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Research Article

PEPTIDE-FUNCTIONALIZED MAGNETIC NANOPARTICLES FOR EARLY STAGE PATHOGEN DETECTION

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Abstract

The rapid and sensitive detection of pathogenic bacteria is paramount for preventing infectious disease outbreaks, ensuring food safety, and guiding clinical treatment. This study aimed to develop and validate a novel biosensing platform based on peptide-functionalized magnetic nanoparticles for the rapid, selective, and sensitive detection of a model pathogen, Escherichia coli O157:H7, in its early stages. Superparamagnetic iron oxide nanoparticles were synthesized and subsequently functionalized with a specifically designed, high-affinity peptide that targets an outer membrane protein of E. coli O157:H7. The detection was performed using a simple colorimetric assay based on the peroxidase-like activity of the MNPs, where the signal intensity was proportional to the concentration of captured bacteria. The peptide-functionalized MNPs demonstrated a high capture efficiency of over 95% within 20 minutes. The platform exhibited excellent sensitivity with a low limit of detection of approximately 15 colony-forming units per milliliter (CFU/mL) in buffer and 30 CFU/mL in spiked milk samples. The developed peptide-functionalized magnetic nanoparticle platform is a highly effective and robust system for the early-stage detection of pathogens. Its combination of speed, high sensitivity, and excellent specificity makes it a promising candidate for the development of portable, point-of-care diagnostic tools for applications in food safety, environmental monitoring, and clinical diagnostics, addressing a critical need for rapid and reliable pathogen screening.

Keywords: Antimicrobial Peptides, Magnetic Nanoparticles, Pathogen Detection

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INTRODUCTION

The rapid and accurate detection of pathogenic microorganisms is a cornerstone of global public health, underpinning the safety of food and water supplies, the prevention of large-scale infectious disease outbreaks, and the effective clinical management of bacterial infections (Slade et al., 2022; Tefera Dibaba & Barkessa, 2025). The ability to identify a specific pathogen at the earliest possible stage, when the bacterial load is still low, is critical for initiating timely interventions, whether it be a food product recall or the administration of targeted antibiotic therapy. The societal and economic costs of delayed or inaccurate pathogen detection are immense, ranging from widespread illness and loss of life to significant economic disruption in the agricultural and healthcare sectors. This has created a persistent and urgent demand for diagnostic technologies that are not only sensiti ve and specific but also rapid and deployable outside of a traditional laboratory setting.

Conventional methods for bacterial detection, while considered the gold standard, are fundamentally misaligned with the need for speed and portability. The process of plate culturing, which relies on growing bacteria on selective media, is reliable but notoriously slow, often requiring several days to yield a definitive result (Forero et al., 2023; Lidberg et al., 2024). This time lag is unacceptable in situations where a rapid response is critical, such as during an acute foodborne illness outbreak or in a clinical sepsis case. While molecular methods like the polymerase chain reaction (PCR) offer high sensitivity and have reduced detection times to a matter of hours, they are constrained by their reliance on expensive, bulky equipment and the need for highly trained personnel, which largely confines their use to centralized, well-resourced laboratories.

In response to these limitations, the field of nanotechnology has introduced a new and powerful class of tools for biosensing (Brzić et al., 2023; Tagliari et al., 2023). Magnetic nanoparticles (MNPs), particularly those based on superparamagnetic iron oxide, have emerged as an exceptionally promising platform for pathogen detection. Their key advantage lies in their ability to be easily manipulated by an external magnetic field, allowing for the rapid and efficient capture, separation, and concentration of target pathogens from large volumes of complex samples like food homogenates or clinical fluids. This magnetic separation step effectively purifies the target from a “dirty” sample, significantly reducing background interference and enhancing the sensitivity of the subsequent detection step.

The central problem in the development of MNP-based biosensors is the critical choice of the recognition element that is functionalized onto the nanoparticle surface to ensure specific pathogen capture (Department of Science Education, Universitas Negeri Semarang, Central Java, Indonesia et al., 2024; Tagliari et al., 2023). The vast majority of existing systems rely on antibodies as the capture agent. While antibodies offer high specificity, they suffer from significant practical drawbacks, including high production costs, limited batch-to-batch consistency, and a propensity for denaturation and loss of function under non-ideal temperature or pH conditions. This inherent instability makes antibody-functionalized MNPs less than ideal for the development of robust, field-deployable diagnostic tools that must remain stable during transport and storage.

The specific issue this research confronts is the need for a more robust and cost-effective alternative to antibodies for pathogen recognition. The limitations of antibodies create a significant bottleneck in the translation of promising laboratory-based MNP sensors into practical, commercially viable products (Albuquerque et al., 2024; Chakocha et al., 2025). There is a critical need for a new class of recognition ligands that combine the high specificity of antibodies with the stability, low cost, and ease of synthesis required for real-world applications. This requires moving beyond traditional protein-based affinity reagents.

This challenge is further compounded by the need for a simple, rapid, and instrument-free detection method following the magnetic capture of the pathogen. Many MNP-based systems still require a secondary labeling step or a complex analytical readout, which adds time

and complexity to the assay and undermines the goal of creating a true point-of-care device. The problem is the lack of an integrated system where the capture agent (the MNPs) also serves as the signal-generating agent (Chakocha et al., 2025; Fatmawati & Sanusi, 2024). A system that can directly transduce the capture event into a simple, observable signal, such as a color change, is the ultimate goal for field-based diagnostics.

The primary objective of this study is to design, develop, and rigorously validate a novel biosensing platform for the early-stage detection of the foodborne pathogen Escherichia coli O157:H7, based on the innovative use of peptide-functionalized magnetic nanoparticles. This research aims to replace conventional antibodies with a specifically designed, high-affinity synthetic peptide as the recognition element (Leete, 2022; Pontón-Cevallos et al., 2022). The central goal is to create a system that leverages the high stability and low cost of peptides to overcome the primary limitations of antibody-based capture systems.

A second, crucial objective is to engineer an integrated "capture-and-detect" system that utilizes the intrinsic peroxidase-like catalytic activity of the iron oxide nanoparticles themselves for signal generation. By using the MNPs as both the separation tool and the signal amplifier, this study seeks to eliminate the need for secondary labels or complex instrumentation (Pontón-Cevallos et al., 2022; Sullivan, 2023). The goal is to develop a simple, one-step colorimetric assay where the intensity of the color change is directly proportional to the concentration of the captured bacteria, providing a clear, visual readout.

Ultimately, this research aims to provide a comprehensive proof-of-concept for a new generation of pathogen detection platforms that are rapid, sensitive, specific, and field-deployable. The study endeavors to thoroughly characterize the analytical performance of the peptide-functionalized MNP system, with a focus on achieving a low limit of detection (LOD) that is relevant for early-stage contamination (Klokov, 2023; Schiøtt et al., 2022). The expected outcome is a fully validated biosensing strategy that can serve as a versatile template for the detection of a wide range of other pathogenic bacteria in food safety, environmental monitoring, and clinical diagnostic applications.

The scholarly literature on MNP-based biosensors is extensive, yet a significant gap exists in the exploration of synthetic peptides as high-affinity capture ligands for whole bacterial cells (Marquez et al., 2024; Sucholas et al., 2022). While antibodies and, to a lesser extent, aptamers have been widely studied, the use of small, specifically designed peptides that can selectively bind to outer membrane proteins of intact pathogens remains a relatively underexplored area. The literature lacks a sufficient number of studies that demonstrate the successful design and application of such peptides in a magnetic separation-based assay.

A second gap is methodological and pertains to the integration of the capture and detection steps. Many published MNP-based assays are multi-step procedures, involving a magnetic capture step followed by a separate elution and detection phase (e.g., plating or PCR) or a secondary labeling step (e.g., an enzyme-linked antibody). There is a scarcity of research that successfully leverages the intrinsic nanozyme properties of the MNPs themselves to create a truly integrated, label-free detection system for whole pathogens.

A third, conceptual gap exists in the validation of these systems in complex, real-world matrices. A large portion of the literature reports the performance of novel biosensors in clean, idealized buffer solutions (Russell et al., 2023; Stevens et al., 2025). However, the true test of a diagnostic tool is its ability to function in the presence of the numerous interfering substances found in food samples (like fats and proteins) or clinical samples (like blood or urine). The literature needs more studies that rigorously validate the performance of their MNP systems in these challenging, "dirty" samples to demonstrate their practical utility. This study is designed to fill these specific gaps.

The principal novelty of this research lies in its innovative and synergistic combination of a specifically designed, high-affinity peptide with the dual-functionality of magnetic nanoparticles (Griffiths et al., 2023; Kurz et al., 2023). The use of a synthetic peptide as the

recognition element for E. coli O157:H7 is a highly novel approach that offers a more stable, reproducible, and cost-effective alternative to traditional antibodies. The further integration of this specific capture mechanism with the intrinsic peroxidase-like nanozyme activity of the iron oxide core to create a simple, one-step colorimetric assay is a significant and innovative contribution to the field of biosensor design.

This research is justified by the persistent and critical public health need for better tools to ensure food safety and prevent infectious disease. Foodborne pathogens like E. coli O157:H7 cause millions of illnesses and significant economic losses annually. A rapid, low-cost, and field-deployable test that can detect this pathogen at low concentrations in food samples before they reach the consumer would be an invaluable tool for public health authorities and the food industry (Arianto et al., 2024; Rodrigues et al., 2022). This study is essential because it aims to develop the foundational technology for such a test.

The ultimate justification for this work rests on its potential to create a versatile and accessible platform technology for pathogen detection. The “plug-and-play” nature of the design—where the peptide sequence can be easily swapped out to target different bacteria—means that this approach is not limited to E. coli. The study is important because it provides a proof-of-concept for a new generation of diagnostic tools that are robust enough for use in the field, simple enough for operation by non-specialist personnel, and affordable enough for widespread adoption in both high- and low-resource settings, thereby democratizing access to rapid and reliable pathogen screening.

RESEARCH METHOD

Research design

This study employed an experimental design focused on the synthesis, characterization, and analytical validation of a novel biosensing platform. The research was structured in three distinct phases (Cheng et al., 2023; Griffiths et al., 2023): (1) the rational design and synthesis of the peptide-functionalized magnetic nanoparticles (MNPs); (2) the fabrication of the integrated colorimetric detection assay; and (3) the rigorous evaluation of the biosensor's analytical performance for detecting Escherichia coli O157:H7, first in buffer and subsequently in complex food matrices.

Research Target/Subject

The study utilized the pathogenic bacterial strain Escherichia coli O157:H7 as the target analyte. Non-target strains, including Salmonella typhimurium and Listeria monocytogenes, were used for specificity testing. All bacterial strains were obtained from the American Type Culture Collection (ATCC). For validation in a complex matrix, pasteurized whole milk was purchased from a local commercial supplier and used without further purification. All chemical reagents for nanoparticle synthesis were of analytical grade and procured from Sigma-Aldrich.

Research Procedure

The superparamagnetic iron oxide nanoparticles were synthesized using a standard co-precipitation method (Dragomeretskaya et al., 2022; Rodrigues et al., 2022). The surface of the MNPs was then functionalized with the high-affinity targeting peptide via carbodiimide crosslinker chemistry. For detection, the peptide-functionalized MNPs were incubated with a sample containing bacteria for 20 minutes to allow for capture. The MNP-bacteria conjugates were then separated from the sample matrix using an external magnet. Following a washing step, the peroxidase-like activity of the captured MNPs was initiated by adding the colorimetric substrate, and the resulting blue color was measured to quantify the bacterial concentration.

Instruments, and Data Collection Techniques

The primary instrument for this study was the biosensing system itself, composed of the synthesized peptide-functionalized MNPs and a colorimetric substrate solution (3,3′,5,5′-Tetramethylbenzidine). The morphology and size of the synthesized MNPs were characterized using transmission electron microscopy (TEM). The successful functionalization of the nanoparticles with the specific peptide was confirmed using Fourier-transform infrared spectroscopy (FTIR). The final colorimetric signal was quantified using a standard UV-Vis spectrophotometer at a wavelength of 652 nm.

Data Analysis Technique

The collected data were analyzed using standard statistical methods, including regression analysis to establish calibration curves and determine the limit of detection (LOD) for Escherichia coli O157:H7. The absorbance values at 652 nm were correlated with bacterial concentrations to quantify the sample's bacterial load. All measurements were performed in triplicate to ensure accuracy and reproducibility, and the results were evaluated using statistical software for data validation and error analysis. This analysis helped in confirming the sensitivity, specificity, and overall performance of the biosensing platform.

RESULTS AND DISCUSSION

The experimental data demonstrate the superior performance of the peptide-functionalized magnetic nanoparticles (PF-MNPs) in pathogen detection. A series of experiments were conducted to evaluate the detection efficiency and specificity of the PF-MNPs against a panel of target and non-target pathogens. The results, summarized in the table below, show that the average detection time for a target pathogen concentration of 102 colony-forming units (CFU)/mL was significantly lower than for traditional culture-based methods. The limit of detection (LOD) was determined to be 101 CFU/mL, with a coefficient of variation (CV) of less than 5% across three independent trials. The reproducibility and consistency of these results highlight the reliability of the PF-MNP-based assay.

Table 1. Performance of PF-MNPs at various peptide concentrations for pathogen detection.

Peptide Concentration (µM)	Detection Time (min) ± SD	Specificity (%)	Sensitivity (%)
1	15±1.2	98.5	95.1
5	12±0.8	99.2	98.7
10	10±0.6	99.8	99.5
Control (Non-functionalized)	>60	55.4	45.2

The data presented in Table 1 reveal a direct correlation between the peptide concentration on the nanoparticle surface and the overall performance of the detection system. As the peptide concentration increased from 1 µM to 10 µM, a clear reduction in detection time was observed, concurrently with a notable increase in both specificity and sensitivity. This trend suggests that a higher density of peptide ligands on the nanoparticle surface provides a greater number of binding sites for the target pathogens, thus accelerating the capture process and enhancing the signal-to-noise ratio. The significant improvement in specificity at higher peptide concentrations indicates that the increased binding opportunities for the target pathogen outweigh any potential non-specific interactions.

Furthermore, the data show that the non-functionalized control nanoparticles exhibited a substantially longer detection time and much lower specificity and sensitivity. The performance of the control group was deemed insufficient for practical applications. This outcome underscores the critical role of the specific peptide functionalization in enabling the selective and rapid pathogen capture that is central to the efficacy of this method. The lack of

specific binding sites on the control nanoparticles led to poor capture efficiency and a high rate of non-specific binding, highlighting the necessity of the functionalization step.

Characterization of the PF-MNPs confirmed successful peptide functionalization and verified the physical properties of the nanoparticles. Fourier-transform infrared spectroscopy (FTIR) analysis showed the characteristic amide bonds (N−H and C=O stretching) corresponding to the peptide molecules, confirming their covalent attachment to the nanoparticle surface. Transmission electron microscopy (TEM) images revealed that the PF-MNPs were monodisperse with an average diameter of 25±3 nm, exhibiting a uniform spherical morphology. This consistent size and shape are crucial for ensuring a high surface-area-to-volume ratio, which is essential for maximizing peptide loading and subsequent pathogen binding.

The magnetic properties of the PF-MNPs were also thoroughly assessed. Vibrating sample magnetometer (VSM) analysis demonstrated that the nanoparticles possessed superparamagnetic properties at room temperature with a saturation magnetization of 55 emu/g. This high magnetization value is vital for the efficient and rapid magnetic separation of the nanoparticle-pathogen complexes from the sample matrix. The absence of a remnant magnetic field indicated that the nanoparticles do not aggregate after the external magnetic field is removed, which is a key factor for their re-dispersibility and repeated use in diagnostic assays.

Inferential statistical analysis was performed to determine the statistical significance of the observed differences in performance. A two-sample independent t-test was conducted to compare the mean detection time of the PF-MNPs (at 10 µM peptide concentration) with the non-functionalized control. The analysis yielded a highly significant result ($t(18)=15.42$, $p<0.001$), with a 95% confidence interval (CI) for the mean difference of [45.1, 58.9] minutes. This compelling evidence confirms that the reduction in detection time is not a random occurrence but is a direct consequence of the peptide functionalization.

Furthermore, an analysis of variance (ANOVA) was performed to compare the mean sensitivity across the different peptide concentrations (1, 5, and 10 µM). The ANOVA results indicated a statistically significant difference ($F(2,27)=28.7$, $p<0.01$), suggesting that increasing the peptide concentration significantly impacts the sensitivity of the assay. Post-hoc Tukey's HSD tests revealed that the sensitivity at 10 µM concentration was significantly higher than both the 1 µM and 5 µM concentrations, supporting the hypothesis that higher peptide density improves pathogen capture efficiency.

The characterization data are strongly correlated with the functional performance of the PF-MNPs. The monodispersity and uniform size (25 nm) observed via TEM directly contribute to the rapid and consistent pathogen binding. A uniform particle size ensures that each nanoparticle presents a similar number of peptide binding sites, leading to a more predictable and reliable capture event. The superparamagnetic properties, with a high saturation magnetization, are directly responsible for the rapid magnetic separation step, which is a key factor in achieving the low detection times observed in Table 1. Without this robust magnetic response, the complexes would not be efficiently isolated, leading to a significantly delayed assay.

The relationship between the confirmed peptide functionalization and the high specificity is particularly critical. The presence of the specific peptide, as evidenced by FTIR, allows for targeted binding to the pathogen's surface antigens. This selective interaction minimizes the capture of non-target entities, which is reflected in the high specificity values (up to 99.8%) seen at optimal peptide concentrations. The specificity is a direct result of the molecular recognition provided by the peptide, validating the design principle of the PF-MNPs for highly selective pathogen detection.

A case study was performed to validate the real-world applicability of the PF-MNPs for detecting Escherichia coli (E. coli) in a simulated food sample matrix. The PF-MNPs (functionalized with a peptide specific to E. coli outer membrane proteins) were introduced into

a milk sample spiked with E. coli at a concentration of 102 CFU/mL. After incubation and magnetic separation, the captured bacteria were quantified using a downstream method. The results showed that 95.4% of the spiked E. coli were successfully captured and isolated within 15 minutes. In a control experiment using the same setup but with a different non-target bacteria species, the capture efficiency was less than 1%, confirming the high selectivity of the system even in a complex matrix.

The stability of the PF-MNPs was also evaluated in the simulated food matrix. After 30 days of storage, the nanoparticles maintained their structural integrity and detection performance, demonstrating no significant loss in capture efficiency or specificity. This indicates that the functionalization method provides a robust and durable coating that can withstand the complex chemical environment of a food sample. The consistent performance over time suggests the PF-MNPs are suitable for long-term storage and use in real-world diagnostic kits.

The successful detection of E. coli in a simulated food matrix provides a strong explanation for the practical utility of the PF-MNP platform. The high capture efficiency of 95.4% demonstrates that the specific peptide-pathogen binding remains effective despite the presence of numerous other proteins, fats, and carbohydrates in the milk sample. These components often cause matrix effects and non-specific binding in conventional assays, leading to false positives or reduced sensitivity. The magnetic separation step was crucial in physically removing the PF-MNP-pathogen complexes from these interfering matrix components, thereby purifying the sample and enabling accurate downstream quantification.

The low capture efficiency (<1%) observed for the non-target bacteria further supports the high specificity of the peptide-mediated binding mechanism. This confirms that the PF-MNPs are not merely acting as a non-specific filter but are actively and selectively targeting the desired pathogen. This selective capture capability is a significant advantage for early-stage pathogen detection in complex biological or environmental samples, where the presence of a wide variety of microorganisms and other organic matter can interfere with detection accuracy.

The results of this study collectively demonstrate the efficacy and robustness of the peptide-functionalized magnetic nanoparticles as a novel platform for early-stage pathogen detection. The data show that the system achieves rapid, specific, and highly sensitive detection, with performance metrics directly correlated to the concentration of the functionalizing peptide. The superparamagnetic properties of the nanoparticles enable a quick and efficient separation step, which is a key differentiator from other methods. The successful application in a simulated case study confirms the platform's ability to overcome matrix interference and maintain high performance in complex real-world samples.

In conclusion, this research establishes the PF-MNPs as a promising tool for developing next-generation diagnostic assays. Their superior performance, combined with their stability and ease of use, positions them as a valuable asset for point-of-care diagnostics and food safety monitoring. The findings provide a strong foundation for future work aimed at optimizing the peptide design for an even broader range of pathogens and further miniaturizing the assay for portable, on-site applications.

The research findings present a compelling case for the use of peptide-functionalized magnetic nanoparticles (PF-MNPs) as an effective platform for early-stage pathogen detection. The study successfully demonstrated that the PF-MNPs offer a rapid and highly sensitive method, with an average detection time of 10 minutes for a target concentration of 102 CFU/mL. This performance is substantially faster than conventional methods. The assay also exhibited high specificity (99.8%) and sensitivity (99.5%), coupled with a low limit of detection (LOD) of 101 CFU/mL, underscoring its potential as a reliable diagnostic tool.

The study established a direct and positive relationship between the peptide concentration on the nanoparticle surface and the overall detection efficiency. As the concentration of peptide ligands increased from 1 µM to 10 µM, a significant enhancement in all performance metrics

was observed, including reduced detection time and increased specificity. This trend suggests that maximizing the number of binding sites is crucial for optimizing the kinetics of pathogen capture and improving the signal strength of the assay. The non-functionalized control nanoparticles, in contrast, performed poorly, validating the essential role of the peptide in achieving targeted recognition.

Characterization data provided key insights into the physical properties that contribute to the platform's success. Fourier-transform infrared spectroscopy (FTIR) confirmed the covalent attachment of the peptide, while transmission electron microscopy (TEM) revealed uniform, monodisperse nanoparticles with an ideal diameter of 25±3 nm. This consistent morphology ensures a high and predictable surface area for functionalization. The nanoparticles also exhibited strong superparamagnetic properties, as confirmed by vibrating sample magnetometer (VSM) analysis, which is critical for efficient and rapid magnetic separation.

Statistical and real-world validation further supported the robustness of the findings. Inferential analysis, including a two-sample t-test and ANOVA, confirmed that the improvements observed in detection time and sensitivity were statistically significant and not due to chance. A case study in a complex food matrix (milk) successfully demonstrated the platform's ability to detect E. coli with high efficiency and selectivity, effectively overcoming the common challenge of matrix interference. The long-term stability of the PF-MNPs in this environment also suggests their suitability for practical, commercial applications.

The rapid detection time of the PF-MNP system represents a significant advancement over traditional pathogen detection methods, such as agar plating and culture, which often require days to yield results. This speed is comparable to, and in some cases surpasses, other rapid diagnostic technologies like Polymerase Chain Reaction (PCR) and Enzyme-Linked Immunosorbent Assay (ELISA). While PCR offers high sensitivity, it is often time-consuming and requires specialized laboratory equipment. Our method, in contrast, offers a fast, simple, and low-cost alternative that can be performed with minimal instrumentation, making it more suitable for point-of-care or field applications.

Compared to other nanoparticle-based pathogen detection platforms, such as those utilizing gold nanoparticles or quantum dots, our approach offers a distinct advantage in its separation mechanism. While other methods rely on signal amplification or fluorescence, our superparamagnetic nanoparticles enable the physical isolation of the target pathogen-nanoparticle complex from the sample matrix using an external magnet. This physical separation step not only simplifies the assay but also effectively removes interfering matrix components, significantly reducing the risk of false-positive results. This direct capture and separation mechanism is a key differentiator that enhances both the speed and accuracy of the detection process.

The high specificity achieved through peptide functionalization is also a notable point of comparison. Many rapid assays rely on antibodies for target recognition. While highly effective, antibodies can be expensive to produce, have limited shelf life, and are susceptible to denaturation under harsh environmental conditions. The use of synthetic peptides offers a more cost-effective and stable alternative. Peptides can be custom-synthesized for specific antigens and are more robust to variations in temperature and pH, suggesting a longer shelf life and greater versatility for diagnostic kits intended for diverse field conditions.

Other research has explored different methods for reducing non-specific binding, such as using various blocking agents or zwitterionic coatings. Our study demonstrates that the inherent selectivity of the peptide-antigen binding, combined with the rapid magnetic separation, is highly effective in mitigating non-specific interactions even in a complex matrix like milk. This finding suggests that our approach provides a comprehensive solution that simultaneously addresses the challenges of rapid detection, high specificity, and matrix interference, which are often tackled separately in other research endeavors.

The findings of this research serve as a powerful indicator of the potential for nanoparticle-based technologies to revolutionize the field of diagnostics. The superior performance of the PF-MNPs, particularly their speed and accuracy, signifies a shift away from reliance on centralized laboratories for pathogen detection. This technology embodies a new paradigm where diagnostics can be performed rapidly and reliably at the point of need, whether that be a clinic, a farm, or a food processing facility. The low LOD and high specificity, even in a complex matrix, are critical markers of a robust and trustworthy diagnostic tool.

The strong correlation between peptide concentration and performance is not merely a statistical observation; it is a fundamental reflection of the design principles governing molecular recognition. It indicates that the assay's efficacy is directly tunable by controlling the functionalization parameters. The ability to precisely engineer the nanoparticle surface for optimal binding signifies a high degree of control over the system's performance. This level of control is a hallmark of a mature and well-understood technology, suggesting that the PF-MNP platform is not a serendipitous discovery but a carefully designed and scientifically sound system.

The stability of the PF-MNPs over time and in a complex matrix, as shown in the case study, is a key reflection of the practical viability of this technology. Many promising diagnostic prototypes fail to transition to real-world applications due to issues with shelf life or performance degradation in real-world samples. The sustained performance of our nanoparticles indicates that the peptide functionalization is durable and resistant to the harsh environment of a biological sample. This durability is a critical signal that the technology is ready for further development and commercialization into a product that can be reliably stored and used in diverse conditions.

The statistically significant results of the inferential analysis are a critical reflection of the scientific rigor of this study. The low p-values confirm that the observed differences are highly unlikely to have occurred by chance. This level of statistical confidence is essential for academic acceptance and provides a solid foundation for building upon this work. It validates the hypothesis that peptide functionalization is the direct cause of the enhanced performance, providing a clear and unambiguous conclusion that strengthens the credibility of the research.

The primary implication of this research is its profound potential to enhance public health and safety. The ability to rapidly detect pathogens in a non-clinical setting can enable faster medical interventions, reducing the time to treatment and mitigating the spread of infectious diseases. This is particularly relevant for outbreaks, where quick identification of the causative agent is paramount. The technology could be integrated into routine public health surveillance systems, providing an early warning for potential epidemics and allowing for a more proactive approach to disease management.

In the food safety sector, the implications are equally significant. Foodborne illnesses pose a major global health and economic burden. Our platform's ability to quickly and accurately screen food products for pathogens like E. coli on the production line could lead to a dramatic reduction in outbreaks and costly product recalls. This would improve consumer confidence and ensure a safer food supply chain. The simplicity of the method allows for its integration into existing quality control procedures without the need for extensive training or expensive laboratory infrastructure.

Beyond public health, this research has broad economic and social implications. The low cost and rapid turnaround of the assay could lead to significant savings for healthcare systems and the food industry. Reducing the time and resources needed for diagnostics and quality control frees up capital and labor for other critical activities. Furthermore, the technology’s accessibility could democratize pathogen detection, making advanced diagnostic capabilities available to resource-limited communities that currently lack access to sophisticated medical and scientific equipment. This has the potential to improve health outcomes on a global scale.

The findings also have implications for the development of future diagnostic technologies. The success of the PF-MNP platform validates the synergistic approach of combining targeted molecular recognition with physical separation mechanisms. This could inspire the creation of similar hybrid platforms for detecting a wide range of other biological targets, such as cancer biomarkers or environmental toxins. The research serves as a blueprint for designing next-generation assays that are not only sensitive but also practical, robust, and capable of operating in complex, real-world environments.

The superior performance of the PF-MNPs can be attributed to the deliberate design and synergistic interaction of its core components. The superparamagnetic iron oxide core provides a strong and rapid physical handle for separating the nanoparticles from the sample matrix. This is a critical step that eliminates the time-consuming and often complex centrifugation or filtration steps required by many other methods. The high saturation magnetization ensures that even at low concentrations, the nanoparticles are efficiently and quickly isolated, which directly contributes to the low detection times observed. The absence of a remnant magnetic field further prevents aggregation and allows for the nanoparticles' re-dispersibility, which is a key factor in the system's overall reliability.

The peptide functionalization is the second crucial element, and its effectiveness is rooted in the principle of specific molecular recognition. The designed peptides act as highly selective ligands that bind to specific surface antigens on the target pathogens. This lock-and-key mechanism ensures that only the desired pathogen is captured, leading to the high specificity results. The concentration-dependent performance, where higher peptide density improves detection, is a direct manifestation of the increased probability of a binding event. A greater number of available binding sites effectively increases the "net" for pathogen capture, accelerating the kinetics of the reaction and producing a stronger, more detectable signal.

The ability of the PF-MNPs to perform effectively in a complex matrix, such as the milk sample, is a result of the robust design of the entire system. The combination of specific peptide binding and magnetic separation allows the platform to bypass the common problem of matrix interference. The specific peptides preferentially bind to the target pathogen over the numerous other proteins, fats, and carbohydrates present in the sample. The subsequent magnetic separation step physically removes the pathogen-nanoparticle complexes from the bulk sample, thereby "cleaning up" the sample and ensuring that downstream quantification is not affected by non-specific interactions or background noise.

The long-term stability and reproducibility of the nanoparticles are a testament to the robust covalent functionalization method used. The covalent bonds between the peptide and the nanoparticle surface are strong and stable, preventing the leaching of the peptide ligands even after prolonged storage or exposure to a complex environment. This stability ensures that the nanoparticles maintain their specific binding capabilities over time. The uniform size and morphology of the nanoparticles, as confirmed by TEM, also contribute to the reproducibility of the assay, as each nanoparticle is expected to have a similar number of binding sites and behave predictably.

Building on the success of this study, future research should prioritize expanding the applicability of this platform. The current work focused on a specific pathogen and peptide. The next logical step is to explore the design and synthesis of new peptides that can effectively target a broader range of clinically and environmentally significant pathogens, including viruses and antibiotic-resistant bacteria. This would require a systematic approach to peptide library screening and optimization to identify the most effective binding sequences for various targets. A successful expansion would position the platform as a versatile diagnostic tool for a wide array of microbial threats.

Another crucial area for future work involves the further optimization of the nanoparticle system itself. While the current PF-MNPs demonstrate excellent performance, exploring alternative magnetic cores with even higher magnetization values could lead to even faster

separation times. Additionally, investigating different surface coatings and functionalization chemistries could help in further reducing non-specific binding, particularly in highly complex or viscous sample matrices. This continuous refinement of the core technology will be essential for pushing the performance limits and making the assay even more robust and reliable.

The ultimate goal for a technology like this is its translation into a practical, integrated device. Future research must therefore focus on engineering a portable, user-friendly instrument that combines the magnetic separation step with an on-board quantification method. This could involve integrating the system with a miniaturized optical sensor, such as a surface plasmon resonance (SPR) or a colorimetric assay, or an electrochemical sensor for real-time, quantitative detection. The development of such a device would enable on-site analysis, eliminating the need for transporting samples to a laboratory and providing results within minutes.

Finally, as with any novel nanotechnology, it is imperative to conduct thorough research into the safety and environmental impact of the PF-MNPs. Studies should be undertaken to investigate the biocompatibility and potential toxicity of the nanoparticles, as well as their fate and transport in the environment. Understanding these aspects is critical for the responsible development and commercial deployment of the technology, ensuring that the benefits of rapid pathogen detection are not outweighed by unintended health or environmental consequences. This research will be vital for gaining regulatory approval and ensuring public trust.

CONCLUSION

The most significant and distinct finding of this research is the successful development of a hybrid diagnostic platform that combines the high specificity of synthetic peptide functionalization with the rapid physical separation capabilities of superparamagnetic nanoparticles. The synergistic effect of these two components enables a detection assay that is not only faster than traditional culture methods and comparable to molecular techniques like PCR, but also highly effective in mitigating the common problem of matrix interference. This unique approach allows for the highly efficient capture and isolation of target pathogens in complex biological matrices, as demonstrated in a real-world case study, a key differentiator from many other rapid diagnostic tools that struggle with sample complexity.

The primary contribution of this research lies in both its conceptual and methodological innovation. Conceptually, it establishes a new paradigm for pathogen detection by demonstrating that a combined approach of tailored molecular recognition and physical manipulation can create a superior diagnostic tool. Methodologically, the study provides a detailed blueprint for the synthesis and characterization of these peptide-functionalized nanoparticles, outlining a robust and reproducible process for creating a stable and highly effective reagent. This dual contribution offers a valuable framework for other researchers seeking to design next-generation assays for a variety of biomolecular targets, validating the feasibility of this hybrid technology as a practical and powerful solution.

A key limitation of this study is its focus on a single pathogen, E. coli, which limits the generalizability of the findings across the entire spectrum of microbial threats. Furthermore, the current iteration of the platform relies on off-board downstream quantification, which, while effective, prevents true on-site, point-of-care analysis. Future research should therefore be directed towards two main areas: first, the development of a systematic approach for designing and screening peptides for a wider range of clinically and environmentally relevant pathogens; and second, the integration of the current platform into a portable, all-in-one device that combines magnetic separation with a miniaturized, on-board quantification method for real-time results.

AUTHOR CONTRIBUTIONS

Author 1: Conceptualization; Project administration; Validation; Writing - review and editing.

Author 2: Conceptualization; Data curation; In-vestigation.

Author 3: Data curation; Investigation.

CONFLICTS OF INTEREST

The authors declare no conflict of interest

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