

Transforming Conventional Polystyrene Microplates into Versatile Platforms for Nucleic Acid Immobilization via One-Step Acid Treatment

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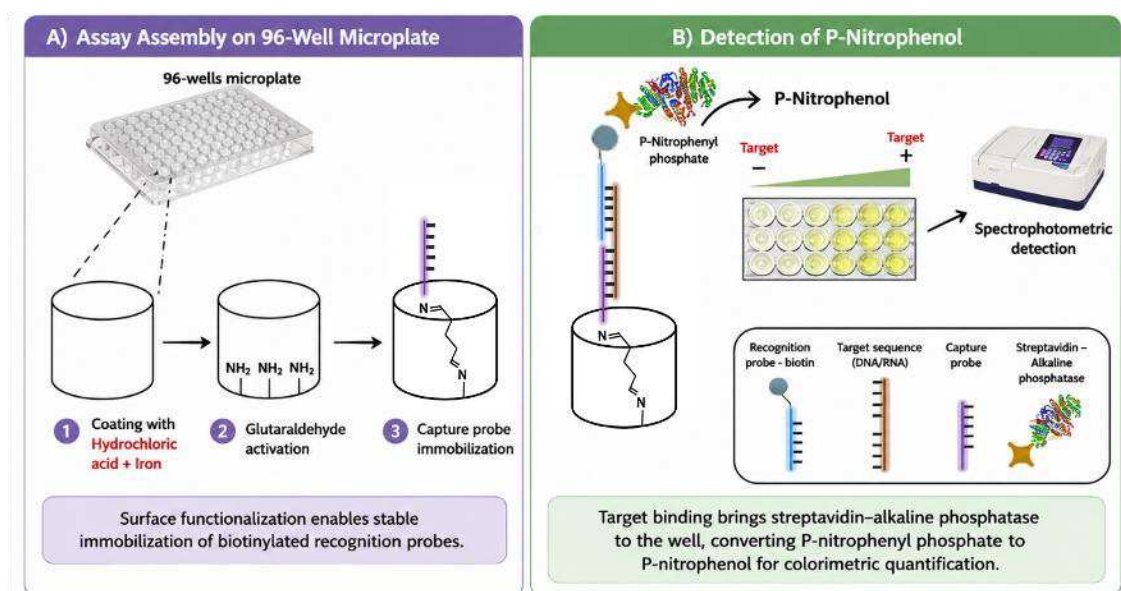
Transforming Conventional Polystyrene Microplates into Versatile Platforms for Nucleic Acid Immobilization via One-Step Acid Treatment

Abstract

In this study, the impact of hydrochloric acid (HCl) activation on the surface chemistry of polystyrene (PS) 96-well microplates was systematically evaluated. The chemical modifications induced by the acidic treatment were analyzed using Fourier transform infrared spectroscopy (FT-IR), X-ray photoelectron spectroscopy (XPS), and contact angle measurements. FTIR and XPS analysis confirmed the successful introduction of amine functional groups onto the PS surface after activation process. On the other hand, a reduction in the water contact angle after acidic treatment indicates a change in surface wettability and reflecting the introduction of new functional groups and an increase in surface hydrophilicity. In addition, SEM characterization revealed noticeable alterations in surface morphology compared to the untreated PS. Based on this surface modification approach, a novel strategy for nucleic acid immobilization was developed using a one-step acidic treatment of 96-well PS microplates as a proof-of-concept. The resulting platform was designed for high-throughput biosensing applications, enabling sensitive detection of oncogenic microRNAs and pathogen-derived DNA sequences. Surface activation of individual wells through HCl treatment facilitated the generation of reactive amine groups on the PS surface. Subsequently, glutaraldehyde was used as a bifunctional crosslinking agent to covalently link the amine-functionalized surface to amine-terminated oligonucleotide probes via imine bond formation. Finally, target nucleic acid recognition was achieved through a sandwich hybridization assay, in which the analyte (either miRNA-222 or an *Escherichia coli*-specific DNA sequence) was captured between an immobilized probe and a complementary detection probe.

Keywords: Polystyrene microplates, immobilization platform, biomarker detection, biosensor, XPS, FTIR.

Graphical Abstract



1. Introduction

Biosensors are increasingly recognized as essential tools for developing rapid, accurate, and user-friendly analytical platforms. Their growing utility in clinical diagnostics, environmental analysis, and biomedical applications is largely driven by their ability to provide real-time, highly sensitive biomarker detection [1, 2]. MicroRNAs (miRNAs) are short endogenous non-coding RNAs (~22 nucleotides) that regulate gene expression at the post-transcriptional level [3–5]. Their dysregulation is closely associated with cancer, with certain miRNAs, such as miRNA-222, showing elevated expression in tumors and serving as potential early diagnostic biomarkers [6, 7]. Although absent in prokaryotes, bacterial pathogens, such as *Escherichia coli*, can be identified by specific genomic sequences, which is essential for ensuring food and water safety and protecting public health [8, 9]. In this context, the performance of DNA-based biosensors largely depends on the efficiency of nucleic acid probe immobilization, as it directly affects sensitivity, specificity, and reproducibility, making the optimization of immobilization strategies and material selection crucial.

Various approaches have been employed for DNA probe immobilization, including physical adsorption [10–12], biotin–avidin affinity binding [13], and covalent coupling [14, 15], [16]. Among these, covalent immobilization is particularly advantageous due to its enhanced stability and strong binding affinity, enabling robust and specific detection of target nucleic acids such as microRNAs (miRNAs) [17, 18].

Alkaline activation of polystyrene (PS) microplates has been extensively employed to enhance surface reactivity prior to silane-based functionalization. For example, treatment with KOH facilitates the generation of surface hydroxyl functionalities (OH) [19], enabling subsequent interaction with (3-aminopropyl)triethoxysilane (APTES) through the formation of silanol (Si–OH) groups and the presentation of amino groups at the interface [20]. These surface functionalities have been exploited for covalent DNA immobilization using glutaraldehyde and for various biosensing applications [21]. To the best of our knowledge, this study is the first report of a one-step acid-activation approach for introducing amine functionalities onto the surface of 96-well PS microplates. The modifications in surface chemistry and textural properties induced by HCl treatment were investigated using X-ray photoelectron spectroscopy (XPS), Fourier transform infrared spectroscopy (FT-IR), scanning electron microscopy (SEM), and water contact angle measurements. This straightforward surface modification strategy yields amine-enriched microplate wells that act as reactive platforms for the covalent immobilization of DNA probes through glutaraldehyde-mediated imine bond formation. Nucleic acid recognition was accomplished using a sandwich hybridization strategy, in which the target sequence and a recognition probe simultaneously hybridized to the immobilized capture strand. Signal readout was achieved via an avidin–alkaline phosphatase conjugate that selectively binds to the biotin of the recognition probe and catalyzes the conversion of para-nitrophenyl phosphate into para-nitrophenol. The formation of this chromogenic product enabled sensitive spectrophotometric monitoring of hybridization events at 405 nm. Validation experiments performed on the functionalized PS high-throughput platform using miRNA-222 and *Escherichia coli*-specific DNA sequences as model targets confirm the feasibility of the proposed strategy and underscore its adaptability for the detection of clinically and environmentally relevant biomarkers, suggesting its broader applicability to diverse nucleic acid analytes.

2. Materials and Methods

2.1 Chemicals and Reagents

Para-nitrophenyl phosphate (PNPP), diethanolamine (DEA), Streptavidin-alkaline phosphatase-conjugated (SA-AP), Tween 20, bovine serum albumin (BSA), Glutaraldehyde, potassium chloride (KCl), and magnesium chloride (MgCl₂) were procured from Sigma-Aldrich, and Hydrochloric acid (HCl) was procured from VWR. All chemicals used for the preparation of the phosphate buffer saline (PBS) (1.8 M KH₂PO₄, 10 mM Na₂HPO₄, 2.7 mM KCl, and 137 mM NaCl, pH 7.4) were purchased from Solvachim (Casablanca, Morocco). All reagents used were of analytical grade. Oligonucleotides, purified by HPLC, were obtained from Eurogentec (Belgium) in a lyophilized state. Distilled water was used to prepare the stock solutions, which were stored at -20 °C. The oligonucleotide sequences are detailed below:

Target miRNA-222

5'-AGCUACAUCUGGCUACUGGGUCUC-3'

Capture probe (complementary to the first part of the microRNA-222 sequence)

5'-NH₂-(CH₂)₆-AAAGAGACCCAGTAG-3'

Recognition probe (complementary to the second part of the microRNA-222 sequence)

5'-CCAGATGTAGCTTTT-biotin-3'

Non-complementary miRNA-21

5'-UAGCUUAUCAGACUGAUGUUGA-3'

Biotin-labeled probe

5'-UCCCUGAGACCCUUUAAC-biotin-3'

Target (E. Coli specific sequence)

5'-TCAGCGGGGAGGAAGGGAGTAAAGTTAATA-3'

Capture probe (complementary to the first part of the specific E. Coli sequence)

5'-NH₂- C₆-AAATATTAACCTTACTCC-3'

Recognition probe (complementary to the second part of the specific E. Coli sequence).

5'-CTTCCTCCCCGCTGATTT-Biotin-3'

2.2 Apparatus

An automated microplate reader measures the absorbance of the enzymatic product at 405 nm (Biotek, USA). The resulting data were processed using Gen5 software, followed by further analysis with OriginPro 8.

Characterization was performed using Fourier transform infrared (FTIR) spectroscopy with an IR Affinity-1S spectrophotometer (PerkinElmer, Llantrisant, UK) in attenuated total reflectance mode. X-ray photoelectron spectroscopy (XPS) is performed on a Thermo Fisher Scientific ESCALAB 250Xi platform equipped with a monochromatic Al K α beam (1486.8 eV) and a hemispherical analyzer. The overall resolution is estimated to be better than 0.5 eV. A 90-degree take-off angle is used for signal capture. The survey spectra are taken with an energy step of 1 eV with a pass energy of 100 eV, and the high-resolution spectra are taken with an energy step of 0.1 eV and a pass energy of 50 eV. The analysis chamber is kept at an ultra-high vacuum (10⁻¹⁰ mbar). All binding energies are calibrated to the C-C single bond signal (C1s) at 284.8 eV. Field emission scanning electron microscope (FE-SEM) from QUANTA FEG 650-Thermo Fisher Scientific (USA) was used to analyze the surface morphology of the PS plate before and after treatment.

2.3 Microplates functionalization

The ELISA microplate modification was initially based on aromatic nitration followed by reduction with HCl/Fe to generate surface amine groups. DNA immobilization using glutaraldehyde showed improved performance. However, optimization results demonstrated that the nitration step was not necessary, as the same response was obtained using only the HCl/Fe treatment. At present, the exact mechanism remains unclear. However, the results obtained from FTIR and XPS clearly indicate the presence of primary amine groups on the surface, as discussed in the rest of this work.

In this study, each well of the microtitration plate was modified in a single step. The wells were exposed to a solution containing a piece of coiled iron wire (2 cm in Length and 1 mm in diameter) and hydrochloric acid (HCl) at an appropriate concentration, in a total volume of 100 μ L, under agitation using a reciprocating shaker (1h 30). Subsequently, the plates were rinsed three times with distilled water. During this treatment step, the surface of the 96-well PS microplates was chemically activated to promote the presence of amine functionalities (NH₂), Confirmed using FTIR and XPS analysis.

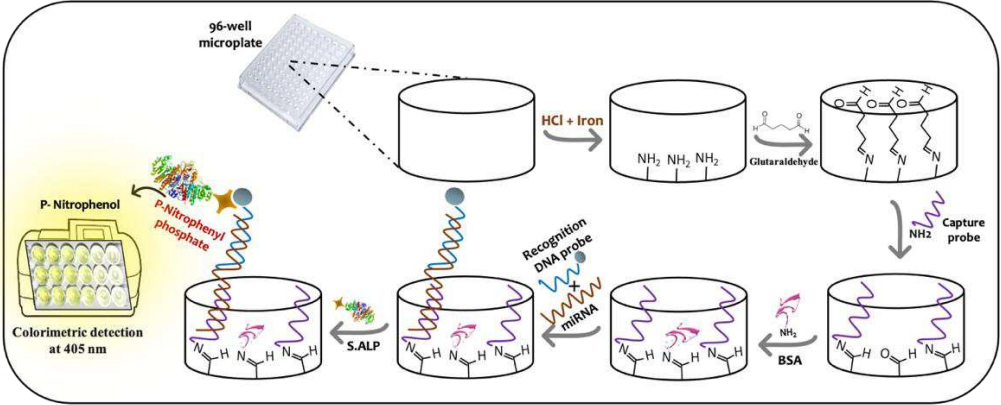
2.4 Capture Probe Immobilization on amine Functionalized Microplates

The immobilization of the capture probe was achieved using glutaraldehyde as a bifunctional crosslinking agent. The amine groups present on the PS microplate surface reacted with one aldehyde moiety of glutaraldehyde to form imine bonds, while the remaining aldehyde group subsequently interacted with the amine-modified capture probe, resulting in covalent imine linkage formation

(DNA-NH₂). In this procedure, a 100 μ L solution containing 5% glutaraldehyde and an appropriate concentration of the capture probe, prepared in PBS buffer (0.1 mM, pH 7.4), was incubated in each well for 1 hour. Following three washes, non-specific binding sites were blocked for 15 minutes with BSA (10 mg/mL). Subsequently, a washing step is required to remove excess (Scheme 1).

2.5 Detection of the complementary target

The detection of various concentrations of complementary targets (miRNA-222) was assessed by hybridization with a biotinylated recognition probe (1 μ M) for 1 hour in each modified well containing capture probe DNA. Each well underwent three washes. Subsequently, each well was incubated with 100 μ L of avidin-labeled alkaline phosphatase (ALP) (1 μ g/mL in DEA buffer, pH 9.8) for 30 minutes. After three washes, 100 μ L of Para-nitrophenyl phosphate (PNPP) (100 mM in DEA buffer, pH 9.8) was incubated at room temperature for 20 minutes. The absorbance of the resulting yellow color was then measured at 405 nm using a microplate reader (Scheme 1).



Scheme 1: Schematic representation of PS microplate functionalization and application for miRNA-222 or an *Escherichia coli*-specific DNA fragment detection

3. Results and Discussion

3.1 Surface Characterizations

The surface morphology of pristine PS, PS treated with HCl, and PS treated with HCl in the presence of Iron (HCl-Fe) was investigated using scanning electron microscopy (SEM), and the obtained results are presented in Fig. 1. The SEM micrograph at 25,000 $\times$ magnification (Fig. 1a) revealed that the untreated PS surface exhibited a smooth, relatively homogeneous structure compared to the other treated samples.

In contrast, chemical treatment with HCl alone and with the HCl-Fe system led to significant alterations in the PS surface morphology. The SEM images of PS-HCl (Fig. 1b) and PS-HCl-Fe (Fig. 1c) clearly show a roughened surface, indicating surface modification after treatment.

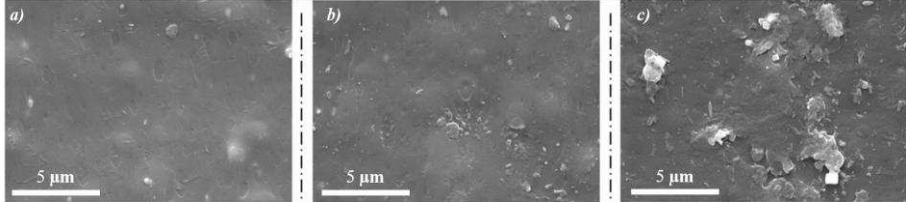


Fig. 1: SEM of a) PS, b) PS/HCl, c) PS/ Iron+ HCl.

Contact angle measurements were performed to evaluate the effect of surface treatments on the wettability of PS. The pristine PS surface exhibited a contact angle of 91.1 $^\circ$ (Fig. 2a), confirming its hydrophobic character. After chemical treatment with HCl in the presence of Iron, a significant decrease in the contact angle to 65.4 $^\circ$ was observed (Fig. 2b). This reduction indicates a marked increase in surface hydrophilicity, attributed to surface chemical modification and the introduction of polar functionalities. The observed change in wettability demonstrates that the applied treatment

effectively alters the surface properties of PS, making it more suitable for subsequent surface functionalization and biomolecule immobilization.

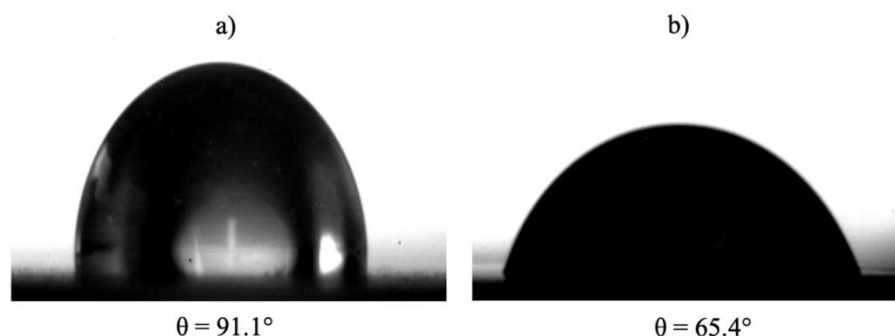


Fig. 2 Contact angle images of a) PS and b) PS/ HCl+iron with water

To determine the nature of the surface functionalities, whether amine ($-\text{NH}_2$) or carboxyl ($-\text{COOH}$) groups, two coupling strategies, glutaraldehyde activation and carbodiimide chemistry, were investigated using the same DNA concentration. Carbodiimide treatment resulted in negligible immobilization efficiency, almost equivalent to the blank value, indicating the absence of carboxyl groups on the surface. In contrast, glutaraldehyde provided significantly improved DNA immobilization, attributed to its ability to react with amine groups through imine bond formation, thereby promoting more effective surface functionalization.

The modification of PS microplates with amine groups after acid treatment was validated by Fourier-transform infrared spectroscopy (FT-IR). The FT-IR spectra of PS and PS treated with HCl in the presence of Iron are shown in Fig. 3. Following the acid treatment, the emergence of a broad absorption band at approximately 3374 cm^{-1} together with a noticeable peak around 1643 cm^{-1} can be attributed to the N-H stretching and bending vibrations, respectively, thereby indicating the successful introduction of amine functional groups on the surface [22, 23].

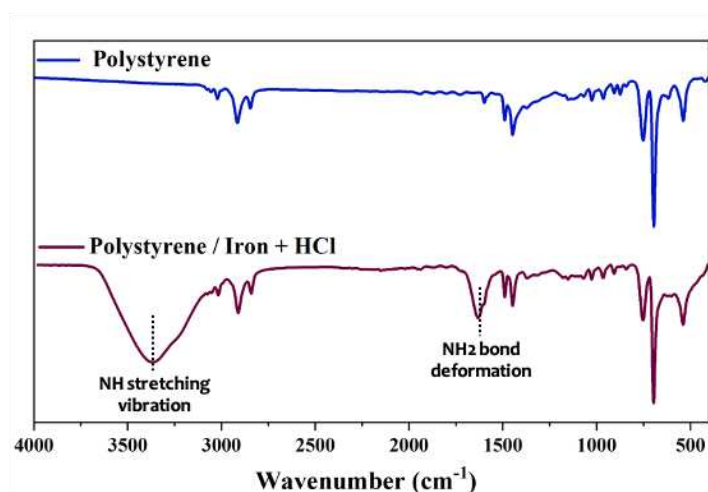


Fig. 3 FT-IR spectra of PS and PS/HCl+iron

The surface atomic compositions of untreated PS as well as PS treated with hydrochloric acid, either alone or in the presence of Iron, were determined by X-ray Photoelectron Spectroscopy (XPS) (Fig. 4.1). The analysis reveals that the pristine PS surface is primarily composed of carbon and oxygen, with trace amounts of heteroatoms, such as sulfur. After acid treatment, additional elements are detected on the modified surfaces. In particular, nitrogen is observed in both treated samples, whereas an iron-related signal is detected exclusively when the treatment is carried out in the presence of metallic Iron.

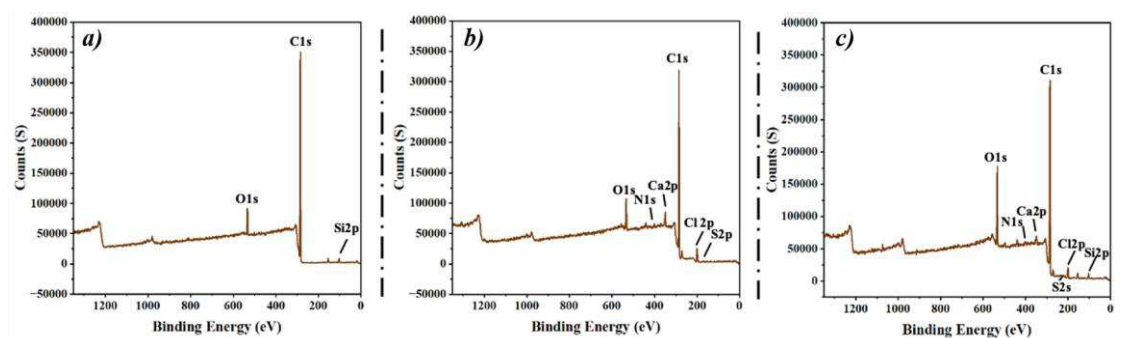


Fig. 4.1 XPS survey of a) PS, b) PS/HCl, c) PS/ HCl+Fe

High-resolution C 1s XPS spectra of untreated and treated PS surfaces are presented in Fig. 4.2. A dominant peak in the spectrum of the untreated surface at approximately 285 eV is attributed to the polymer's carbon backbone. A secondary contribution ~ 286 eV, overlapping with the main carbon signal, may be associated with C–N type environments [24]. In addition, a peak near 291.4 eV is assigned to π – π^* shake-up transitions characteristic of aromatic rings. Following acid treatment, the contribution attributed to C–N environments is no longer observed.

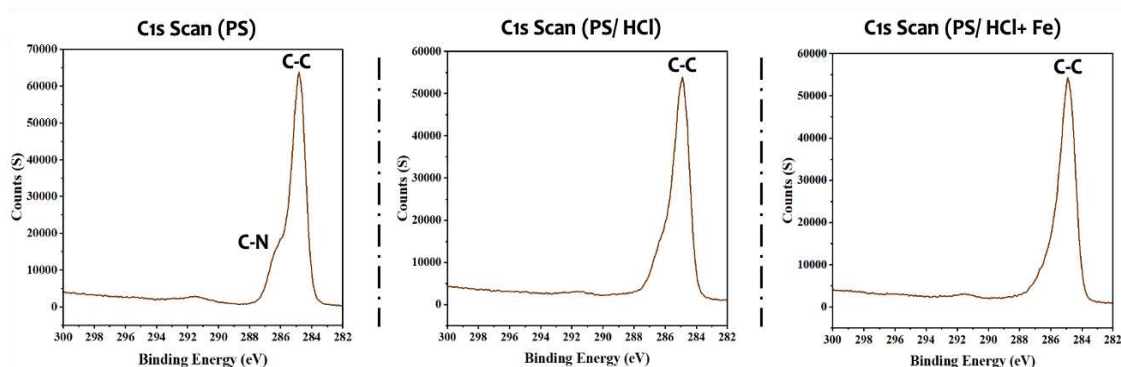


Fig. 4.2 C1s scan of a) PS, b) PS/HCl, c) PS/ Iron+ HCl.

The high-resolution N1s spectra of the investigated surfaces are shown in Fig. 4.3. A nitrogen-related signal centered around 401 eV appears only after treatment with hydrochloric acid, either in the absence or presence of Iron. This binding energy is consistent with the characteristic signal of amine-type functional groups ($-\text{NH}_2$), indicating the introduction of amine functionalities onto the modified PS surface [25].

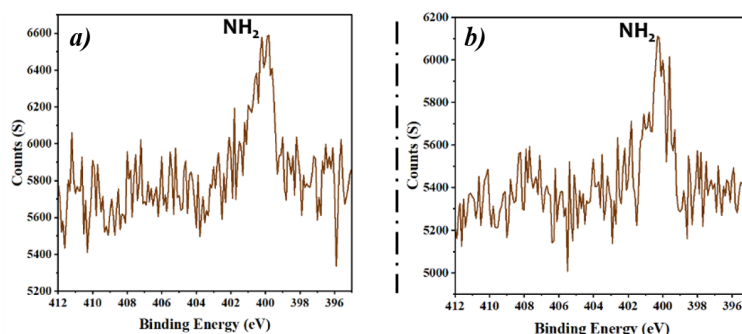
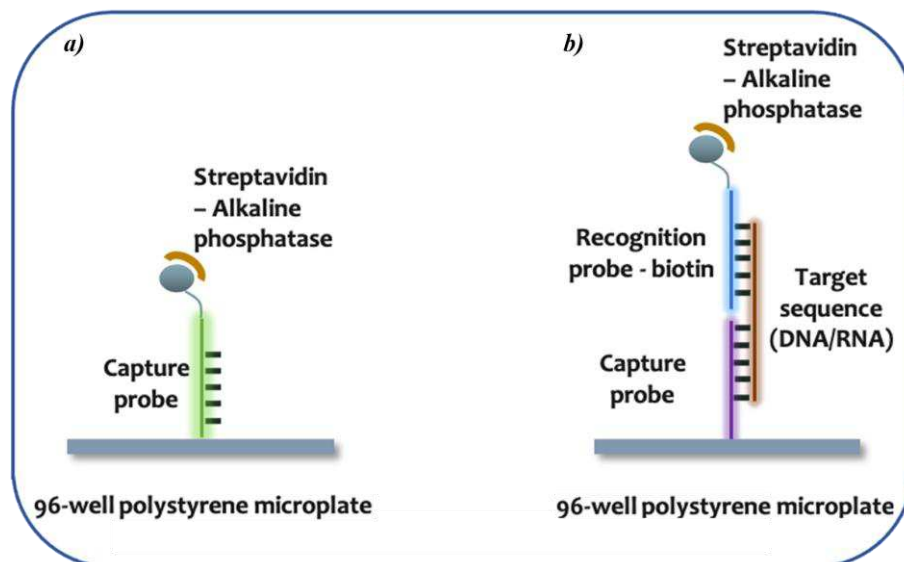


Fig. 4.3 N1s scan of a) PS/HCl, b) PS/HCl+iron

3.2. Optimization of parameters affecting the immobilization platform

To enable more efficient optimization of the immobilization platform, we used a simplified method compared to the one presented in Scheme 1, based on the same principle but without the hybridization step. We directly immobilized a nucleic sequence labeled with biotin by attaching the amine groups of the nitrogenous bases to the functionalized wells of PS microplates using glutaraldehyde (Scheme 2a). The model nucleic sequences of our probe will be used later (Scheme 2b).



Scheme 2: *a) Simplified method for DNA immobilization. b) Complementary Target Detection Using a Complete Sandwich System*

3.2.1. Effect of HCl concentration and iron presence on microplate modification and immobilization efficiency

Fig. 5a shows the effect of HCl concentration on the modification of PS microplates with amine groups using HCl. The maximum immobilization rate was observed at HCl concentration of 9M. Therefore, 9 M HCl was chosen as the optimal concentration for the remaining experiments.

Different acids have been studied as functionalization agents for PS microplates for their use as immobilization platforms, including sulfuric acid (H_2SO_4), nitric acid (HNO_3), and hydrochloric acid (HCl), within the development of biodetection systems. Fig. 5b presents the colorimetric results obtained with different acids used to modify PS microplate wells. Although the biotin-labeled probe concentration was identical for all wells, a notable difference in the colorimetric response was observed for each acid. This variation can be attributed to differences in the amount of amine groups at the surface. Among the tested acids, hydrochloric acid exhibited the most pronounced colorimetric response. Therefore, hydrochloric acid was selected as the optimal modification agent for the polystyrenePS microplates, promoting the immobilization of nucleic sequences and the detection of target strands.

To demonstrate the significance of adding Iron during the modification of wells with amine groups using hydrochloric acid, a biotin-labeled probe was immobilized on the wells of PS microplates that had been modified solely with hydrochloric acid overnight. This setup was compared with the response of the same sequence immobilized with the addition of Iron during the hydrochloric acid modification. As indicated in Fig. 5c, a higher absorbance signal was observed when Iron was added during the hydrochloric acid treatment compared to hydrochloric acid treatment alone. These results emphasize the role of iron addition, which, through its interaction with HCl, may promote an increased availability of amine groups at the surface.

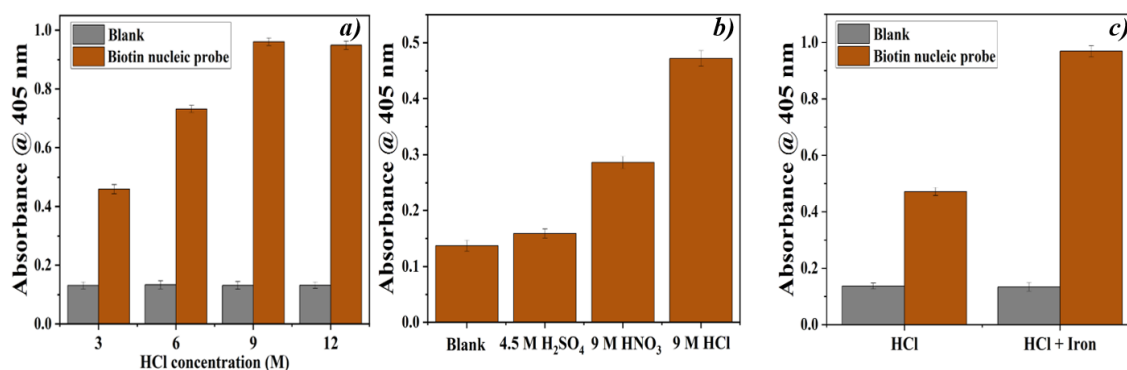


Fig. 5 *a) Effect of HCl Concentration on immobilization efficiency. b) absorbance results of*

enzymatic product after PS Microplate Well Modification Using Different Acids (H_2SO_4 , HNO_3 , and HCl). C) Immobilization Efficiency by Using 9M HCl only, and 9M HCl in the presence of Iron

3.2.2. Optimization of the Modification Time and the Glutaraldehyde Concentration

The HCl concentration and reaction time was optimized to maximize immobilization rate. As shown in Fig. 6a, the immobilization of a fixed concentration of a biotin-labeled probe on PS microplates modified with 9 M HCl increases with increasing modification time. Based on this observation, 1.5 hours was selected as the optimal modification time for the subsequent manipulations.

The biotin-labeled probe is immobilized using glutaraldehyde as a cross-linking agent, which facilitates attachment of nucleic acid sequences to the amine-activated platform via its aldehyde functionalities. A range of glutaraldehyde concentrations (1% to 10%) was tested, and the optimal immobilization rate was achieved with a 5% glutaraldehyde solution (Fig. 6b).

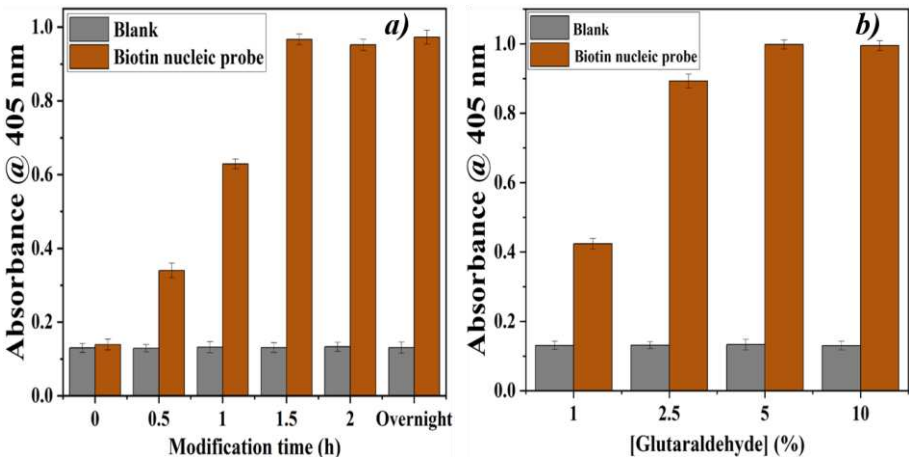


Fig. 6 Optimization of Factors Influencing the Immobilization Platform Based on the Enzymatic Product Response in the Presence and Absence of a Biotin-Labeled Nucleic Acid Sequence. **a)** Modification time with HCl in the presence of Iron. **b)** Glutaraldehyde concentration

3.2.3. Developed Platform Stability under Harsh Conditions

To assess the robustness of the immobilization platform, biotin nucleic probe was immobilized on the developed platform after exposure to extreme conditions, including incubation for 20 minutes at different pH levels (1, 7, and 14) (Fig. 7a), exposure to 0.1 M surfactants (CTAB, Tween, SDS) (Fig. 7b), treatment with 1 M $NaCl$ (20 minutes), repeated washing (10 cycles) with distilled water, and washing with hot water at $90^\circ C$ (Fig. 7c). No significant changes in the immobilization platform's performance were observed under these conditions, except for SDS, which had a noticeable effect on stability.

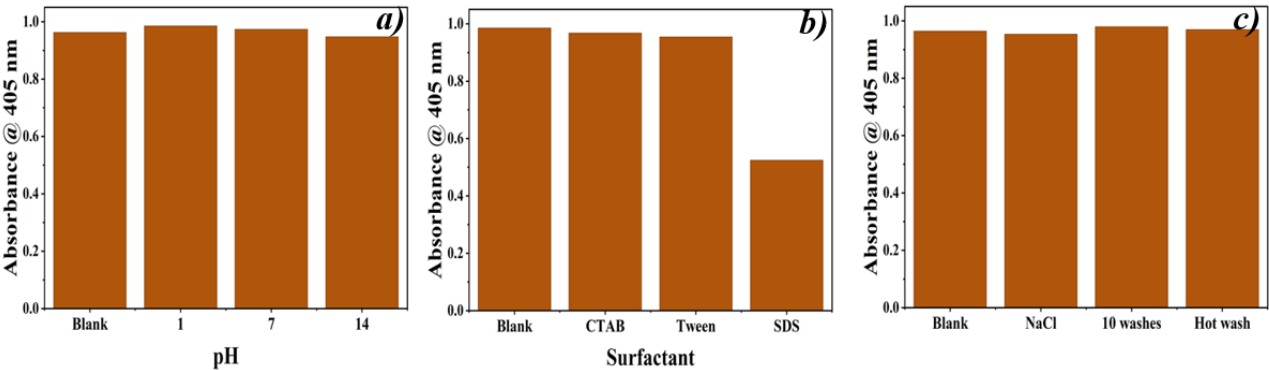


Fig. 7 Stability evaluation of the developed platform under harsh conditions: **a)** pH 1, 7, and 14; **b)** 0.1 M surfactants (CTAB, SDS, Tween); and **c)** 1 M $NaCl$ exposure, repeated washing at room temperature, and hot-water washing ($90^\circ C$)

The storage stability of the developed platform was evaluated by immobilizing the same concentration

of biotin nucleic probe on two separate microplates and monitoring their performance under different conditions. One microplate was stored at 4 °C, while the other was kept at ambient temperature. The activity of the DNA platform was assessed on different days through incubation with alkaline phosphatase and PNPP. The results show that when stored at 4 °C, the DNA sensors retained approximately 90% of their initial response after 25 days, demonstrating their robustness for practical applications. In contrast, storage under room temperature resulted in retention of only about 79% of the initial response over 16 days (Fig. 8)."

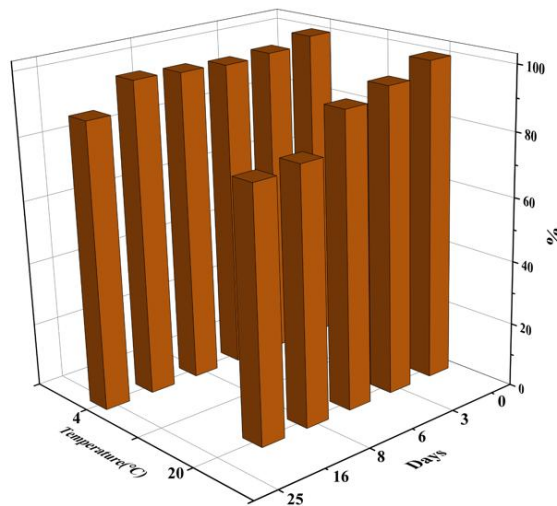


Fig. 8 Storage stability of the developed platform at 4 °C and room temperature over varying durations

3.2.4. Comparative assessment of platform modification methods

We compared two methods for modifying PS microplates (Fig. 9): a two-step method using KOH and APTES [21] and the single-step acid treatment using HCl and Iron proposed in this work. Both platforms were tested by immobilizing biotin-labeled probe using the same concentration of glutaraldehyde. The results showed that the microplates modified by acid treatment in the presence of Iron exhibited a higher absorbance, indicating significantly higher immobilization rates. This superior performance can be attributed to the enhanced surface properties imparted by the acid treatment, which facilitate more efficient immobilization of the nucleotide sequence.

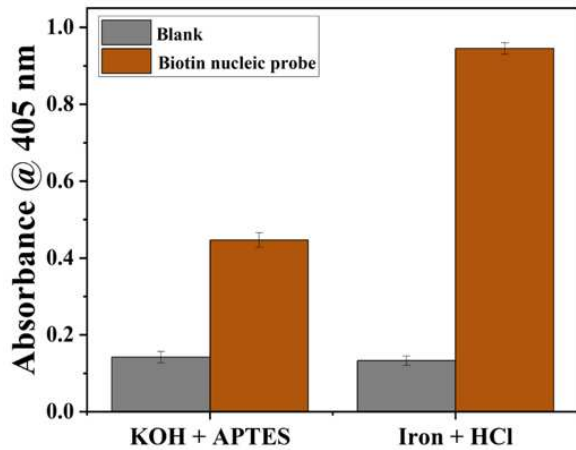


Fig. 9 Absorbance measurements after Modification of PS Microplates with KOH+APTES and HCl+Iron

3.3. Analytical application of the developed platform for the detection of the complementary Target

To test the analytical performance of the developed biosensor, after immobilizing a fixed concentration of the immobilization probe (complementary to the first part of the microRNA-222 sequence) on the

platform (Scheme 2b), various concentrations of the target miRNA-222 were incubated. One part of the miRNA strand hybridizes to the probe immobilized on the platform, while the other hybridizes to the recognition strand labeled with biotin. Then, the proposed colorimetric detection method is applied, resulting in a color change that varies with the concentration of complementary miRNA hybridized to the developed platform. This coloration has been measured using an ELISA reader.

Consequently, the color intensity of the enzymatic product increases with the concentration of the complementary miRNA-222, ranging from 0,5 nM to 20 nM (Fig. 10a). As observed, a linear relationship exists between miRNA-222 concentration and color intensity. Furthermore, the detection limit of the developed biosensor was estimated to be 0,38 nM for a signal-to-noise ratio of 3. The linear regression equation obtained is $Y = 0.038 [\text{miRNA-222}] (\text{nM}) + 0.157$, with a correlation coefficient of $R^2 = 0.976$.

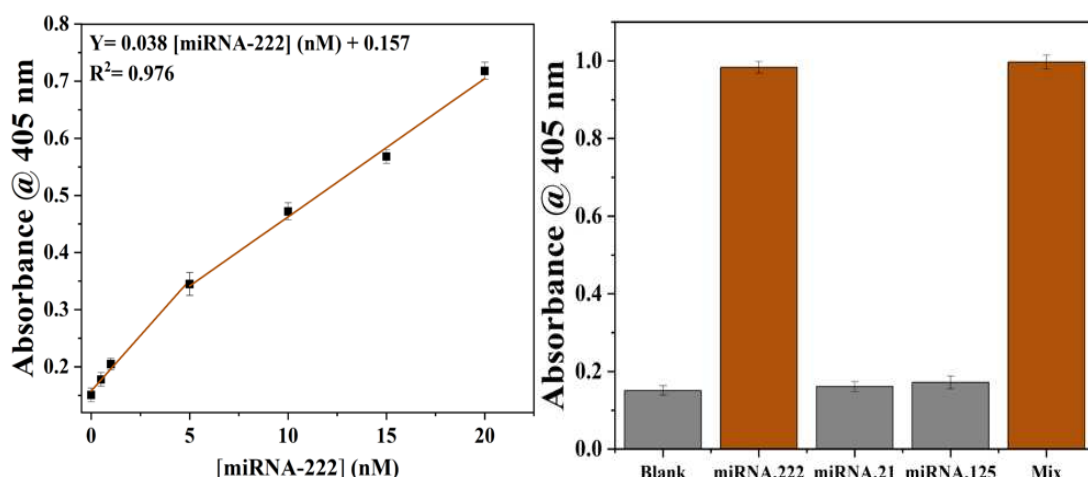


Fig. 10: a) Calibration curve of absorbance values measured at 405 nm for different concentrations of target miRNA-222 and b) the selectivity of the developed biosensor for 50 nM of complementary miRNA (miRNA-222) compared to non-complementary miRNAs (miRNA-21 and miRNA-125).

To assess the selectivity of the developed biosensor, we tested a complementary miRNA (miRNA-222) and two non-complementary miRNAs (miRNA-125 and miRNA-21). The results indicate that our platform can specifically detect miRNA-222 even in the presence of other types of miRNAs. This demonstrates the absence of non-specific adsorption and the good selectivity of the developed platform (Fig. 10b).

However, many alternative platforms often require additional time, higher temperatures, multiple reagents, and complex modification steps to achieve effective immobilization. Similarly, methods that rely on commercial gold nanoparticles (AuNPs) or printed circuit board gold electrodes are generally more expensive, further increasing costs and complexity. In contrast, our approach, which involves modifying ELISA microplates with hydrochloric acid, offers significant advantages in terms of simplicity, availability, cost-effectiveness, multiplexing capabilities, and standardization. Furthermore, the immobilization on a 96-well microplate offers high-throughput analysis.

Table 1 Comparison of Different Platforms for DNA Immobilization and RNA Detection

Immobilization Platform*	Immobilization Technique	Chemical Interaction	Comments	LOD	Ref
Printed circuit board gold electrode (PCBGE)	Covalent	Au-thiol bond	Use of commercially available gold-based electrodes: These are expensive and increase the platform's overall cost.	$0.5 \times 10^{-6} \text{ M}$	[26]
GO@ AgNCs		Cytosine-AgNCs silver nanoclusters	The synthesis of nanoparticles requires specific reagents, various acids, extended durations, elevated temperatures, and centrifugation processes.	$3 \times 10^{-10} \text{ M}$	[27]
AuNPs		Au-thiol bond	Use of commercial AuNPs: These nanoparticles are costly and require centrifugation during preparation or use.	$5 \times 10^{-6} \text{ M}$	[28]

Au gold@ CoNi-MOF	Adsorption	Hydrogen bonds, π - π stacking, and hydrophobic interactions	Use of commercially available gold-based electrodes. Need for toxic reagents, long synthesis time, and elevated temperatures.	$0,14 \times 10^{-15}$ M	[29]
CdTe@ GDS	Covalent	Cd-thiol bond Te-thiol bond	Need of toxic reagents, long synthesis time, and elevated temperatures.	$2,52 \times 10^{-9}$ M	[30]
Elisa microplate@ HCl	Covalent	imine bond	The proposed approach relies on a simple, readily available modification of the microplate, offering cost-effectiveness, multiplexing capabilities, standardization, and a simple alternative.	3.8×10^{-10} M	This work

*Au: Gold; GO: Graphene Oxide; Ag: Silver; Co: Cobalt; Ni: Nickel; MOF: Metal-Organic Framework; Cd: Cadmium; Te: Tellurium; GDS: Quantum Dots.

Another application of the developed biosensor is the detection of E. Coli bacteria. The biosensor was tested with different concentrations of a specific sequence from E. Coli bacteria. The capture probe (complementary of the first part of specific E. Coli sequence) was immobilized on the developed platform, and different concentrations of E. coli specific sequence target DNA (E. Coli) (5, 10, and 15 nM) were added simultaneously with the recognition probe labeled with biotin (complementary of the second part of specific E. Coli sequence) as scheme 1. After the hybridization step, the streptavidin-enzyme was introduced into the wells, and after the addition of PNPP as a substrate, the enzymatic product was measured at 405 nm. The results are presented in Fig. 11, clearly indicating that the absorbance increased with increasing concentrations of the E. coli-specific DNA sequence, thereby confirming the success of the developed platform for detecting E. coli.

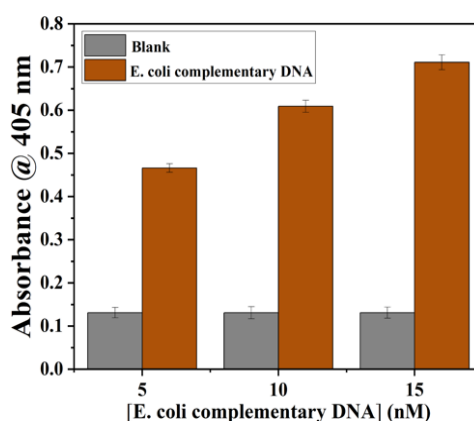


Fig. 11: Application of the developed platform for the detection of a specific DNA sequence (E. Coli bacteria)

4. Conclusions

This study demonstrates a simple, one-step acid-assisted strategy for transforming conventional polystyrene (PS) microplates into reactive platforms for nucleic acid immobilization. Surface characterization by FT-IR, XPS, SEM, and contact angle measurements confirmed that treatment with HCl in the presence of iron substantially altered the surface chemistry and morphology of PS, resulting in enhanced hydrophilicity and the formation of surface amine functionalities. These modifications enabled efficient covalent immobilization of oligonucleotide probes through glutaraldehyde-mediated coupling.

The developed platform exhibited superior immobilization performance compared with the conventional KOH/APTES modification approach while requiring fewer processing steps and more readily available reagents. The resulting microplate-based biosensing platform showed excellent stability under a wide range of chemical and physical conditions and retained high activity during storage.

As a proof-of-concept, the platform was successfully applied to the colorimetric detection of both miRNA-222 and an Escherichia coli-specific DNA sequence via a sandwich

hybridization assay. The biosensor achieved sensitive and selective detection of miRNA-222 with a detection limit of 0.38 nM while maintaining compatibility with standard ELISA readers and high-throughput 96-well formats.

Overall, the proposed surface modification strategy provides a low-cost, scalable, and versatile alternative to more complex immobilization platforms. Its simplicity, robustness, and adaptability make it a promising platform for future nucleic acid biosensing applications in clinical diagnostics, environmental monitoring, and other bioanalytical fields.

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