitor plates are reportedly used in phage deposition in an external electric field to form chemically bonded and organized phage layers to construct sensing layers [14]. These gold surfaces allow chemical modifications that increase the semicircle diameter in EIS measurements, corresponding to larger surface areas available for phage attachment [19]. The modification ensures the improvement of electrochemical properties of the surface, ensuring an increase in charge transfer resistance and, in turn, enabling better immobilization of the phages due to the increase in the binding sites or a rise in desorption resistance. Accordingly, EIS measurement was used and showed phage-immobilized gold microelectrodes to greatly outperform the antibody-immobilized interdigitated ones in terms of signal generation, thus proving that the phages are effective for reaching a stable and high-density immobilization. [4].

Arya et al. employ dithiobis (succinimidyl propionate) (DTSP) self-assembled monolayer, serving as a covalent binding agent for T4 phage using SPR technology [17]. Bovine serum albumin was applied as a blocking layer to inhibit unspecific bacterial adherence. Crosslinkers such as glutaraldehyde are used on the gold substrate in cysteine and cysteamine SAMs mediated covalently attachment of T4 phages to detect *E. coli* K12 [16]. Several cleaning solutions, such as piranha solution, are used to clean the surface of the gold substrate and subsequently with Ar-plasma [55].

Salmonella-specific M13 phage immobilized on gold electrode surface previously coated with glutaraldehyde crosslinked electrodeposited layer of polytyramine (Pty) detected *Salmonella spp*. Singh et al. fabricated a biosensing platform for the recognition and capture of *S. typhimurium* using the engineered tail spike proteins (TSPs) of P22 phage, which showed 6-fold higher bacterial capture compared to intact P22 phages as well as wild-type TSPs (wtTSPs) [9]. The TSPs tagged N-terminal cysteine, and C-terminal cysteine were immobilized on gold-coated Si substrates using thiol-chemistry. Additionally, SPR was used to extend the immobilized chemistry to gold-coated SF-10 glass substrates for real-time analytical detection of the host bacteria.

A biosensor based on *Salmonella*-specific M13 phage/Pty/gold electrode showed reasonable specificity in detecting *Salmonella spp*. The modified electrodes were reproducible, and the flow system facilitated easy regeneration of the electrode surface, enabling its reuse and thereby lowering the cost of analysis [56]. The planar gold-coated QCM was used to covalently attach the M13 phages through SAM of N-hydroxy-succinimide thioctic ester and added the BSA as a blocking agent [18]. This would increase the attachment of T4-phage and bovine serum albumin (BSA) layers, increasing the diameter of the semicircle in the Nyquist plot that describes the relation between impedance and frequency. An increased semicircle diameter represents an increased resistance to electron transfer, and thus against the migration of a redox probe chemical elective indicator of electron transfer to the electrode surface. The biotin-terminated alkyl chain is used to functionalize the gold surfaces, enhancing the binding of streptavidin and reducing the unwanted adsorption of the host bacterium [15]. Such streptavidin-functionalized surfaces have a higher tendency to capture phages, particularly with biotinylated capsid heads, compared to wild-type phages [15]. Cysteamine hydrochloride (Cyst), 1,4-phenylene diisothiocyanate, activates the cysteamine-modified gold electrode. It increases the diameter of the semicircle because of the increase in chain length increase of the SAMs and the generation of neutral charge after the association of the amino groups of the cysteamine with the thiocyanate group. Thus, the activated electrode has a large area for T4-phage molecules attachment and acts as an insulating layer due to having the negatively charged (pI4−5) at the working pH 7.4 [19]. Subsequently, the diffusion of the redox probe toward the electrode surface is hindered by an insulating layer of phage, which increases the electron-transfer barrier and enlarges the diameter of the semicircle.

3.3. *Silicon/glass in PBPDBs*

The optical biosensors preferably use silica glass or other silicon-based compounds. Silicon oxide and metal oxide substrates are highly compatible with surface silanization, a gentle chemical reaction that facilitates the formation of strong covalent bonds between the phage and the substrate. Phages can be covalently immobilized through silanization of glass using the (3-aminopropyl) triethoxysilane. Consequently, the resulting surface retains its smoothness and chemical uniformity [12]. The phage-based optical biosensors, including those based on SPR, employ silicon and glass substrates because of their optical transparency [9], while microfabricated biosensors utilize silicon wafers. Silicon oxide and metal oxide substrates allow covalent solid bonds between phage and substrate, and the resultant surface maintains smoothness and chemical homogeneity [12]. The glass used in the phage-based sensing platform can be treated with 3-aminopropyltrimethoxysilane and P22 phages before immobilization onto the sensor through a conventional EDC/NHS reaction [12]. The surface reaction was enhanced using the bifunctional aminosilane linkers and the sulfo-NHS and this combination also allowed the permanent attachment of phage to the substrate. EDC/NHS provides a higher surface coverage and prevents aggregate formation. The 3-aminopropyltrimethoxysilane modified P22 PBPDBs used for the detection of *S. typhimurium* showed high selectivity towards *S. typhimurium* when its selectivity was compared with *E. coli* (strain 33780) and *Listeria monocytogenes* [12]. It was suggested that TSP and bacterial membrane receptors have an affinity for the immobilized P22 layer and can discriminate among different bacterial types that are determined by O-antigenic receptors in the outer membrane of *S. typhimurium*.

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Glass and silicon-based substrates, particularly silicon oxide and metal oxide, are highly suitable for phage-based optical biosensors due to their excellent compatibility with surface silanization. This compatibility enables strong covalent bonding between the phages and substrate while preserving the surface's smoothness and chemical uniformity. Additionally, the optical transparency of these materials makes them ideal for optical biosensor applications, including SPR. The use of 3-aminopropyltrimethoxysilane and EDC/NHS reactions further enhances surface coverage and prevents aggregate formation, leading to a stable and selective phage immobilization. These characteristics make glass and silica substrates particularly advantageous for creating effective and reliable biosensors with high selectivity and sensitivity, as demonstrated in the detection of *S. typhimurium*.

3.4. *Carbon-based substrates in PBPDBs*

The electrochemical enhancements of the sensor increase with the use of carbon nanotubes, provide a high surface area for phage immobilization, and maintain the high load of phage particles. Zhou et al. employed polyethyleneimine (PEI)-functionalized multiwall carbon nanotubes to immobilize the phages through electric field-induced (EFI) that allows the achievement of high phage particles on electrode surface [29]. This EFI method creates an oriented electric field that aligns the phage particles in a way such that their orientations are appropriate for binding. Since the binding efficiency is maximized, this increases the sensitivity of the biosensor. Further, this method of immobilization enhances not only the stability of the immobilized phages but also rather high-density-oriented phage attachment, thus considerably enhancing the overall biosensor performance. Using EFI immobilization, T2 phages could be oriented on positively charged functionalized CNT matrices to enable the chemical anchoring of phage onto the nanostructured electrode [29]. The infectivity of *E. coli* cells was noticed after 25 minutes of incubation.

The multi-walled carbon nanotubes, gold nanoparticles, and tungsten oxide nanostructures were employed to fabricate a nanocomposite as the sensor platform for phage immobilization. SAMs of 4-amino thiophenol (4-ATP) on the nanocomposite surface modify the sensor surface for effective covalent immobilization of lytic t-4 phage [2]. Subsequently, a glutaraldehyde (2%) chain was used to activate the surface and to form a well-organized monolayer for covalent immobilization of the page which also increases the insulating property of the electrode surface [56]. Additionally, nanocomposite accelerated electron transfer from the solution interface to the electrode surface, leading to a reduction in the impedimetric signals. The presence of nanomaterials increased sensitivity, while the immobilized active phage achieved high specificity when tested against various non-targeting bacterial strains. [2]. T4 phage L91 displays biotin, and the wild-type T4 phage is covalently attached to arrays of screen-printed carbon electrodes for the direct impedimetric detection of *E. coli* K12 cells. Screen-printed carbon electrode microarrays can be modified with gamma phage and can be used to detect *Bacillus anthracis* [61]. The vegetative cells of *B. anthracis Sterne* were detected with Electrochemical Impedance Spectroscopy.

Quintela et al. employed streptavidin-coated screen-printed carbon electrode (SPCE) to directly immobilize the phages to fabricate a sandwich-type amperometric biosensor for the detection of shiga toxin-producing *E. coli* (STEC) [4]. A 40 mM of hydrogen peroxide, a substrate for HRP, and ferrocene dicarboxylic acid as a mediator were added to SPCEs to shuttle electrons between the redox reaction center and the working electrode [62]. After the immobilized phages captured the STEC cells, a detection solution that contained the same type of HRP-tagged phages (same phages) was introduced to speed up the catalytic reaction and bonded with gold nanoparticles to enhance signal strength. The added detection element solution lets the sandwich capture STEC cells and phage-host binding interactions that contain HRP, resulting in STEC/phage-SHRP complex formation. HRP was used to label the phages for luminescence-based assay, and efficient binding of the HRP-labeled phage to its host cells enhanced biosensor sensitivity [4]. The screen-printed graphene electrodes (SPEs) can be conveniently integrated with modern portable biosensing instrumentation and functionalization with COOH groups which is important for achieving a covalent linkage between the electrode surface and the phage head [63]. The surface area and electron mobility of graphene electrodes are high. Bhardwaj et al. immobilized phages on oxidized graphene electrodes for impedimetric sensing of *S. arlettaei* [63]. The carboxyl (eCOOH) groups were generated by graphene electrodes through electrochemical oxidization in chronoamperometry mode and then exposed to 0.1 M EDC in 0.12 N HCl for 10 min to activate eCOOH functional groups. In biosensors, chronoamperometry mode is used to apply a constant voltage and measure current over time, enabling real-time monitoring of biochemical reactions on the sensor surface. Followed by incubation of electrodes with enriched phage solution for 60 minutes at 37 °C to allow covalent immobilization of phages. These PBPDBs showed a quantitative and fast response for *S. arlettae*. A quick response time was 2 min, low LOD was 2 CFU, while broad detection range was 2.0–2.0 $\times$ 10^6 CFU.

Additionally, the sensor was stable over a prolonged period and did not show a response for *E. coli* and *Staphylococcus aureus,* indicating the sensor's high specificity. The practical utility of the developed sensor has been demonstrated by the analysis of *S. arlettae* in spiked water and apple juice samples. Recently, the nanosheets of reduced graphene oxide were used to immobilize a nonlytic and highly stable M13 phage to fabricate a chemiresistor biosensor to detect coliform bacteria. The interdigitated gold electrode chips were fabricated on a p-type silicon substrate with SiO2 insulating oxide layer deposited on top, followed by deposition of Cr (5 nm) and Au (50 nm) layers deposited on the substrate. The hydroxyl groups were introduced by using the 30% ammonium hydroxide and activated by 6.0 mM EDC and 6.0 mM NHS in 100 mM 2-(Nmorpholino) ethanesulfonic acid (MES) buffer (pH 5.5) before incubating with 25 mL of M13 phage solution at 4 C for 180 minutes. The sensor detected *an E. coli* strain with a LOD of 45 CFU/mL and did not show a response to other than the targeted bacteria. The sensor is simple, selective, specific, and holds promising potential for economic and accurate on-site detection of fecal coliforms. A phage tail spike proteins (TSPs) coated graphene biosensor based on the field-effect transistor was fabricated for real-time monitoring of *E. coli*. The sensor

showed promising advantages such as facile operation, low cost, selectivity, and high sensitivity [64].

3.5. *Magnetic particles as substrate in PBPDBs*

Magnetic substrates are an innovative element for phage immobilization. Functionalizing magnetic substrates with different chemicals increases the bacterial capture efficiency of immobilized phages or their particles. Wang et al. coated magnetic nanoparticles with phage long-tail-fiber proteins (LTF4-a) to specifically separate and concentrate the *S. typhimurium* in artificially spiked food samples such as milk, lettuce, and egg [52]. The clinically meaningful pathogenic bacteria such as *Acinetobacter baumannii* and *Pseudomonas aeruginosa* were successfully detected using the phage TFPs can be a good candidate for bio-probes [52]. Phage long-tail fiber proteins are rarely reported for their bacterial detection properties on magnetic nanoparticles

Wang et al. employed gold-coated strips of the ME alloy as bare ME sensor platforms to adsorb the filamentous E2 phage [57]. In phage solution, the gold-coated strips of the ME alloy were immersed and rotated for the uniform attachment of phages and to minimize the non-specific binding of *Salmonella* to the biosensors, different blocking agents such as BSA, casein, and Superblock) were used. Consequently, the ME biosensor technique was a reliable and effective method for detecting foodborne pathogens in fresh produce. *S. typhimurium* phage-based magnetoelastic beads [38] and *Salmonella* antibody-coated immunomagnetic bead biosensors [58] for detection of *Salmonella*. The capture efficiency of phage-based magnetoelastic beads was high. Suppose phages are to be immobilized on magnetic particles to capture and separate bacteria. In that case, the avidity between the phage and particle must allow for pulling a relatively larger bacterium [42]. Liebana et al. employed P22 phage-modified magnetic particle conjugate (P22-MP) for the electrochemical magnetogenosensing of bacteria [5]. This approach identifies as few as 3 CFU/mL of *Salmonella* in Luria-Bertani (LB) media within a 4-hour. It showcases the superior stability, cost-effectiveness, and advantage of using phages, which are produced without harming animals, as biorecognition elements in biosensing devices. The P22-MP was fabricated using the tosyl-activated magnetic particles for the covalent coupling of native P22 phage through the reaction of aminated aminoacidic lysine moieties of the main capsid monomeric protein an amine linkage [59]. The Bradford test determined the binding efficiency of capsid protein and magnetic particle, which measure the viral protein amount before and after the immobilization steps. This method captured the bacteria through the host interaction with high specificity and efficiency, and a double-tagging polymerase chain reaction (PCR) was then performed to amplify the DNA of the captured bacteria. Additionally, electrochemical magnetogenosensing was used to detect the double-tagged amplicon.

A novel method was developed employing the genetically modified phages expressing affinity tags such as biotin carboxyl carrier protein and cellulose-binding module on their heads to enable uniform immobilization on streptavidin-coated magnetic beads and microcrystalline cellulose beads [60]. These affinity tags produce a powerful, almost irreversible interaction with their receptors, streptavidin. The affinity tags were introduced on the head of the T4 phage by the phage display method to fabricate effusively vigorous recombinant phages expressing biotinylated affinity tags that were recognized by the respective receptors and did not allow the change in the host range of the T4 phage. [60]. It was suggested that this highly active biosorbent, due to oriented immobilization of phage, could open new avenues in developing biosensors for rapid and user-friendly bacterial detection. Choi et al. use ferromagnetoelastic (FME) biosensor to immobilize the lytic, tailed Bacillus cereus-specific phage by employing a two-step chemical linkage involving 11-mercapto-1-undecanoic acid (11-MUA) combined with a crosslinking agent, specifically N-(3-dimethyl aminopropyl)-N'-ethylcarbodiimide hydrochloride and N-hydroxysuccinimide (EDC/NHS). The covalent bond between the phage and the sensor surface is formed by the latter chemical approach, resulting in a stable and specific attachment of the phage. It was observed that phage density increases with the increase in phage concentration. 11-MUA and EDC/NHS were chosen because this combination significantly improved the density and orientation of the phages on the surface, increasing it by as much as 4.3 times.

4. Principle of phage attachment and immobilization strategies

Phage immobilization plays a vital role in the efficient detection of bacteria. Phages are immobilized on the material surface through their functional groups of amino acid side chains [42]. The high density of phage particles on the surface enhances bacterial binding domains. However, this increased density can also lead to potential inactivation of the phage film, which may affect bioactivity and requires careful optimization[27]. Tailed phages are immobilized through electrophoretic and electrostatic interaction due to their negative charge. While the surface of the phage head is negatively charged, the tail region has a slightly positive charge[65]. Tail edges are the region of functional receptors of phages that help them bind to their host bacteria [4]. When phages are attached via their head capsid protein to the surface of microsensors, their tail fibers are oriented toward the medium. This alignment enhances the phages' ability to capture bacteria [15]. Initial studies used to attach the phages to the sensor surface through physical adsorption; however, such techniques could not provide enough phage surface coverage. On the other hand, the sensor's surface on which phages are attached through chemical means has high phage surface coverage, improving such sensors' performance [16]. Phage-based biosorbents, fabricated with chemical biotinylation of phages, are more efficient than simple physical adsorption of phages [15].

The surface coverage of physically absorbed phage is low and desorbed during the analytic detection [66]. Physical adsorption of phages relies on weak forces, such as van der Waals forces or electrostatic interactions, to adhere phages to the biosensor's surface without needing chemical bonding or more complex attachment strategies. The physically adsorbing phages on the sensor's surface are frequently utilized to fabricate phage-immobilized ME biosensors [67]. Phages are also immobilized through random physisorption onto physical substrates, which is an easy, single-step, rapid method and does not require any chemical modifications such as biotinylation or crosslinking [68]. The bacterial capture by physisorbed phages can be monitored through SPR [55]. Mejri et al. functionalized transducers with phages through physisorption due to their straightforwardness [68]. Microelectrodes were attached to phages by incubation at 37 °C for 1.5 hours. Then, they were washed, and bovine serum albumin was used for physical blocking to prevent unwanted non-target components. The phage-based pathogen-detecting platform may reduce its sensitivity due to the low surface charge often observed in the case of physical adsorption of phages [11].

Low surface charge minimizes the electrostatic interactions between the phages and the target pathogens, leading to less efficient binding and hence lower detection sensitivity. Without the stability of covalent or electrostatic attachment, physical adsorption provides a greater likelihood for desorption and suboptimal orientation of the immobilized phages, thereby reducing sensitivity again. Such considerations emphasize the need for alternative immobilization strategies to improve surface charge enhancement and increase binding efficiency to maintain high sensitivity in pathogenic detection.

Chemical functionalization strategies for phage immobilization on biosensor surfaces have replaced the physical adsorption methods to increase the number of deposited phages, which increases the PBPDBs' efficiency. It suggested that the bond of chemically immobilized phages with the sensor's surface is powerful compared to physical adsorption and has fewer chances of virion detachment [69]. Niyomdecha et al. use a glutaraldehyde crosslinked electrodeposited layer of polytyramine (Pty) for the immobilization of *Salmonella*-specific M13 phage on a gold electrode surface [56]. This phage-based gold electrode detected

Salmonella in the chicken meat sample solution by binding to the amino acid groups of its filament. It has been observed that compared to the density of glutaraldehyde-immobilized phages (8 ng mm−2) on a gold surface, the surface density of the L-cysteine immobilized phages cysteine alone (12 ng mm−2) is high [70]. Dithiobis (succinimidyl propionate) (DTSP) self-assembled monolayer mediated attached T4 phage on SPR surface could be regenerated with 20 mM NaOH for repeated use, an approach not viable with physically adsorbed phages [17]

Before phage immobilization, the positively charged polyelectrolyte poly-L-lysine (PLL) can be used for pre-coating the sensor surface to form a favorable surface for phage deposition, as demonstrated by [27]. Subsequently, different phage suspensions of tailed phage specific for *P. aeruginosa* and non-tailed phage specific for *S. typhimurium* have flowed over the sensor surface to achieve surface immobilization. The phages deposited PLL-coated QCM with Dissipation Monitoring (QCM-D) sensors were employed to capture *P. aeruginosa* and *S. typhimurium*. Different surface densities for both phages were tested to determine the optimum phage surface densities. Similarly, in another study, QCM-D sensors were coated with phage spheroid monolayers using the Langmuir–Blodgett (LB) technique [20]. This technique is a method for depositing monolayers of material onto a substrate could provide valuable context. The sensor was tested in MRSA and MSSA containing water followed by penicillin-binding protein (PBP 2a) specific antibody conjugated latex beads exposure to discriminate between methicillin-resistant and sensitive *S. aureus* strains. A nanosensor based on covalently immobilized phage that is cross-linked with SAMs of 4-Aminothiophenol (4-ATP) and glutaraldehyde showed increased sensitivity with the limit of detection of 3.0 CFU/mL [2].

The amine coupling method can be used to immobilize *Salmonella*-specific MSal020417 phage on biosensor surfaces [41] under different pH to find the optimum pH level. The highest binding of *Salmonella*-specific MSal020417 phage was observed at pH 3.0, and an approximate rise of 2900 in the response units (RU) was detected post-immobilization [41]. Liu et al. immobilized 12-amino acid peptide displaying phage probe M13 on an 11-MUA chip, which was ultrasonically rinsed using acetone, methanol, and alcohol [71]. This is followed by allowing the self-assembly carboxyl terminated monolayer on the gold surface via immersing the chip in an 11-MUA solution for 24 hours. After immersing, to activate the carboxyl groups and immobilize the phage, the chip was placed inside the BIAcore3000. This method is very effective as a chip was functional even after 50 times of use, thus implying that it can regenerate and be reused.

Rippa et al. fabricated *Tbilisi*-phage immobilized surface-enhanced Raman scattering (SERS) nanosensors using the SAM of multilayer octupolar metastructures that serves as a dual-functional sensing chip, combining SERS and SPR capabilities. It highly detects local refractive index changes (to levels lower than 17 femtomolar) and enhances Raman signals [43]. This phage-immobilized SERS showed high molecular selectivity and provided label-free detection of *Brucella*. The single-cell measurements were performed for rapid identification, identifying bacteria in less than 60 minutes without any precultivation step. The SERS-SPR probe and nonlinear optical detection could form a multi-probing platform that provides further insights into the targeted species' polarizability and chirality (handedness). This octupolar coupling platform can also be immobilized with different phages to identify their medically relevant bacterial pathogens at concentrations below 104 CFU/mL. Richter et al. developed a technique for creating chemically bonded and organized phage layers to construct sensing layers [14]. The phages were deposited in an external electric field, essentially a capacitor plate comprising the solid support's gold surface and an isolated copper electrode. The screening of the applied field was limited to facilitate the process of orientation of the virions, which increases the sensitivity of this phage-based biosensor platform.

4.1. Immobilization of genetically modified phages

Genetic modification allows site-specific immobilization for capsid-modified phages, facilitated by the fusion of a gene encoding a protein affinity domain incorporated into phage genomes or expressed exogenously. The CRISPR/Cas9 system further greatly increases the efficiency with which precise, targeted changes are made in the genetic material of phages [42]. Compared to the other traditional methods of genetic engineering, CRISPR/Cas9 provides high accuracy for specific editing in DNA, which allows one to design phages that target certain bacterial strains with far greater efficiency. This system enables the construction of phages with enhanced targeting capabilities and hence represents top candidates for an application in biosensors. The genetically engineered phages are subsequently coated onto the free-standing ME resonator acting both as a biomolecular recognition component and a signal transducer [44]. According to the principle, detection is based on monitoring changes in the resonator frequency, which is proportional to the sensor mass changes (Fig. 2).

The genetically modified phages expressing biotin on capsid were attached to the sensor surface using streptavidin, resulting in attachment densities of 4.4 phages/m2, a 15-fold increase over simple physisorption [15]. Recently, Carmody et al, fused monomeric streptavidin with a phage capsid protein to allow site-specific self-assembly of up to several fusion proteins per capsid. The phages fused monomeric streptavidin was immobilized on biotic coated magnetic nanoparticles for detection of *E. coli* and achieved a detection limit of less than ten CFU in 100 mL of water [42]. Vinay et al. developed a portable phagosensor, a phage-based *Salmonella enterica Typhimurium* detecting biosensor using the HK620 and P22 prophages, which were genetically modified with gfpmut2 and kanamycin resistance genes [72]. They combined the fluorescence as an output signal and the flow cytometer as a rapid and real-time detection device to achieve high sensitivity and reduce the detection time. Retrospectively, such a flow cytometer was fabricated for detecting pathogen spores in deserts in the US and widely used for HIV field detection campaigns in Africa [73,74]. This portable phagosensor detected as little as ten bacterial cells, which is one of the vital achievements and comparable to the levels specified in the European directive on microbiological contamination. However, most phage-based biosensors detect 10^2 to 10^4 bacterial cells per ml. Additionally, this phagosensor can detect bacteria, even in diluted samples or mixed co-cultures.

Edgar et al. also employed genetically modified T7 phages, which express a small peptide on the major capsid protein, and the modified phages were conjugated with streptavidin-coated QDs [51]. This modification enables the peptide to be biotinylated by the biotin-ligase protein (BLP) found in the host bacterium [51]. Specific lysine residue in the tagged peptide was targeted for the attachment of biotin post-translationally via BLP. The affinity of the streptavidin/biotin system is leveraged by genetic biotinylation that facilitates the attachment of phages on the surface [16]. This phage-based assay significantly reduced the amplification time due to releasing 10–1000 phage from each infected bacterium. The phage head can be modified using the sulfosuccinimido-biotin that reacts with the amino groups of the coat proteins [75]; this modification led to phages immobilized with their tails pointing upwards, enabling effective recognition and capture of their targets [15].

4.2. Phage orientation on the sensor surface

The orientation of immobilized phages depends on the type of phages. For example, T4 phages are oriented as "head-down" and "tail-up," which expose the tail fiber to target the host bacterial cells. At the same time, there is no preferred orientation for asymmetrical phages, which generally exhibit low bacteria capture efficiency [29]. Additionally, the appropriate phage orientation is governed using an optimized concentration of phages with certain distances also to avoid phage

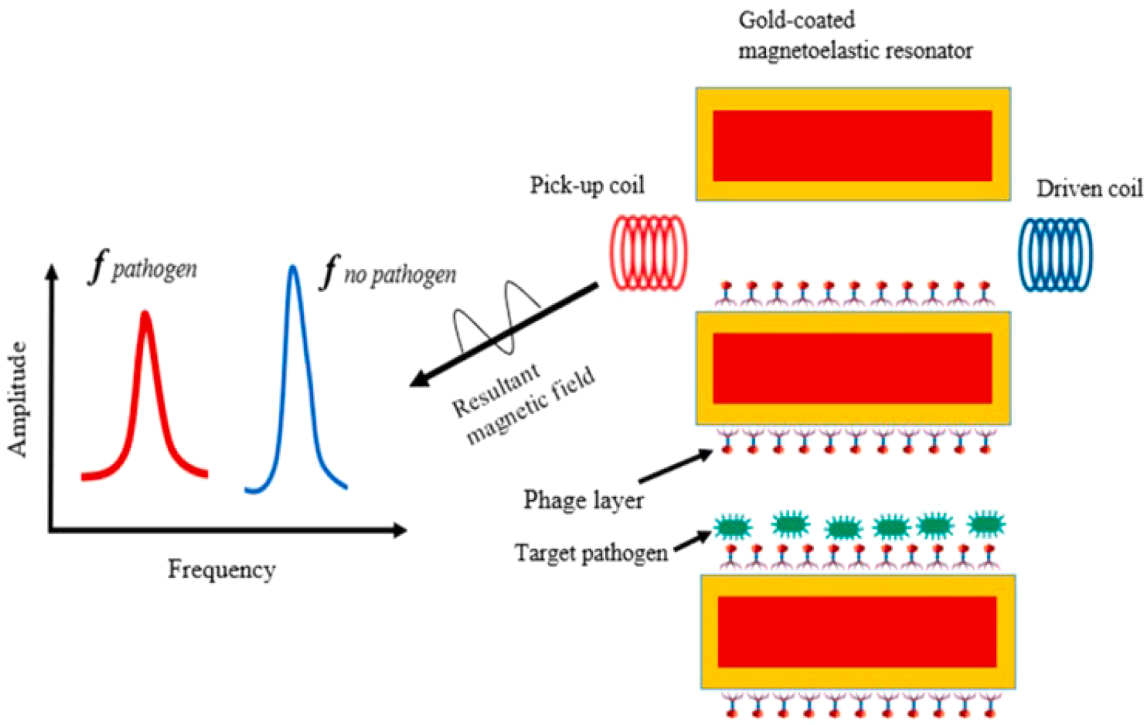

Fig. 2. This plotting shows the resonance frequency shift in the presence of target pathogens. The red peak pathogen binding, whereas the blue peak does not show any such binding. Schematics of the structure of the biosensor including a gold-coated magnetoelastic resonator, a phage layer, and associated magnetic field detection elements are also given together with the graph. This layer-by-layer assembly provides a path for the effective specific binding of the target pathogens, leading to a corresponding frequency shift as the indication of the presence of pathogens Copyrights 2022, MDPI [6].

aggregation. An orientation toward the analyte in the solution influences the efficiency and performance of phage-based biosensors. In phage-based biosensors, efficiency can significantly be taken aback by immobilized phage orientation, where wrong orientations point toward reduced available binding sites for bacterial capture, hence a lowered performance of the sensor [60,50]. It is worth noting that phage orientation must be appropriate because proper phage orientation enhances its capability to effectively capture target bacteria. For example, methods such as immobilization under an electric field, using chemical functionalization with either SAMs or PEI coatings, have been employed to orient the phages in such a way as to optimize their binding contact. These approaches and strategies of orientation improve bacterial capture efficiency and raise the sensitivity and reliability of biosensor performance. The phages on the sensor surface are positioned as "tail-up" and "head-down," and this is how they are ideally immobilized [10] and efficiently bind to the host bacterium that must be free and oriented perpendicular to the surface [11]. This tail-up orientation allows the tailspike proteins to recognize the O-antigenic repeating units on the cell surface of the pathogen [12]. The estimated phage surface density with high bacterial capturing affinity is 19 phages $\mu m^{-2,}$ and affinity reduces if this coverage exceeds [13]; however, the density of phage surface coverage with 100 phages μm−2 may still have bacterial capturing affinity if phages are positioned in a tail-up orientation [14]. It has been reported that a narrow range of phage surface densities has less activity. The T4 phage surface density increase from 19 to 28 phages μm significantly reduces the detection of *E. coli* K12 [13]. Additionally, the surface density of phages can be increased using purification agents such as Sephacryl S-1000, which was reported to increase phages surface density to 54 ± 7 phages/mm2 [13]. The density of purified P22 phage improved to 199 ± 2 phages/mm2, which was 25-fold higher than unpurified P22 phages and showed a jamming coverage of 0.13 at 40 °C. It has been observed that compared to the density of glutaraldehyde-immobilized phages (8 ng mm−2) on a gold surface, the surface density of the L-cysteine immobilized phages cysteinealone (12 ng mm−2) is high [70].

The desired phage orientation can be achieved by applying a positive voltage, as phages have negatively charged heads and positively charged tail fibers [53]. To attain excellent phage coverage and improved orientation, Xu et al. investigated the density of deposited phage biosensor and its bacteria capture efficiency by studying the effects of electric field and chemical functionalization [53]. The sensitivity of the bare gold surface was increased 4-fold upon applying an external electric field. It has been reported that 20-fold sensitivity increases with a chemically functionalized surface compared to the case without an electric field. The using of an appropriate electric field may result in the correct orientation of phages, which boosts the efficiency of bacterial capture and the sensitivity of the device by allowing the bacterial recognition proteins (BRPs) on tail fibers to work proficiently [76].

The external electric field induces polarization, allowing the phages' "head-down and tail-up" orientation because of their electric dipole property, as demonstrated in Lorentz force in electromagnetics theory [14,25]. In the study by Richter et al. an alternating current (AC) electric field generated from pulses was used instead of a direct current (DC) voltage. This approach was chosen because the strength of the AC field could extend across the entire suspension during the intervals between pulses. Furthermore, it remained effective within the electric double layer, constrained by the L^D, thereby lengthening the duration phages were exposed to the effective electric field [14]. It revealed that the Debye length between the sensor's surface and the sample solution allows for practical phage orientation. The external electric field's effect on phages is small when its size is higher than Debye's length. In these cases, electrostatic interactions between the positively charged electrode surface and negatively charged heads prompt the orientation [53]. However, the phage orientation occurs along the electric field lines within the volume limited by the surface and the L_D when the phage size is smaller than the Debye length [25]. Thus, the densities of deposited phages depend on the effect of L_D and phage concentrations. The RBPs do not significantly capture bacteria by horizontally orientated phages,

but entropy favors such orientation. The number of available receptors is significantly high with the increased random-oriented virions.

Richter et al. corrected the spatial arrangement of the virions to increase the number of available receptors, resulting in four times increased sensitivity compared to disordered phages [25]. The phages were treated as asymmetrical colloidal particles using a non-zero permanent dipole moment, allowing the phages to be oriented on the solid substrate. Additionally, the correct ordering of the phages on the sensor surface overcomes its inactivation problem due to increased surface coverage [13]. Therefore, sensors' performance can be improved by exploiting the orientation of phages. It has been reported that using glutathione SAM allows the oriented attachment of the RBP of Campylobacter phage NCTC onto SPR surfaces, which exhibited a detection limit of as102 CFU/mL [46].

5. The detection efficiency of PBPDSs

Many surface plasmon resonance (SPR)-based sensors, with lytic phage, T4, and M13 phages, detect pathogens such as *S. aureus, E. coli*, and *Salmonella* mainly within the LOD range of 104 to 107 CFU/mL [77, 30]. Similarly, T4 phage-based SPR sensors were employed to detect the coliform marker in *E. coli* known as β-galactosidase (β-gal) [36]. *E. coli* cells were detected at a concentration range of 7×10^2 to 7×108 CFU mL^{-1}. *E. coli* and methicillin-resistant *S. aureus* (MRSA) were also detected by phase-sensitive SPR based on T4 and BP14 phages with a LOD of 103 CFU/mL for both species [37].

Karoonuthaisiri et al. use 12-mer peptides expressing filamentous M13 phages in an SPR-based assay to detect the foodborne bacterium *Salmonella* [41]. In optimizing this assay, immobilization and interaction buffers were used, including citric acid, to enhance phage binding and bacterial capture efficiency. Fig. 3 A schematic representation showing the operating principle of phage-based SPR biosensors.

Moving from SPR to another detection platform, *Tbilisi*-phage immobilized surface-enhanced Raman scattering (SERS) nanosensors showed high molecular selectivity and provided free label-free detection of *Brucella*. The single-cell measurements were performed for rapid identification, identifying bacteria in less than 60 minutes without need for precultivation step. This octupolar coupling platform can also be immobilized with different phages to identify their medically relevant bacterial pathogens at concentrations below 10^4 CFU/mL.

E. coli has also been detected by phage-based SPR biosensor within 15 min, with an LOD of around 100 CFU/mL [17]. Applying surface activation techniques, such as cysteamine + glutaraldehyde, DTSP, and electric field orientation, up to a 10-fold sensitivity enhancement was obtained with the phage layer. These two methods generate significant synergetic effects, resulting in a 50- to 64-fold rise in the quantity of *E. coli* cells captured by the T4 phage-based sensor. Dithiobis (succinimidyl propionate) (DTSP) self-assembled monolayer mediated attached T4 phage on SPR surface could be regenerated with 20 mM NaOH for repeated use, an approach not viable with physically adsorbed phages [17].

Quartz Crystal Microbalance with Dissipation Monitoring (QCM-D) has also employed phage-based detection techniques. Phage deposited on PLL-coated QCM-D sensors was used to capture *P. aeruginosa* and *S. typhimurium*. By testing various phage surface densities, studies found 7.1×10^9 CFU/mL as an ideal tailed phage concentration and 8.6×10^9 pfu/mL as an ideal non-tailed phage concentration for the optimization of bacterial capture efficiency [27]. By physical adsorption, a QCM sensor immobilizing *S. typhimurium* phage on piezoelectric transducers with response time as fast as within 3 minutes was able to detect cells as

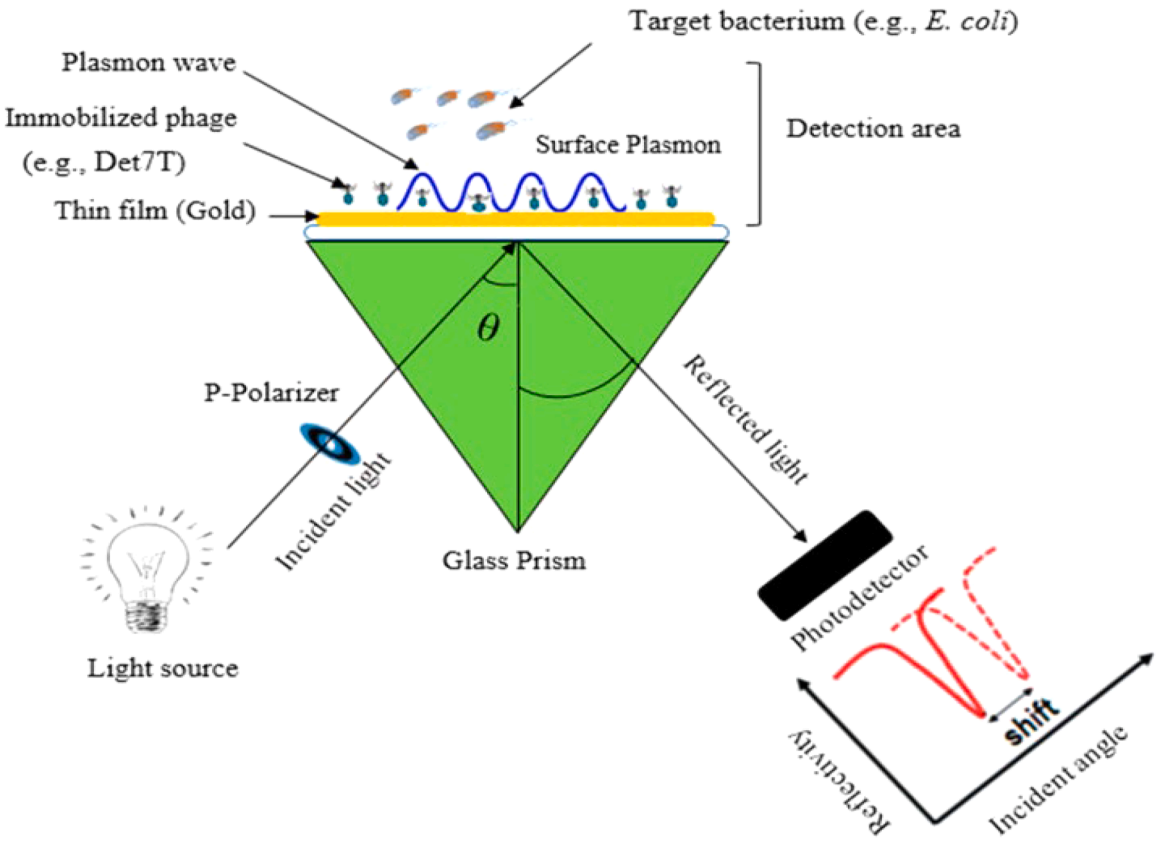

Fig. 3. The figure illustrates a phage-based SPR biosensor for bacterial detection. Incident light passes through a glass prism with a thin gold film, exciting surface plasmons. Immobilized phages on the gold surface bind specifically to target bacteria like *E. coli*, altering the mass and refractive index at the sensor surface. This binding event causes a detectable shift in the SPR signal, proportional to the bacterial concentration. This design leverages the specificity of phages for sensitive, quantitative pathogen detection in various samples Copyrights 2022, MDPI [6].

low as 100 cells/milliliter and was effective in identifying bacteria at concentrations from 10 to 10 million cells/milliliter [78].

The multifunctionality of phage-based biosensors can also be extended to the detection of more than one pathogen in a single platform. For instance, Huang et al. developed the use of magnetoelastic - ME - biosensor coated with distinctive phages for *S. typhimurium* and *B. anthracis* [79]. Each corresponding host bacterium is detected because the capture of bacteria leads to a reduction in resonance frequency due to an increase in sensor mass. For instance, the use of phages with conjugation to QDs of different emission wavelengths has made multiplexed detection possible during one assay [56,51].

New designs also form part of the advances in phage-based SPR biosensors. An SPR biosensor using a tail protein fragment of phage lambda was used for the label-free, rapid detection of *E. coli* by Shin et al. Using the C-terminal tail protein J fragment that binds *E. coli* K-12 via its LamB receptor, this biosensor had a range of detection from 2×10^4 to 2×10^9 CFU/mL, with its lower detection limit of 2×10^4 CFU/mL [50]. Hyeon et al. developed the SPR biosensors one step further by preparing the wild-type Det7 tail protein as a sensing element to detect *E. coli* K12 cells. Upon lysis of *E. coli* cells, β-D-galactosidase is released and allowed to interact with its substrate, named p-aminophenyl-β-D-galactopyranoside (βPAPG), to produce a measurable signal in the form of p-aminophenol (PAP) [31], Further, phage-immobilized interdigitated gold microelectrodes were used for the detection of E. The quantification range has been 10^4–10^7 7 CFU/mL for *E. coli*, with little or no interference from non-target bacteria such as Lactobacillus, proving the specificity of this method [49].

5.1. Real-time detection of pathogens through PBPDBs

The PBPDBs also detected pathogens in real-time in different samples, including milk, fruits, water, and meat. Park et al. compared the efficiency of the phage-based ME biosensor method with Q-PCR in detecting *S. typhimurium* directly grown on tomato surfaces. The LOD of phage-based ME biosensors was 0.944 and 3 log CFU, while 0.977 and 2 log CFU for Q-PCR [80], showing the LOD of the ME biosensor method one order more than that of Q-PCR. Advancing research on phage-based ME biosensors, the same team conducted on-site and field tests of the biosensor's ability to identify *S. typhimurium* cultured on spinach leaves [26]. The LOD for the phage-based ME biosensor was 2.17 for the adaxial and 1.94 log CFU/spinach for the abaxial surface, higher than qPCR (1.37 log CFU/spinach). Some other studies reported these phage-based biosensors also include E2 and M13 phages, which can detect *Salmonella* on various food surfaces with respectable LODs as low as 200 CFU/mL [39]. Whereas phage C4-22 probes-based ME sensor platform coupled with the frequency scanning devices detected low Salmonella concentration of 7.86×10^3 CFU/mm^2 on raw chicken breast filets [81]. The phage-adsorbed ME biosensor platforms detected an initial increase of 100 CFU/25 g *S. typhimurium* on spinach leaves with a confidence level exceeding 95% ($p < 0.05$).

The JRB7 phage-coated magnetoelastic wireless and label-free sensor was evaluated for the real-time detection of *B. anthracis* spores in vitro with straightforward application in flowing liquids without requiring intricate circuit configurations [82]. Using a 1 mm sensor at a flow rate of 40 μL/min, real-time monitoring of *B. anthracis* spores suspended in water (at a concentration of $\times 10^8$ spores/ml) revealed a continuous decrease in the sensor's resonance frequency until the point of binding saturation was reached. In water suspension tests for *B. anthracis* spores using a 1 mm sensor, the detection threshold was identified to be approximately 10^3 spores/ml, with a linear response observed across spore concentrations ranging from 5×10^3 to 5×10^7 spores/ml. The sensor was characterized for first-order resonance frequency, and it was found that its frequency versus mass sensitivity was in mL and μm, and with the decrease of sensor length, it increases. Additionally, the high-frequency sensitivity in response to mass changes enables the sensor to detect slight variations in mass when it captures the target pathogens [82]. The decrease in physical dimensions of phage-based-magnetoelastic sensors increases their sensitivity, and the order of magnitude and sensitivity enhance the LOD of the pathogen [38].

Decreasing the particles' size can further enhance phage-based-magnetoelastic sensors' sensitivity, allowing for the detection of smaller quantities of spores, as a smaller sensor size leads to an increase in resonance frequency [83]. Choi et al. use ferromagnetoelastic (FME) biosensor to immobilize the lytic, tailed B. cereus-specific phage to detect B. cereus on the food surface [10]. Biolayer interferometry-based LysGH15 detected *S. aureus* directly in food samples through phage lysin (LysGH15) and long tail fibers (ice cube and light soy sauce) with a LOD of 13CFU/mL with a binding time of 12min. Interestingly, LysGH15 retained recognizing and binding ability even after C54A mutation [84].

E. coli was also detected in water samples through amperometric measurements using the phiX174 phage as a detection probe [32]. The phages such as B1-7064 and D29 effectively detected the cells of *B. cereus* and *M. smegmatis*, respectively, through combined activity of phage typing and cell marker enzyme activity such as alpha-glucosidase and beta-glucosidase [33]. The T4 phage immobilized on an optical fiber surface through ultra-sensitive long-period fiber gratings (LPFGs) detected *E. coli* as low as 10^3 CFU/mL with an experimental accuracy greater than 99% [85]. The external refractive index alters the resonance wavelength (λR), which LPFGs can sense. With telecommunication wavelengths (~1.56 um), the LPFGs sensing platform can achieve more accurate *E. coli* detection and improve LOD to 10^3 CFU/mL [31].

A T4 phage-based micro electrochemical sensor (T4B-MES) achieves a meager limit of detection of 14±5 CFU/mL of viable *E. coli* B cells. It potentially could be used for antibiotic susceptibility testing, water quality monitoring, and the food industry if integrated with microfluidics [53]. SPCE-immobilize phages-based sandwich-type amperometric biosensor detected several strains of the STEC, such as STEC O26, O157, and O179, in fresh ground beef and pasteurized apple juice with a LOD of 10–10^2 CFU/g or mL[4]. The nanosensors based on covalently immobilized phage that is cross-linked with SAMs of 4-Aminothiophenol (4-ATP) and glutaraldehyde showed increased sensitivity with the limit of detection of 3.0 CFU/mL [2]. The nanosensors were highly specific and accurately identified the targeted foodborne pathogen in food samples but did not show detection of the various non-targeting bacterial strains. It achieved an impressive recovery rate between 90.0% and 108% [2]. Therefore, the newly developed phage-based sensor is recommended as a disposable label-free impedimetric biosensor for quick real-time food quality and quantity monitoring.

Similarly, in another study, QCM-D sensors were coated with phage spheroid monolayers using the Langmuir–Blodgett (LB) technique [20]. The sensor was tested for MRSA and MSSA-containing water. Both strains were inhibited with immobilized phages. The lytic activity and bacterial capture efficiency of phages increased after transforming into spheroids. This work introduces a concept for creating a rapid biosensor technology to detect and distinguish between MRSA and MSSA strains through simultaneous measurements using lysergic phage and PBP2a antibodies. A biosensor based on *Salmonella*-specific M13 phage/Pty/gold electrode showed reasonable specificity in detecting *Salmonella* spp. 1.0×10^7 CFU/mL in chicken meat samples with a wide linear range, a low detection limit, and a relatively short detection time. Compared to non-target bacteria, there was an eight times higher capacitance change for *Salmonella*. Additionally, compared to the LOD of other *Salmonella* spp-specific biosensors using E2 phage [38–40], the current *Salmonella*-specific M13 phage/Pty/gold electrode exhibited high LOD. Even the LOD of this *Salmonella*-specific M13 phage/Pty/gold electrode was higher than the biosensor based on the same M13 phage [41]. *S. typhimurium* phage-based magnetoelastic beads detected *Salmonella enteritidis* at 5×10^3 CFU/mL in 25 g of food sample [38]. Fig. 4 shows the detection of bacteria by phage-based biosensors

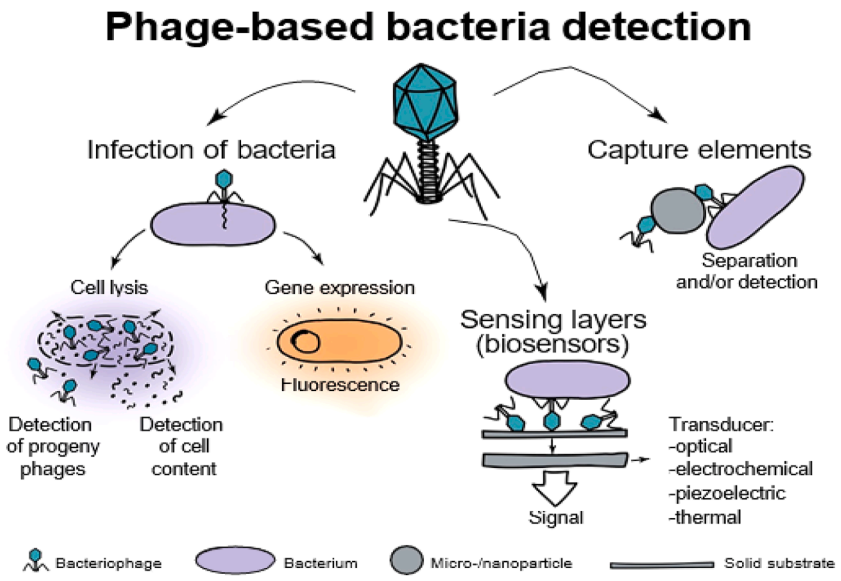

Fig. 4. Schematic illustration of the detection of bacteria through phage-based biosensors. Bacteria exposed to immobilized phage lyses release cell contents that can be detected. Phages can also deliver foreign genes into bacteria, marking the host with a fluorescent, colorimetric, or optical label. Additionally, phages can be employed to construct sensing layers in biosensors. When attached to micro- or nanoparticles, bacteriophages act as capturing agents in various tests. Copyrights Elsevier 2018 [86].

5.2. Bacterial detection through genetically modified phages-based biosensor

Genetically modified phages enhance detection by increasing enzyme expression, improving fluorescence imaging, and overall detection sensitivity. released when infecting bacteria. The expression of such phage enzymes can be increased by genetic modifications, according to the study of Wisuthiphaet et al. [47]. They showed that engineered phages released many phages, enabling fluorescence imaging and resulting in high detection sensitivity, allowing for identifying as few as 100 bacteria per gram within a 6-hour timeframe [65]. Neufeld et al. fabricated a phagemid system combining the nonlytic phage M13KO7 and a commercial plasmid, pFLAG-ATS-BAP, having gene code for alkaline phosphatize enzyme, for the detection of *E. coli* TG1 cells via the activity of alkaline phosphatase [34]. In the phagemid system, the enzymes are overproduced due to the phages' nonlytic behavior, which increases the assay's sensitivity as 1 CFU/mL bacterial cell could be detected within 180 minutes.

Similarly, the alkaline phosphatases are also overexpressed by genetically engineered T7 phage, which codes for *pho* responsible for overexpression of alkaline phosphatase. These enzymes catalyzed The electrochemical reactions, which quantify the pathogenic *E. coli* with a LOD of 1 CFU/mL post-enrichment on spinach leaves [35]. The phage head can be modified using the sulfosuccinimido-biotin that reacts with the amino groups of the coat proteins [75], which makes the recognition and capture of the target bacteria [15]. Edgar et al. employed genetically modified T7 phages, which express a small peptide on the major capsid protein, and the modified phages were conjugated with streptavidin-coated QDs [51]. The QDs can easily detect the releasing phages [51]. Quantum dots have high quantum yield and brightness. They can emit intense fluorescence even when excited with low-intensity light, thus enhancing the sensitivity of phage-based biosensors and can detect low concentrations of pathogens. Streptavidin-functionalized surfaces have a significantly higher tendency to capture phage, particularly with biotinylated capsid heads, compared to wild-type phages [15].

6. The probabilities of false-positive results in PBPDBs

The phage-based method of detection relies on the sensitivity of phages toward target bacteria with very high specificity, thereby reducing drastically the potential for false-positive results, considering the nature of phage infection and attachment to target bacteria [21]. This is because phages recognize and attach to specific receptors on the surface of their target bacteria, usually absent in other organisms besides the hosts. This selectivity in binding entails that only the choice of target bacteria produces a detectable result, hence increasing the diagnostic value in terms of accuracy and reliability. The use of phages in combination with other diagnostic methods, such as qPCR, has been employed as a means of further minimizing the possibility of false-positive results. Each technique, when combined, then exploits those advantages that it has: in this case, phages have a high degree of specificity attributed to the interaction with bacterial unique receptors; qPCRs confirm the target DNA sequence, hence increasing the confidence level for this method. The application of both approaches will not only confirm phage-based detections but also will point to those bacterial stains resistant to phages, which is one more step toward better diagnostic accuracy and reliability [22].

Moreover, phage cocktails, cocktail mixtures of different phages, may also be employed to capture a wider range of target bacteria in the event of encountering phage-resistant bacteria [87]. However, while the cocktails enhance capture by increasing the possibility of capturing phage-resistant strains, they may broaden the host range, thereby reducing specificity both to an individual species and to an increase in the risk of false-positive results. However, it will always have one trade-off, which is that the added phages may bind to the receptors of non-target bacteria as well, hence decreasing overall selectivity. This trade-off may be lessened if the selection and optimization of the phages within the cocktail are expertly done, focusing on those that have very little cross-reactivity. Besides that, resistance to phages can develop in bacteria rather seldomly because, regularly, there is no consistent selective pressure of phages on bacteria. Most phages would denature without bacterial hosts; thus, they could not survive for a long period without target bacteria hosted in an environment [87]. Unlike other detection methods, PBPD [3]. This feature is a serious advantage when compared to other diagnostic methods like PCR and immunoassays,

which can detect DNA or antigens, respectively, from dead bacteria and give positive results even though they may be dead. Such phage-based biosensors will only identify the viable bacteria; hence, it reflects their real presence in their active state, thereby underlining one of their most important benefits in medical diagnosis. FASTPlaqueTB commercial kits have been reported to generate false-positive results in the detection of *Mycobacterium tuberculosis*. This might be a partial virucidal inactivation of introduced phages while the residual ones give a positive signal. Besides that, respiratory samples are very complex and can contain non-tuberculous mycobacteria that may interfere with this assay and cross-react. Such improvements may include sample preparation protocol optimization, and enhancement of the virucidal step to ensure that complete phage neutralization has taken place to reduce false positives. Further improvement in assay accuracy may be achieved by increasing the specificity of the phages used in the kits for more exclusive targeting of *Mycobacterium tuberculosis* [87]. Denis et al. suggested that false positive readings may generated due to inhibition due to containing toxic compounds in the absence of phages [88]. Such toxic compounds may interfere with the accuracy of the assay due to their apparent inhibitory response, which could be misinterpreted as positive results. Additionally, the presence of diverse phage populations with varying rates of infection within the samples could hinder precise calibration and limit the assay's use for qualitative analysis. In practical terms, this diversity in infection rates may cause fluctuations in signal output, which affects the assay's accuracy in detecting specific target organisms or quantifying bacterial load. For example, in a biosensor setup, if some phages are faster or more efficient at binding and lysing bacteria, the signal may peak prematurely or vary unexpectedly, making it difficult to correlate signal strength with bacterial concentration. This issue is particularly problematic in assays that rely on precise, quantitative measurements rather than just detecting the presence or absence of a target. To overcome these challenges, standardization protocols and calibration techniques are essential. For instance, using a well-characterized phage population with known infection kinetics could improve consistency. Additionally, implementing calibration curves specific to each phage variant or developing mathematical models that account for phage variability could help refine the assay's quantitative capabilities. These measures would enhance assay reliability, making it more robust for both qualitative and quantitative applications.

7. Shortcomings and limitations in PBPDSs and possible solutions

The genetic instability of phages can undergo multiple passaging that may introduce, over time, mutations that can alter the host range, hence the specificity and/or long-term reliability of phage biosensors. Modifications that could cause loss of diagnostic precision, wherein phage stability and specificity may be ensured through control storage conditions or genetic modification [87]. The large size of phage particles, along with their sensitivity to drying, is one of the main obstacles in the fabrication of nanoscale phage-based biosensors. It can reduce binding efficiency in sensor surfaces. Thus, these problems related to size and stability may interfere with signal transduction and hence may suggest alternative strategies, such as using phage proteins, that are further aimed at improving sensor performance at the nanoscale [3].

In pathogen-detecting biosensors, the large size of whole phages often compromises the measurement signal in its path by disturbing signal transduction. Specific phage components, such as tail fibers or receptor-binding proteins, represent a more stable and compact alternative that may be better suited to yield higher performance in biosensors. These components can give rise to improved binding efficiency but may also involve some trade-offs regarding specificity or binding strength [89]. This may be augmented by the loss of binding capability of the tail fibers during drying. Bacterial lysis in intact phage-based systems will release materials that can interfere with signal stability. These may be overcome with strategies including the use of stabilizing agents or the use of alternative phage-derived proteins, thus ensuring sensor performance will be reliable over time [9]. Some phages even degrade the receptors on the surface of the host bacteria enzymatically to break the signal variability from the biosensor platform [90]. Another alternative is the use of phage tail proteins that, as indeed noted by [8], usually enhance stability due to their heat-resistant nature. Most of the separation applications require the use of streptavidin-functionalized particles because of the very tight binding of the biotinylated recognition element/phage via the streptavidin-biotin interaction (Kd $\approx 10^{-15}$ nM)[8]. The scalability of functionalization and cost become huge concerns with the purified proteins, as well as the functionalizing of large surface areas on magnetic nanoparticles that can span several square meters.

QDs coupled to streptavidin-coated phages have been used in biosensor applications in bacterial detection with some success [51]. The problem remains concerning the nanotoxicity of QDs, as they can result in environmental harm and even cytotoxic effects in cases of accumulation [91]. Therefore, research focuses on safer alternatives nowadays, like silicon-based nanomaterials, at the expense of reduced risks rather than fluorescent properties.

High densities are often actually found to cause agglomeration of immobilized phages, in which the over-immobilization of phage leads to the satirical hindrance effect, thus occluding active binding sites and reducing bacterial capture efficiency. Such effects could be better optimized using controlled deposition techniques or spacer molecules on sensor surfaces [9,17]. Although piranha solution is effective for surface cleaning, it is dangerous to health due to its corrosive nature. Other safe methods such as UV-ozone cleaning shall reduce hazards yet effectively prepare sensor surfaces.

Till now, impedance and conductivity measurements are considered troublesome in biosensing due to the optimization of culture media required besides contaminations. Some of these challenges can be overcome by indirect impedimetric approaches consisting of adding potassium hydroxide, which removes the CO_2 produced by bacterial growth; this simplifies measurement with improved accuracy [92]. With this kind of approach, the high-specificity detection of *L. monocytogenes, S aureus, Salmonella enterica, Campylobacter* spp., *E. coli*, and *Enterococcus faecalis* has been performed within four hours [92]. The LOD with *Salmonella* M13 phage biosensor was at a low of 200 CFU/mL and could be decreased by using enrichment media like peptone, therefore increasing sensitivity up to 1.9–3.9-fold. This further decreases the limit of detection if the incubation time can be more than 24 hours [56]. Immobilization also is susceptible to pH variations. Normally, citric acid-Na_2HPO_4 buffers can be used in a normal pH range of 3.0–7.4 for modifying pH to achieve better electrostatic interaction and for phage stability [56,41,60].

8. Conclusion and future remarks

There is a high level of variety in the development of phage-based biosensors for rapid, sensitive, and specific methods of detection. Phage-based biosensors could introduce new frontiers in diagnostic technologies. The unique characteristics of phages include their attack on only live bacteria, the possibility of tailoring them by genetic modification, and the combination with the leading sensor platforms in ways that emphasize the key role in the development of diagnostics.

With infectious diseases on the rise, there has never been a time when timely diagnosis was so important for competent infection management. Phage-based biosensors meet these challenges with rapid detection and point-of-care analysis, particularly needed in low-resource situations. The combination of phage specificity with recent improvements in sensor technology makes the route to diagnostic tool realization promising for meeting urgent needs for rapid and reliable detection.

Therefore, such high specificity of phages means that their application in biosensors will be associated with a decrease in false positives

and an increase in diagnostic accuracy. Further support for the effective immobilization of phages is given by innovations in the materials used for sensors, such as gold, magnetic-based, and carbon-based substrates. Each of these substrates has different advantages in terms of sensitivity, scalability, and integration, which will realize the full potential of phage-based sensors.

Given these strengths, phage stability and sensitivity to drying have been the challenges. With modification techniques' advent such as genetic and chemical, besides material sciences that progress, these weaknesses started to be overcome. It would be possible to continue future research beyond the already achieved phage stability under fluctuating environmental conditions and new materials to optimize phage immobilization for consistent performance.

The promising availability of complex analytes denotes the ability of multiplexed biosensors, which have the potential to detect multiple pathogens simultaneously through immobilization with different phages. The systems are designed based on several technical considerations, such as reducing cross-reactivity and signal interference. However, they hold great promise for clinical diagnostics, enabling comprehensive detection in a single assay.

Finally, the broad challenges to the development and wide diffusion of phage-based biosensors remain. These extend into critically needed regulatory approvals, phage stability under varying conditions, and integration into user-friendly, cost-effective platforms. Indeed, simplification of regulatory pathways, reduction of production costs, and improvement of commercialization strategies will be crucial in advancing phage-based biosensors from bench to real applications in health and beyond.

Funding

Not applicable.

CRediT authorship contribution statement

Ihtisham Ul Haq: Writing – review & editing, Writing – original draft, Data curation, Conceptualization. **Kashif Rahim:** Formal analysis, Conceptualization. **Sajida Maryam:** Resources, Writing – review & editing. **Najeeba Parre Paker:** Resources, Writing – review & editing.

Declaration of competing interest

I confirm that there are no relationships or activities that could appear to have influenced the submitted work. Neither I nor any of my co-authors have any financial interest or personal relationship with other people or organizations that could inappropriately influence our work.

Acknowledgments

All the authors of the manuscript thank and acknowledge their respective universities and institutes. Ul Haq also thanks to 38/2022 Postgraduate development program strategic partnerships in the states III Capes/Fapemig for the scholarship.

Data availability

No data was used for the research described in the article.

References

[1] K. Paramasivam, Y. Shen, J. Yuan, I. Waheed, C. Mao, X. Zhou, Advances in the development of phage-based probes for detection of bio-species, Biosensors 12 (2022), https://doi.org/10.3390/bios12010030.

[2] N. Abdelhamied, F. Abdelrahman, A. El-Shibiny, R.Y.A. Hassan, Bacteriophage-based nano-biosensors for the fast impedimetric determination of pathogens in food samples, Sci. Rep. 13 (2023) 1–11, https://doi.org/10.1038/s41598-023-30520-3.

[3] K.B. Shivaram, P. Bhatt, M.S. Verma, K. Clase, H. Simsek, Bacteriophage-based biosensors for detection of pathogenic microbes in wastewater, Sci. Total Environ. (2023) 165859, https://doi.org/10.1016/j.scitotenv.2023.165859.

[4] I.A. Quintela, V.C.H. Wu, A sandwich-type bacteriophage-based amperometric biosensor for the detection of Shiga toxin-producing Escherichia coli serogroups in complex matrices †, RSC Adv. 13 (2020) 35765–35775, https://doi.org/10.1039/d0ra06223e.

[5] S. Liébana, D.A. Spricigo, M.P. Cortés, J. Barbé, M. Llagostera, S. Alegret, M. I. Pividori, Phagomagnetic separation and electrochemical magneto- genosensing of pathogenic bacteria, Anal. Chem. 85 (2013) 3079–3086.

[6] R.R. Al-hindi, A.D. Teklemariam, M.G. Alharbi, I. Alotibi, S.A. Azhari, I. Qadri, T. Alamri, S. Harakeh, B.M. Applegate, A.K. Bhunia, Bacteriophage-based biosensors : a platform for detection of foodborne bacterial pathogens from food and environment, Biosensor (2022) 1–22.

[7] Y. Takakusagi, K. Takakusagi, F. Sugawara, K. Sakaguchi, Using the QCM biosensor-based T7 phage display combined with bioinformatics analysis for target identification of bioactive small molecule. Plant Chem. Genomics, 2018, pp. 159–172.

[8] M. Walter, C. Fiedler, R. Grassl, M. Biebl, R. Rachel, X.L. Hermo-parrado, A. L. Llamas-saiz, R. Seckler, S. Miller, M.J. Van Raaij, J.V. Irol, Structure of the receptor-binding protein of bacteriophage Det7 : a podoviral tail spike in a myovirus ๑, J. Virol. 82 (2008) 2265–2273, https://doi.org/10.1128/JVI.01641-07.

[9] A. Singh, S.K. Arya, N. Glass, P. Hanifi-moghaddam, R. Naidoo, C.M. Szymanski, J. Tanha, S. Evoy, Bacteriophage tailspike proteins as molecular probes for sensitive and selective bacterial detection, Biosens. Bioelectron. 26 (2010) 131–138, https://doi.org/10.1016/j.bios.2010.05.024.

[10] I.Y. Choi, J.H. Park, K.M. Gwak, K.-P. Kim, J.-H. Oh, M.-K. Park, Studies on lytic, tailed Bacillus cereus -specific phage for use in a ferromagnetoelastic biosensor as a novel recognition element, J. Microbiol. Biotechnol. 28 (2018) 87–94.

[11] N. Tawil, E. Sacher, R. Mandeville, M. Meunier, Strategies for the immobilization of bacteriophages on gold surfaces monitored by surface plasmon resonance and surface morphology, J. Phys. Chem. (2013).

[12] H. Handa, S. Gurczynski, M.P. Jackson, G. Auner, J. Walker, G. Mao, Recognition of Salmonella typhimurium by immobilized phage P22 monolayers, Surf. Sci. 602 (2008) 1392–1400, https://doi.org/10.1016/j.susc.2008.01.036.

[13] R. Naidoo, A. Singh, S.K. Arya, B. Beadle, N. Glass, J. Tanha, Evoy, Surface-immobilization of chromatographically purified bacteriophages for the optimized capture of bacteria, Bacteriophage 2 (2012) 15–24, https://doi.org/10.4161/bact.19079.

[14] Ł. Richter, K. Bielec, A. Les, Ł. Marcin, J. Paczesny, Dense layer of bacteriophages ordered in alternating electric field and immobilized by surface chemical Modification as sensing element for bacteria detection, ACS Appl. Mater. Interfaces 9 (2017) 19622–19629, https://doi.org/10.1021/acsami.7b03497.

[15] L. Gervais, M. Gel, B. Allain, M. Tolba, L. Brovko, M. Zourob, R. Mandeville, M. Griffiths, S. Evoy, Immobilization of biotinylated bacteriophages on biosensor surfaces, Sensors Actuators B 125 (2007) 615–621, https://doi.org/10.1016/j.snb.2007.03.007.

[16] A. Singh, N. Glass, M. Tolba, L. Brovko, M. Griffiths, S. Evoy, Immobilization of bacteriophages on gold surfaces for the specific capture of pathogens, Biosens. Bioelectron. 24 (2009) 3645–3651, https://doi.org/10.1016/j.bios.2009.05.028.

[17] S.K. Arya, A. Singh, R. Naidoo, P. Wu, T. Mcdermott, S. Evoy, Chemically immobilized T4-bacteriophage for specific Escherichia coli detection using surface plasmon resonance, Analyst (2011) 486–492, https://doi.org/10.1039/c0an00697a.

[18] L.M.C. Yang, J.E. Diaz, T.M. McIntire, G.A. Weiss, R.M. Penner, Covalent virus layer for mass-based biosensing, Anal. Chem. 80 (2008) 933–943, https://doi.org/10.1021/ac071470f.

[19] C. Tlili, E. Sokullu, M. Safavieh, M. Tolba, M.U. Ahmed, M. Zourob, Bacteria screening, viability, and confirmation assays using bacteriophage-impedimetric/loop-mediated isothermal amplification dual-response biosensors, Anal. Chem. 85 (2013) 4893–4901.

[20] R. Guntupalli, I. Sorokulova, E. Olsen, L. Globa, O. Pustovyy, T. Moore, B. Chin, J. Barbaree, V. Vodyanoy, Detection and identification of methicillin resistant and sensitive strains of Staphylococcus aureus using tandem measurements, J. Microbiol. Methods 90 (2012) 182–191, https://doi.org/10.1016/j.mimet.2012.05.003.

[21] S.A. Ranveer, V. Dasriya, F. Ahmad, H.S. Dhillon, M. Samtiya, E. Shama, Positive and negative aspects of bacteriophages and their immense role in the food chain, Npj Sci. Food. 8 (2024) 6753, https://doi.org/10.1038/s41538-023-00245-8.

[22] Z. Wang, Development of bacteriophage based detection technique for food safety and environment monitoring, 2016.

[23] C.M.A. Brett, Electrochemical impedance spectroscopy in the characterisation and application of modified electrodes for electrochemical sensors and biosensors, Molecules (2022) 27, https://doi.org/10.3390/molecules27051497.

[24] L. Xu, X. Bai, Current state of development of biosensors and their application in foodborne pathogen detection, J. Food Prot. 84 (2021) 1213–1227, https://doi.org/10.4315/JFP-20-464.

[25] Ł. Richter, K. Matuła, A. Leśniewski, K. Kwaśnicka, M. Łoś, J. Łoś, R. Hołyst, Ordering of bacteriophages in the electric field: application for bacteria detection, Sensors Actuators B Chem. (2016) 233–240, https://doi.org/10.1016/j.snb.2015.09.042.

[26] M.K. Park, J.W. Park, H.C. Wikle, B.A. Chin, Evaluation of phage-based magnetoelastic biosensors for direct detection of Salmonella Typhimurium on

spinach leaves, Sensors Actuators, B Chem. 176 (2013) 1134–1140, https://doi.org/10.1016/j.snb.2012.10.084.
[27] A.L.J. Olsson, A. Wargenau, N. Tufenkji, Optimizing bacteriophage surface densities for bacterial capture and sensing in quartz crystal microbalance with dissipation monitoring, ACS Appl. Mater. Interfaces 8 (2016) 13698–13706, https://doi.org/10.1021/acsami.6b02227.
[28] W. Hussain, M. Wajid, U. Farooq, A. Aziz, S. Wang, Biosensors and Bioelectronics Bacteriophage-based advanced bacterial detection : concept, mechanisms, and applications, Biosens. Bioelectron. 177 (2021) 112973, https://doi.org/10.1016/j.bios.2021.112973.
[29] Y. Zhou, A. Marar, P. Kner, R.P. Ramasamy, Charge-directed immobilization of bacteriophage on nanostructured electrode for whole cell electrochemical biosensors charge-directed immobilization of bacteriophage on nanostructured electrode for whole cell electrochemical biosensors nano electrochemist, Anal. Chem. 89 (2017) 5734–5741, https://doi.org/10.1021/acs.analchem.6b03751.
[30] E. Goldman, S. Walper, Phage-displayed single domain antibodies as recognition elements, in: B. Lin, B.R. Ratna (Eds.), Virus Hybrids as Nanomaterials: Methods and Protocols, Methods in Molecular Biology 1108 (2014) 201–210, https://doi.org/10.1007/978-1-62703-751-8_15.
[31] S. Roy, F. Arshad, S. Eissa, M. Safavieh, S.G. Alattas, M.U. Ahmed, M. Zourob, Recent developments towards portable point-of-care diagnostic devices for pathogen detection, Sensors Diagnos. 1 (2022) 87–105, https://doi.org/10.1039/d1sd00017a.
[32] T. Neufeld, A. Schwartz-Mittelmann, D. Biran, E.Z. Ron, J. Rishpon, Combined phage typing and amperometric detection of released enzymatic activity for the specific identification and quantification of bacteria, Anal. Chem. 75 (2003) 580–585, https://doi.org/10.1021/ac026083e.
[33] E. Sokullu, A. Ng, Phage-Based Biosensors for Food Analysis, in: M.U. Ahmed, M. Zourob, E. Tamiya (Eds.), Food Biosensors, The Royal Society of Chemistry, 2016, pp. 432–462, https://doi.org/10.1039/9781782623908-00432.
[34] T. Neufeld, A.S. Mittelman, V. Buchner, J. Rishpon, Electrochemical phagemid assay for the specific detection of bacteria using Escherichia coli TG-1 and the M13KO7 phagemid in a model system, Anal. Chem. 77 (2005) 652–657, https://doi.org/10.1021/ac0488053.
[35] A.Y. El-moghazy, N. Wisuthiphaet, X. Yang, G. Sun, N. Nitin, Electrochemical biosensor based on genetically engineered bacteriophage T7 for rapid detection of Escherichia coli on fresh produce, Food Control 135 (2022) 108811, https://doi.org/10.1016/j.foodcont.2022.108811.
[36] E.R.. Goldman, J.L.. Liu, R.D.. Bernstein, M.D.. Swain, S.Q.. Mitchell, G. P. Anderson, Ricin detection using phage displayed single domain antibodies, Sensors 9 (2009) 542–555, https://doi.org/10.3390/s90100542.
[37] T. Ktari, H. Baccar, M.B. Mejri, A. Abdelghani, Calibration of surface plasmon resonance imager for biochemical detection, Int. J. Electrochem. (2012), https://doi.org/10.1155/2012/421692, 2012.
[38] R.S. Lakshmanan, R. Guntupalli, J. Hu, D. Kim, V.A. Petrenko, J.M. Barbaree, B. A. Chin, Phage immobilized magnetoelastic sensor for the detection of Salmonella typhimurium, J. Microbiol. Methods 71 (2007) 55–60, https://doi.org/10.1016/j.mimet.2007.07.012.
[39] S. Li, Y. Li, H. Chen, S. Horikawa, W. Shen, A. Simonian, B.A. Chin, Direct detection of Salmonella typhimurium on fresh produce using phage-based magnetoelastic biosensors, Biosens. Bioelectron. 26 (2010) 1313–1319, https://doi.org/10.1016/j.bios.2010.07.029.
[40] M.K. Park, H.C. Wikle, Y. Chai, S. Horikawa, W. Shen, B.A. Chin, The effect of incubation time for Salmonella Typhimurium binding to phage-based magnetoelastic biosensors, Food Control 26 (2012) 539–545, https://doi.org/10.1016/j.foodcont.2012.01.061.
[41] N. Karoonuthaisiri, R. Charlermroj, M.J. Morton, M. Oplatowska-Stachowiak, I. R. Grant, C.T. Elliott, Development of a M13 bacteriophage-based SPR detection using Salmonella as a case study, Sensors Actuators, B Chem. 190 (2014) 214–220, https://doi.org/10.1016/j.snb.2013.08.068.
[42] C.M. Carmody, S.R. Nugen, Monomeric streptavidin phage display allows efficient immobilization of bacteriophages on magnetic particles for the capture, separation, and detection of bacteria, Sci. Rep. (2023) 1–10, https://doi.org/10.1038/s41598-023-42626-9.
[43] M. Rippa, R. Castagna, M. Pannico, P. Musto, G. Borriello, R. Paradiso, G. Galiero, S.B. Censi, J. Zhou, J. Zyss, L. Petti, Octupolar metastructures for a highly sensitive, rapid and reproducible phage-based detection of Bacterial pathogens by SERS octupolar metastructures for a highly sensitive, rapid and reproducible phage-based detection of Bacterial pathogens by SERS, ACS Sensors 2 (2017) 947–954.
[44] C.A. Grimes, S.C. Roy, S. Rani, Q. Cai, Theory, instrumentation and applications of magnetoelastic resonance sensors: a review, Sensors 18 (2011) 2809–2844, https://doi.org/10.3390/s110302809.
[45] M. Schmelcher, M.J. Loessner, M. Schmelcher, M.J. Loessner, Application of bacteriophages for detection of foodborne pathogens Application of bacteriophages for detection of foodborne pathogens, Bacteriophage 7081 (2014) e28137, https://doi.org/10.4161/bact.28137.
[46] A. Singh, D. Arutyunov, M.T. Mcdermott, M. Szymanski, S. Evoy, Specific detection of Campylobacter jejuni using the bacteriophage NCTC 12673 receptor binding protein as a probe, Analyst (2011) 4780–4786, https://doi.org/10.1039/c1an15547d.
[47] N. Wisuthiphaet, X. Yang, G.M. Young, N. Nitin, Rapid Detection of Escherichia coli in Beverages Using Genetically Engineered Bacteriophage T7, AMB Express, 2019, https://doi.org/10.1186/s13568-019-0776-7.
[48] B.B. Watson, The application of the phage-fluorescent antiphage staining system in the specific identification of Listeria, 1965.
[49] J. Wang, M. Hofnung, A. Charbit, The C-terminal portion of the tail fiber protein of bacteriophage lambda is responsible for binding to LamB, its receptor at the surface of Escherichia coli K-12, J. Bacteriol. 182 (2000) 508–512, https://doi.org/10.1128/JB.182.2.508-512.2000.
[50] H.J. Shin, W.K. Lim, Rapid label-free detection of E. coli using a novel SPR biosensor containing a fragment of tail protein from phage lambda, Prep. Biochem. Biotechnol. 48 (2018) 498–505, https://doi.org/10.1080/10826068.2018.1466154.
[51] R. Edgar, M. Mckinstry, J. Hwang, A.B. Oppenheim, R.A. Fekete, G. Giulian, C. Merril, K. Nagashima, S. Adhya, High-sensitivity bacterial detection using biotin-tagged phage and quantum-dot nanocomplexes, Natl. Acad. Sci. United States (2006), https://doi.org/10.1073/pnas.0601211103.
[52] L. Wang, H. Lin, J. Zhang, J. Wang, Talanta Phage long tail fiber protein-immobilized magnetic nanoparticles for rapid and ultrasensitive detection of Salmonella, Talanta 248 (2022) 123627, https://doi.org/10.1016/j.talanta.2022.123627.
[53] J. Xu, C. Zhao, Y. Chau, Y. Lee, The synergy of chemical immobilization and electrical orientation of T4 bacteriophage on a micro electrochemical sensor for low-level viable bacteria detection via Differential Pulse Voltammetry, Biosens. Bioelectron. (2019) 111914, https://doi.org/10.1016/j.bios.2019.111914.
[54] Z.A. Ahovan, A. Hashemi, L.M. De Plano, M. Gholipourmalekabadi, A. Seifalian, Bacteriophage based biosensors : trends, outcomes and challenges, Nanomaterials 10 (2020) 501.
[55] S. Balasubramanian, I.B. Sorokulova, V.J. Vodyanoy, A.L. Simonian, Lytic phage as a specific and selective probe for detection of Staphylococcus aureus — A surface plasmon resonance spectroscopic study, Biosens. Bioelectron. 22 (2007) 948–955, https://doi.org/10.1016/j.bios.2006.04.003.
[56] S. Niyomdecha, W. Limbut, A. Numnuam, P. Kanatharana, R. Charlermroj, N. Karoonuthaisiri, P. Thavarungkul, Phage-based capacitive biosensor for Salmonella detection, Talanta 188 (2018) 658–664, https://doi.org/10.1016/j.talanta.2018.06.033.
[57] F. Wang, S. Horikawa, J. Hu, H.C. Wikle III, I.-H. Chen, S. Du, Y. Liu, B.A. Chin, Detection of Salmonella typhimurium on spinach using phage-based magnetoelastic biosensors, Sensors 17 (2) (2017) 386, https://doi.org/10.3390/s17020386.
[58] S.J. Favrin, S.A. Jassim, M.W. Griffiths, Application of a novel immunomagnetic separation-bacteriophage assay for the detection of Salmonella enteritidis and Escherichia coli O157:H7 in food, Int. J. Food Microbiol. 85 (2003) 63–71, https://doi.org/10.1016/S0168-1605(02)00483-X.
[59] S. Kang, A.M. Hawkridge, K.L. Johnson, D.C. Muddiman, P.E. Prevelige, Identification of subunit - subunit interactions in bacteriophage P22 procapsids by chemical cross-linking and mass spectrometry research articles, J. Proteome Res. 5 (2006) 370–377.
[60] M. Tolba, O. Minikh, L.Y. Brovko, S. Evoy, M.W. Griffiths, A. Tg, Oriented immobilization of bacteriophages for biosensor applications ↭, Appl. Environ. Microbiol. 76 (2010) 528–535, https://doi.org/10.1128/AEM.02294-09.
[61] A. Shabani, C.A. Marquette, M.F. Lawrence, Carbon microarrays for the direct impedimetric detection of Bacillus anthracis using Gamma phages as probes †, Analyst (2013) 1434–1440, https://doi.org/10.1039/c3an36830k.
[62] Y. Lin, S. Chen, Y. Chuang, Y. Lu, T.Y. Shen, C.A. Chang, C. Lin, Biosensors and Bioelectronics Disposable amperometric immunosensing strips fabricated by Au nanoparticles-modified screen-printed carbon electrodes for the detection of foodborne pathogen Escherichia coli O157 : H7, Biosens. Bioelectron. 23 (2008) 1832–1837, https://doi.org/10.1016/j.bios.2008.02.030.
[63] N. Bhardwaj, S.K. Bhardwaj, J. Mehta, G.C. Mohanta, A. Deep, Bacteriophage immobilized graphene electrodes for impedimetric sensing of bacteria (Staphylococcus arlettae), Anal. Biochem. 505 (2016) 18–25, https://doi.org/10.1016/j.ab.2016.04.008.
[64] X. Tan, M. Yang, L. Zhu, G. Gunathilaka, Z. Zhou, P.Y. Chen, Y. Zhang, M.M. C. Cheng, Ultrasensitive and selective bacteria sensors based on functionalized graphene transistors, IEEE Sens. J. 22 (2022) 5514–5520, https://doi.org/10.1109/JSEN.2022.3147229.
[65] I.A. Quintela, T. Vasse, C. Lin, Advances, applications, an limitations of portable and rapid detection technologies for routinely encountered foodborne pathogens, Front. Microbiol. 13 (2022) 1054782, https://doi.org/10.3389/fmicb.2022.1054782.
[66] J. Xu, Y. Chau, Y. Lee, Phage-based electrochemical sensors : a review, Micromachines 10 (2019) 855.
[67] S. Horikawa, S. Li, D. Bedi, W. Shen, I.-H. Chen, Y. Chai, M. Auad, M. Bozack, J. Barbaree, V. Petrenko, B. Chin, Effects of surface phage coverage on the performance of wireless phage-immobilized magnetoelastic biosensors, ECS Trans. 33 (2010) 41–48, https://doi.org/10.1149/1.3484105.
[68] M.B. Mejri, H. Baccar, E. Baldrich, F.J. Del Campo, S. Helali, T. Ktari, A. Simonian, M. Aouni, A. Abdelghani, Biosensors and Bioelectronics Impedance biosensing using phages for bacteria detection : Generation of dual signals as the clue for in-chip assay confirmation, Biosens. Bioelectron. 26 (2010) 1261–1267, https://doi.org/10.1016/j.bios.2010.06.054.
[69] V. Nanduri, I.B. Sorokulova, A.M. Samoylov, A.L. Simonian, V.A. Petrenko, V. Vodyanoy, Phage as a molecular recognition element in biosensors immobilized by physical adsorption, Biosens. Bioelectron. 22 (2007) 986–992, https://doi.org/10.1016/j.bios.2006.03.025.
[70] N. Tawil, E. Sacher, R. Mandeville, M. Meunier, Biosensors and bioelectronics surface plasmon resonance detection of E . coli and methicillin-resistant S . aureus using bacteriophages, Biosens. Bioelectron. 37 (2012) 24–29.

[71] F. Liu, Z. Luo, X. Ding, S. Zhu, X. Yu, Phage-displayed protein chip based on SPR sensing, Sensors Actuators, B Chem. 136 (2009) 133–137, https://doi.org/10.1016/j.snb.2008.11.031.
[72] M. Vinay, N. Franche, G. Grégori, J. Fantino, Phage-based fluorescent biosensor prototypes to specifically detect enteric bacteria such as E . coli and Salmonella enterica Typhimurium, PLoS One (2015) 1–17, https://doi.org/10.1371/journal.pone.0131466.
[73] S. Barbesti, L. Soldini, G. Carcelain, A. Guignet, V. Colizzi, B. Mantelli, A. Corvaglia, T. Tran-minh, F. Dorigatti, B. Autran, A. Lazzarin, A. Beretta, Short communication a simplified flow cytometry method of CD4 and CD8 cell counting based on thermoresistant reagents : implications for large scale monitoring of HIV-infected patients in resource-limited settings, Cytom. Part B Clin. Cytom. 51 (2005) 43–51, https://doi.org/10.1002/cyto.b.20068.
[74] H.B. Steen, Flow cytometer for measurement of the light scattering of viral and other submicroscopic particles, Cytom. Part B. 99 (2004) 94–99.
[75] W. Sun, L. Brovko, M. Griffiths, FOOD-BORNE PATHOGENS Use of bioluminescent Salmonella for assessing the efficiency of constructed phage-based biosorbent, J. Ind. Microbiol. Biotechnol. 27 (2001) 126–128.
[76] Z. Hosseinidoust, T.G.M. Van De Ven, N. Tufenkji, Bacterial capture efficiency and antimicrobial activity of phage-functionalized model surfaces, Langmuir 27 (2011) 5472–5480.
[77] S. Roy, N.F. Mohd-Naim, M. Safavieh, M.U. Ahmed, Colorimetric nucleic acid detection on paper microchip using loop mediated isothermal amplification and crystal violet dye, ACS Sens. 2 (2017) 1713–1720, https://doi.org/10.1021/acssensors.7b00671.
[78] E.V Olsen, I.B. Sorokulova, V.A. Petrenko, I. Chen, J.M. Barbaree, V.J. Vodyanoy, Affinity-selected filamentous bacteriophage as a probe for acoustic wave biodetectors of Salmonella typhimurium, Biosens. Bioelectron. 21 (2006) 1434–1442, https://doi.org/10.1016/j.bios.2005.06.004.
[79] S. Huang, H. Yang, R.S. Lakshmanan, M.L. Johnson, J. Wan, I. Chen, H.C.W. Iii, V. A. Petrenko, J.M. Barbaree, B.A. Chin, Sequential detection of Salmonella typhimurium and Bacillus anthracis spores using magnetoelastic biosensors, Biosens. Bioelectron. (2009) 1730–1736, https://doi.org/10.1016/j.bios.2008.09.006.
[80] M. Park, J.W. Park, H.C. Wikle, B.A. Chin, Comparison of phage-based magnetoelastic biosensors with Taqman- based quantitative real-time PCR for the detection of Salmonella typhimurium directly grown on tomato surfaces, Biosens. Bioelectron. 3 (2012) 1–8, https://doi.org/10.4172/2155-6210.1000113.
[81] I. Chen, S. Horikawa, K. Bryant, R. Riggs, B.A. Chin, J.M. Barbaree, Bacterial assessment of phage magnetoelastic sensors for Salmonella enterica Typhimurium detection in chicken meat, Food Control 71 (2017) 273–278, https://doi.org/10.1016/j.foodcont.2016.07.003.
[82] W. Shen, R.S. Lakshmanan, L.C. Mathison, V.A. Petrenko, B.A. Chin, Chemical Phage coated magnetoelastic micro-biosensors for real-time detection of Bacillus anthracis spores ☪, Sensors Actuators B. Chem. 137 (2009) 501–506, https://doi.org/10.1016/j.snb.2009.01.027.
[83] M.L. Johnson, J. Wan, S. Huang, Z. Cheng, V.A. Petrenko, D. Kim, I. Chen, J. M. Barbaree, J. Wook, B.A. Chin, A wireless biosensor using microfabricated phage-interfaced magnetoelastic particles, Sensors Actuators A 144 (2008) 38–47, https://doi.org/10.1016/j.sna.2007.12.028.
[84] X. Liu, C. Huang, C. Qiu, Z. Wang, M. Cheng, Y. Zhang, Biosensors and Bioelectronics Rapid and sensitive detection of Staphylococcus aureus using biolayer interferometry technology combined with phage lysin LysGH15, Biosens. Bioelectron. 198 (2022) 113799, https://doi.org/10.1016/j.bios.2021.113799.
[85] S.M. Tripathi, W.J. Bock, P. Mikulic, R. Chinnappan, A. Ng, M. Tolba, M. Zourob, Biosensors and Bioelectronics Long period grating based biosensor for the detection of Escherichia coli bacteria, Biosens. Bioelectron. 35 (2012) 308–312, https://doi.org/10.1016/j.bios.2012.03.006.
[86] Ł. Richter, M. Janczuk-richter, J. Niedzió, J. Paczesny, R. Ho, Recent advances in bacteriophage- based methods for bacteria detection, Drug Discov. Today. (2018) 23, https://doi.org/10.1016/j.drudis.2017.11.007.
[87] D.A. Schofield, N.J. Sharp, C. Westwater, Phage-based platforms for the clinical detection of human bacterial pathogens, Bacteriophage 2 (2012) 105–121.
[88] R. Denis, X. M-B, J. Mas, Fast phage detection and quantification : An optical density-based approach, PLoS One 15 (2019) e0216292, https://doi.org/10.1371/journal.pone.0216292.
[89] F. Mi, C. Hu, Y. Wang, L. Wang, F. Peng, P. Geng, M. Guan, Recent advancements in microfluidic chip biosensor detection of foodborne pathogenic bacteria : a review, Anal. Bioanal. Chem. (2022) 2883–2902, https://doi.org/10.1007/s00216-021-03872-w.
[90] S.H. Hyeon, W.K. Lim, Novel surface plasmon resonance biosensor that uses full-length Det7 phage tail protein for rapid and selective detection of Salmonella enterica serovar Typhimurium, Biotechnol. Appl. Biochem. 68 (2001) 5–12, https://doi.org/10.1002/bab.1883.
[91] N. Bhardwaj, S.K. Bhardwaj, J. Mehta, K.H. Kim, A. Deep, MOF-bacteriophage biosensor for highly sensitive and specific detection of staphylococcus aureus, ACS Appl. Mater. Interfaces. 9 (2017) 33589–33598, https://doi.org/10.1021/acsami.7b07818.
[92] G. Guła, P. Szymanowska, T. Piasecki, G. Sylwia, T. Gotszalk, Z. Drulis-kawa, The application of impedance spectroscopy for pseudomonas biofilm monitoring during phage infection, Viruses 12 (2020) 407.