Exploring Bacterial Attachment on Modified Polycaprolactone-Polyaniline-Gelatin Nanofibers for Innovative Detection Strategies

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Abstract

Introduction: Detecting pathogenic bacteria quickly and accurately is essential for food safety and clinical diagnostics. Biosensors offer a fast, cost-effective alternative to traditional microbial identification. This study describes a novel nanofiber-based biosensor designed to sensitively detect *Escherichia coli (E. coli).*

Materials and Methods: Electrospun nanofibers composed of polycaprolactone (PCL), gelatin (Gel), and polyaniline (PANi) were fabricated to create a biocompatible scaffold. Biotinylated bacteriophages, serving as highly specific bioreceptors for *E. coli*, were covalently immobilized onto the surface of the prepared nanofibers. This immobilization strategy employed avidin-biotin interactions mediated by EDC/NHS crosslinking chemistry, which activated carboxyl groups on the nanofiber surface to enable stable and oriented bioconjugation. The capture efficiency and specificity of the functionalized platform were subsequently evaluated against target *E. coli* cells using scanning electron microscopy (SEM) and atomic force microscopy (AFM).

Results: Raman spectral analysis confirmed the presence of characteristic peaks corresponding to amide and amine (NH) functional groups on the nanofiber surface following phage immobilization, verifying successful chemical modification. Qualitative analysis using SEM and AFM provided direct visual evidence of bacterial attachment onto the bacteriophage-functionalized nanofiber mats, demonstrating the platform's capacity for effective microbial capture.

Conclusion: The integration of conductive PANi with a biocompatible PCL/Gel scaffold, combined with the specificity of phage functionalization, establishes an effective interface for bacterial detection. This innovation signifies a pivotal advancement in pathogen detection methodologies and biosensor technologies, offering a promising tool for applications requiring rapid and reliable identification of microbial contaminants.

Keyword: *Bacteriophage T4, Biosensor, E. coli, Electrospinning, PANi*

1. Introduction

Microorganisms, including bacteria and viruses, are ubiquitous in the environment, and varied microbial populations flourish in both natural and extreme stress conditions, such as soil, water bodies, the human digestive system, hydrothermal vents, acidic mine drainage, and petroleum reservoirs [1]. This underscores the necessity for detection methods aimed at identifying pathogens in food, water, and air to protect public health. Furthermore, many standard methods demand intricate equipment that may not be feasible for in-situ utilization [2].

Though generally considered commensal, certain pathotypes of *E. coli* are serious human pathogens that can cause life-threatening conditions, such as bloody diarrhea and kidney failure [3]. It is imperative to establish rapid and accurate methodologies for the detection and quantification of *E. coli* in food products, environmental surveillance, and clinical diagnostics. Emerging technologies such as isothermal amplification, biosensors, surface-enhanced Raman spectroscopy, paper-based diagnostics, and smartphone-based methods represent new approaches for diagnosing *E. coli* [4]. Biosensors provide precise measurements of chemicals and microorganisms in samples. Their scalability, effectiveness, and versatility render them suitable for use in medicine, industry, and agriculture [5].

Electrically Conducting Polymers (CPs) are functional polymers that are applied in the development of electrospun nanofibers, which feature properties that can be tailored. These nanofibers have a large surface area, facilitate electron flow, and enhance biosensor functionality [6]. PANi has been identified as an exceptionally versatile material in various key areas of science and technology, particularly in tissue engineering [7], electrochromic devices [8], bio-actuators [9], solar cells [10], and biosensors [11]. Polycaprolactone (PCL) provides excellent mechanical strength and biocompatibility, while Gelatin (Gel) introduces natural binding motifs that enhance bio-interactions. The incorporation of Polyaniline (PANi) significantly boosts the electrical conductivity of the scaffold. Together, the PCL/Gel/PANi composite forms an ideal, highly functionalized nanofibrous matrix for biosensor applications.

In this study, we aimed to develop bacteriophage-modified PCL/Gel/PANi nanofibers as an effective biosensing platform for the detection of *E. coli*. To achieve this, bacteriophages were immobilized onto the nanofiber surface through an avidin–biotin interaction, enabling the selective capture and entrapment of *E. coli*. The resulting nanofiber–phage sensor provides a promising strategy for the development of targeted nano/micro-patterned systems with potential applications in electrical and biomedical sensors, actuators, and tissue engineering materials.

2. Materials and Methods:

2.1 Materials

Linker biotin-N-hydroxysuccinimide (NHS) (MW 341.38), Avidin, 1-ethyl-3-(3-dimethylaminopropyl) carbodiimide (EDC) (MW 155.24), and 2-(N-morpholino) ethanesulfonic acid (MES) buffer (MW 951.24) were sourced from Sigma, USA. A 0.1 M phosphate buffer (pH 7) was used as a supporting electrolyte, with all solutions having been prepared using ultrapure water from a Millipore-Milli Q system (18 MΩ cm). Reagents were used as received, and experiments were conducted at room temperature (25°C). *E. coli* (ATCC: 25922) and bacteriophage T4 (ATCC: 11303) were obtained from the Medical Sciences Research Center. PANi (emeraldine base) with a 100,000 MW was used for this research epxeriment.

2.2 Methods

2.2.1 Electrospinning

The methodology used in this study has been previously described and validated in earlier research [12]. In summary, to fabricate nanofibers, 1.8 g of PCL was dissolved in 9 ml of formic acid, and 0.1 g of PANi was added after being dissolved in 1 ml of formic acid. Simultaneously, 0.8 g of gelatin was dissolved in 5 ml of acetic acid. Each sample was loaded into a syringe and bi-electrospun at a distance of 15 cm from the collector, with a flow rate of 0.4 ml/h and an RPM of 500.

2.2.2 Preparation of bacteriophage/avidin/nanofibers

The immobilization protocol used in this study was adapted from a previously established and validated approach [13]. The preparation of bacteriophage/avidin/nanofibers involved three steps. First, biotinylated bacteriophage was created by dissolving NHS-Biotin in DMSO at 22 mg/mL. It was then added to the bacteriophage, and incubated at room temperature for 4 hours. In the second step, avidin was immobilized on the PCL/Gel/PANi scaffold by suspending 0.7 mg of avidin in 1.5 mL of MES buffer, adding NHS and EDC solutions while stirring, and allowing the mixture to react for 45 minutes. The activated avidin was then dropped onto the nanofibers and left to react for 2 hours before being washed with MES buffer. Lastly, biotinylated bacteriophage was immobilized on the

avidin/nanofibers by capping 300 µL of the solution on it and keeping it in the dark overnight at room temperature, followed by being washed with PBS buffer. As a result of this process, bacteriophages attached themselves to nanofibers. The schematic representation of this process can be found in Fig. 1.

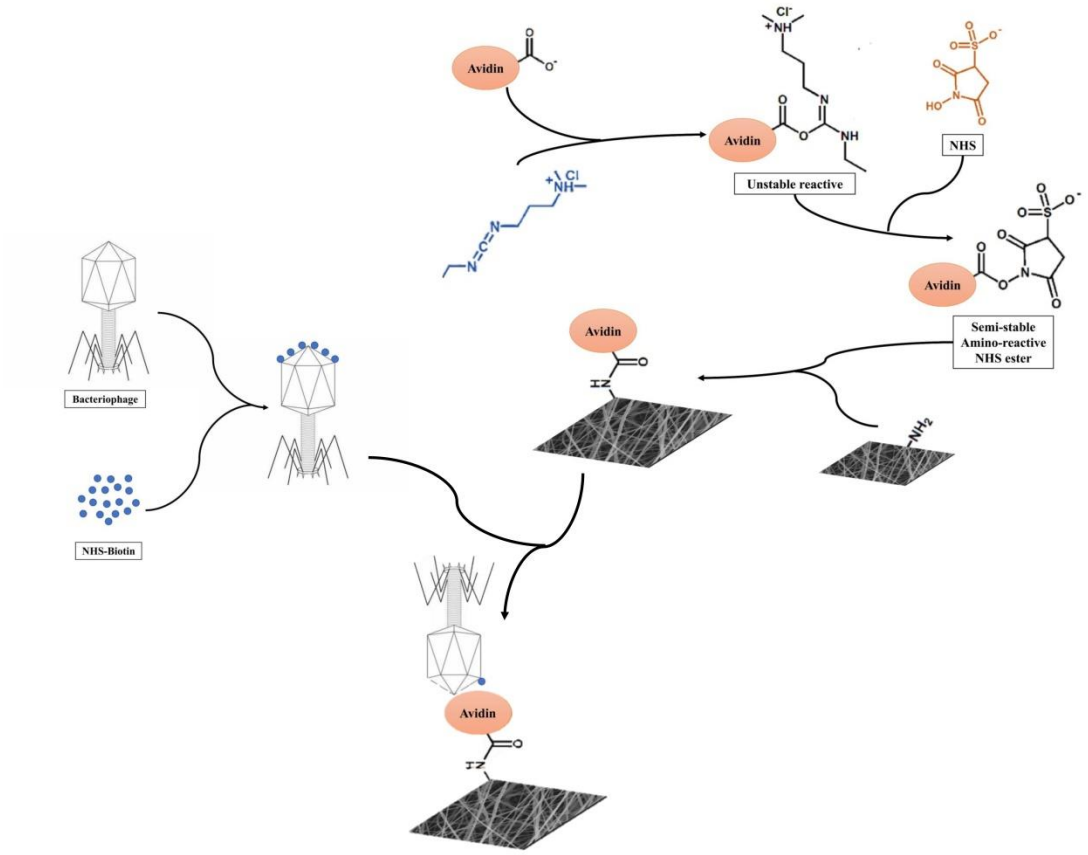

Fig. 1 Schematic of bacteriophage-modified nanofiber preparation. The process involves three main steps: (1) biotinylation of the bacteriophage, (2) immobilization of avidin onto the electrospun PCL/Gel/PANi nanofibrous scaffold, and (3) attachment of the biotinylated bacteriophages to the avidin-coated scaffold via strong avidin-biotin affinity.

2.2.3 Raman spectroscopy

Molecular structure changes in the nanofibers, confirmed by Raman spectroscopy [14], were obtained using a Nicolet Raman spectrometer. Raman spectra were captured within the spectral span of 400–4000 cm^{-1}.

2.2.4 Scanning electron microscopy (SEM)

In order to evaluate the structure of fibers, detect phage and *E. coli* on nanofibers, and measure the diameter of the fibers, scanning electron images were obtained using a KYKY-EM3200 scanning electron microscope (SEM) [15] from Japan. The samples underwent treatment with a gold sputter coating, and Image J software was utilized to analyze the thickness of the fibers, specifically measuring the thickness of 30 randomly selected fibers.

2.2.5 Atomic Force Microscopy

The surface morphology and topographic features of the nanofibers were investigated using Atomic Force Microscopy (AFM) [16]. Measurements were performed with an Ambios Technology Q-Scope™ 250 atomic force microscope operating in contact mode under ambient laboratory conditions (room temperature).

3. Results:

3.1 Raman Spectroscopic characterization of PCL/Gel/PANi nanofibers

To analyze the molecular composition and ensure the successful incorporation of polyaniline (PANi) into the polymeric matrix, we performed Raman spectroscopy. This technique is both sensitive and non-destructive, providing extensive information about molecular vibrations, which makes it especially effective for studying chemical structure, crystallinity, and molecular interactions in composite biomaterials [17].

Fig 2 shows the Raman spectrum of the PCL/Gel/PANi nanofibers, highlighting characteristic vibrational bands that confirm the chemical integrity and successful hybridization of the blend components. Notably:

A prominent peak at 1588 cm^{-1} corresponds to N–H bending vibrations, indicating the presence of amine functionalities in the emeraldine salt form of PANi. This peak reflects the conductive nature of polyaniline and confirms its electroactive state. Another distinct band observed at 1473 cm^{-1} is attributed to the C–N stretching mode of PANi, further confirming the structural presence and chemical integration of PANi within the nanofiber network.

The presence of both quinoid and benzenoid structures in the Raman spectrum suggests that PANi preserves its distinctive redox-active form. This characteristic is crucial for achieving electrical conductivity in biomedical scaffolds, particularly in applications like neural interfaces, biosensors, and stimuli-responsive tissue engineering scaffolds [18]

The Raman spectral data collectively corroborated the successful establishment of a chemically integrated nanofibrous scaffold created from PCL, gelatin, and PANi. The conservation of both structural and functional groups suggests that this material is viable for applications that necessitate a combination of mechanical strength, biocompatibility, and electroactivity.

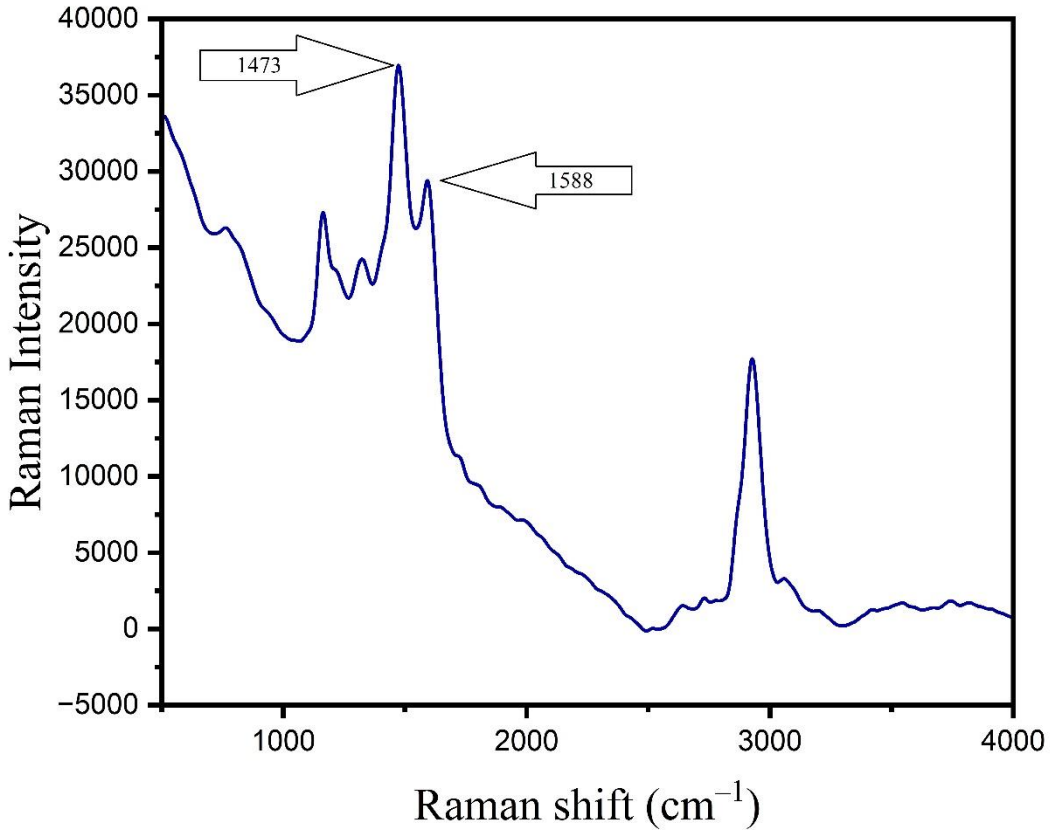

Figure. 1 Raman spectra of PCL/Gel/PANi nanofibers. Key spectral features include the characteristic peaks at 1473 cm^{-1} indicating the C=N, and at 1588 cm^{-1} corresponding to the C=C stretching

3.2 Scanning Electron Microscopy Imaging

To evaluate the morphology, surface functionalization, and antibacterial interactions of electrospun nanofibrous scaffolds, we utilized Scanning Electron Microscopy (SEM). SEM offers high-resolution imaging that allows for precise visualization of the nanofiber architecture, fiber diameter, and interactions with biological agents like bacteria or bacteriophages. Fig 3a displays the baseline morphology of the PCL/Gel/PANi electrospun nanofibers, illustrating a uniform and randomly oriented network with fiber diameters ranging from approximately 300 to 400 nm. Figs 3b and 3c show the PCL/Gel/PANi scaffolds after surface functionalization with bacteriophages. As indicated by the arrows, distinct spherical nanoparticle-like clusters are visible on the surface of the nanofibers.

These structures are related to the immobilized bacteriophages, which are attached through a biotin–avidin linker system. This system is commonly applied to maintain phage activity and realize targeted antibacterial action. The successful existence of these phage clusters substantiates the biofunctionalization of the nanofibers without compromising their structural integrity. Figs 3d, 3e, and 3f provide crucial insights into the biological interaction between the scaffolds and bacterial cells. In Fig 3f, bacterial cells appear to be well-adhered to the surface of the modified nanofibers. Additionally, Fig 3d and 3e reveal morphological signs of bacterial lysis, such as membrane rupture and structural deformation, following exposure to the bacteriophage-functionalized scaffolds. This lytic effect confirms the bactericidal action of the immobilized phages, which operate through specific recognition and infection of target bacterial cells, ultimately leading to their breakdown. Notably, the altered morphology of bacteria in these panels, evidenced by membrane perforation and debris, supports the hypothesis that phage-modified surfaces can serve as sensors.

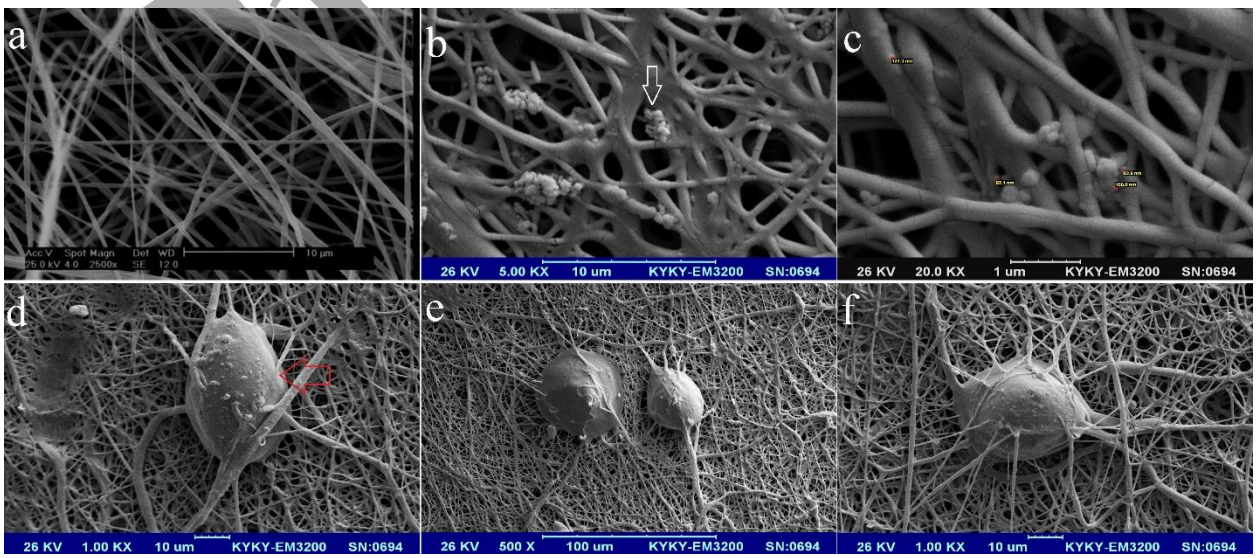

Figure. 2 SEM images of the electrospun PCL/Gel/PANi nanofibers (a), the nanofibers modified with bacteriophages (b, c), and bacteria trapped on the nanofibers (d, e, f).

3.3 Atomic Force Microscopy Analysis

For the purpose of obtaining high-resolution insights into the topographical and morphological aspects of the created PCL/Gel/PANi nanofibers, we utilized Atomic Force Microscopy (AFM). AFM offers nanoscale visualization of surface structures and enables a thorough evaluation of topographic features, surface roughness, and interfacial interactions between biomaterials and biological entities.Figs 3a–c show AFM topographic images of the nanofiber scaffolds under various conditions: Fig 3a illustrates the pristine surface morphology of the unmodified PCL/Gel/PANi nanofibers. The image illustrates a closely woven fibrous network that exhibits a relatively uniform thickness of strands and notable surface complexity. The visible peaks and troughs in the topology indicate a high degree of surface roughness, which can significantly influence the surface area.

In Fig 3b, the nanofiber surface has been functionalized with bacteriophages, as indicated by the arrow. The image clearly shows localized protrusions or clusters that represent phage entities immobilized onto the fiber surface. This successful visualization of the structures confirms that biotin–avidin-based conjugation has effectively modified the surface. The nanoscale distribution of phages on the scaffold is crucial for ensuring targeted antimicrobial activity and maintaining high surface coverage.

Fig 3c depicts the interaction between bacterial cells and the scaffold surface. The arrow points to a bacterial cell that is tightly bound to the nanofiber matrix. The morphological characteristics of the bacterial cell and its integration into the fibrous network demonstrate that the scaffold promotes bacterial adhesion. Notably, the changes in the surface surrounding the bacterium may indicate localized disruption, suggesting bacteriophage-induced lysis.

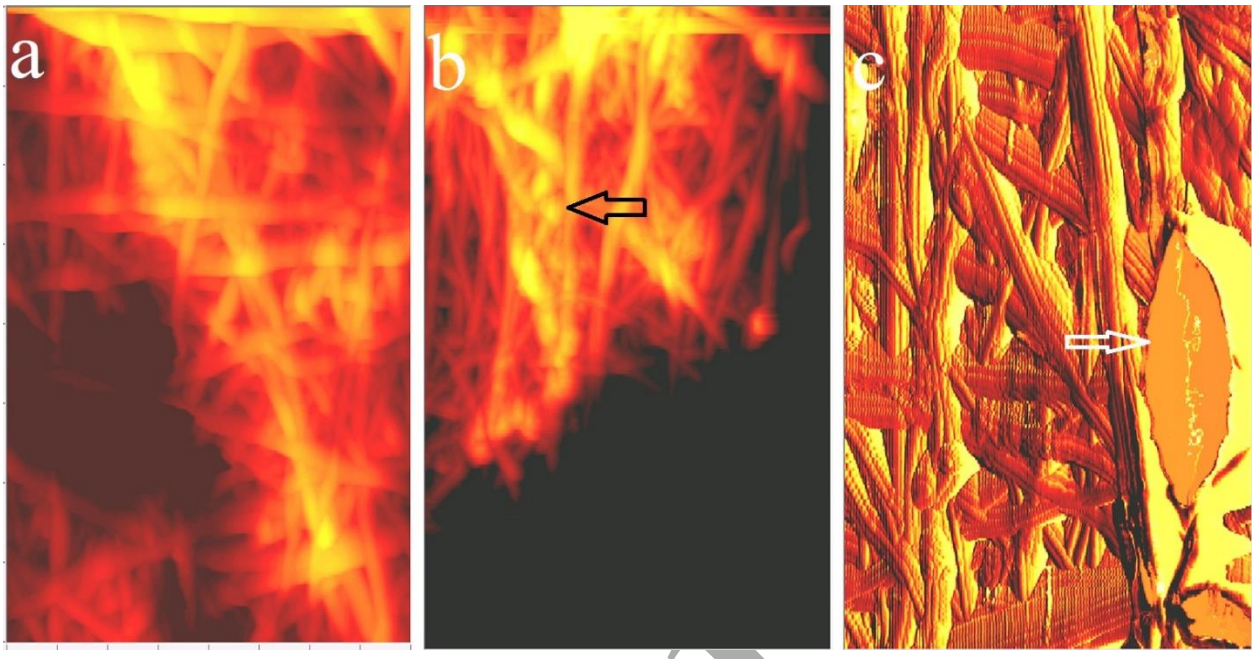

Fig. 3 shows AFM images of the electrospun PCL/Gel/PAN nanofibers (a), the nanofibers modified with bacteriophages (b), and the bacteria trapped on the nanofibers (c).

4. Discussion:

Conventional approaches to bacterial identification frequently fall short in terms of accuracy and speed. While genetic techniques like PCR offer good sensitivity and specificity, they tend to be complex and challenging [19, 20]. Therefore, there is a necessity for identification approaches that are simpler, more rapid, and more accurate, a challenge that nano biosensors can effectively address [20]. To meet this need, electrospinning serves as a potent approach for the generation of target-specific nanofibers utilizing diverse polymers [21]. In our research, we implemented electrospinning to successfully design and assemble these nanofibers. We selected this particular composite because of its relevance in biological and surface characteristics, specifically capitalizing on the N-H group provided by the PANi polymer for subsequent functionalization.

Mohamad et al. [22] analyzed chemically modified PANi and graphene (GP) composite nanofibers as an electrochemical DNA biosensor for the detection of *Mycobacterium tuberculosis*. Their findings highlighted the

exceptional electrochemical performance of PANi/GP composite nanofibers, which is due to their remarkable sensitivity and accuracy when interacting with synthetic DNA.

When bacteriophages attach to chemically functionalized sensor surfaces, they generally form stronger and more stable interactions than those achieved through physical adsorption, thereby reducing the risk of detachment during measurement processes. Accordingly, this strategy has been increasingly employed for the immobilization of phages in electrochemical biosensors. In the study by Shabani et al. screen-printed carbon electrodes (SPEs) were electrochemically oxidized to generate carboxyl groups, which were subsequently activated using EDC under acidic conditions. The successful immobilization of T4 bacteriophages onto the electrode surface was attributed to the formation of amide bonds between the primary amine groups (–NH2) of the phage protein coating and the activated carboxylic groups (–COOH) on the SPE surface. Similarly, Bhardwaj et al. [24]. applied the same chemical approach for the immobilization of lytic bacteriophages against Staphylococcus arlettae onto electrochemically oxidized graphene electrodes containing surface carboxyl groups. Furthermore, Gervais et al. [25] engineered wild-type T4 bacteriophages to express a biotin-binding domain on their capsid through phage display technology, enabling the oriented immobilization of biotinylated phages on streptavidin-modified surfaces.

In the present study, Raman spectroscopy confirmed the presence of N–H functional groups on the scaffold surface as a result of PANi incorporation, indicating successful surface functionalization of the nanofibers. The interaction between avidin and NHS likely resulted in amide bond formation between avidin and the nanofiber surface, while the biotin–NHS complex could further interact with the primary amine groups of the phage membrane proteins, thereby enhancing bacteriophage immobilization and surface activation. The results demonstrated that bacteriophage-functionalized nanofibers could effectively capture and detect E. coli cells. SEM analysis was employed to investigate the morphology and surface characteristics of the electrospun nanofibers, as well as to evaluate bacterial attachment and phage distribution on the scaffold surface. The electrospun fibers exhibited a random and defect-free alignment (Fig. 2a), which is advantageous for the homogeneous dispersion of bacteriophages across the nanofibrous matrix. Similar SEM-based investigations of biological agents immobilized on nanowire biosensors have also been previously reported [26]. In addition to SEM, AFM was used to further

confirm the binding of bacteriophages to the nanofibers and the identification of E. coli. AFM provides nanoscale topographical information and high-resolution surface imaging, making it a valuable complementary technique for evaluating nanoscale surface modifications and biological interactions. Compared with SEM, AFM can reveal subtle surface features and localized structural changes that may not be easily distinguishable through electron microscopy alone. Therefore, the combined use of Raman spectroscopy, SEM, and AFM provided strong evidence for the successful immobilization of bacteriophages on the nanofibrous scaffold and supported the effectiveness of the developed biosensing platform.

E. coli has previously been detected using optical, electrochemical, and mass-sensitive biosensors incorporating bioreceptors such as antibodies, aptamers, bacteriophages, and DNA probes [27]. The findings of the present study further demonstrate the potential of bacteriophage-modified nanofibers as a sensitive and selective platform for bacterial detection and trapping applications.

In the previous studies, polyaniline demonstrated its potential for surface modification by effectively binding bacteriophages, which facilitated the identification of bacteria [28]. Building on this foundation, our research aspires to design and fabricate innovative nanofibers that include polyaniline. By enhancing the surface characteristics of these nanofibers, we seek to improve the identification of bacteria. Realizing that pure polyaniline nanofibers by themselves are inadequate [12], we have strategically integrated it with other polymers to develop a robust nanofibers that exhibits exceptional characteristics for future studies.

Our study demonstrates that it is achievable to design and implement high-precision, targeted sensors by altering nanofibers. Furthermore, by changing the configuration of these nanofibers and utilizing a variety of polymers, one can produce nanofibers with exceptional traits suited for specialized uses.

Despite the promising results, a limitation of the current study is that the sensor was primarily tested under controlled laboratory conditions. Future research should evaluate the detection efficacy of the PCL/Gel/PANi-bacteriophage biosensor in complex, real-world biological or environmental matrices (e.g., wastewater or blood samples) [29]. Additionally, future studies should focus on the long-term stability and commercial scalability of these functionalized nanofibers [30].

5. Conclusion

The integration of nanotechnology with biosensor technologies has profoundly enhanced the detection of bacterial pathogens, such as *E. coli*, in food and water supplies. Emerging biosensors alongside electrospun nanofibers provide rapid, cost-effective, and sensitive alternatives for pathogen detection. A notable advancement is the PCL/Gel/PANi nanofibers functionalized with bacteriophages, which achieves high specificity and rapid response for *E. coli* detection. This innovation has the potential to enhance the capabilities of biosensors and to underscore the promise of nanotechnology in biomedical and environmental applications. Future research should prioritize enhancing the scalability and stability of biosensors to broaden their usability in various contexts.

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Ethical statement

There is none to be disclosed.

Authors' contribution

AKM, AD, and KM: preparing the draft; managing data; conceptualizing; designing experiments; validating the study; writing and evaluating the paper; and analyzing data. MA, DA, and MHF: Writing and reviewing the article. RFM: Conceptualization, Trials design, providing materials and equipment, study justification, supervision, reviewing the article.

Conflict of interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

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