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Novel Nitric Oxide-Releasing Polyvinyl Alcohol Films for Disrupting *Pseudomonas aeruginosa* Biofilms

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Abstract

Novel nitric oxide (NO)-releasing polyvinyl alcohol (PVA) films were prepared by blending sodium nitroprusside (SNP), which serves as an NO donor, into a PVA solution. The microtiter plate (MTP) was used to measure the biofilm-forming capacity of eight *Pseudomonas aeruginosa* isolates on PVA film-coated MTP, MTP coated with nitric oxide-releasing PVA films, and catheters coated with NO-releasing PVA films. The qPCR was employed to determine the effect of PVA, nitric oxide, and NO mixed with PVA on the gene expressions of *pslA*, *pelA*, *algD*, *nirS,* and *bdlA* genes. All isolates developed strong biofilms on uncoated MTP, with a highly significant decrease on NO- releasing-PVA film-coated MTP and catheters coated with NO-releasing-PVA film compared to PVA film-coated MTP and uncoated MTP. Significant downregulation of *pelA* and *pslA* expression was determined upon exposure to the SNP-PVA and SNP alone. However, *algD* displayed insignificant downregulation after exposure to the SNP-PVA blend and SNP alone versus the PVA solution. Furthermore, *nirS* and *bdlA* exhibited insignificant upregulation after exposure to SNP-PVA and SNP alone versus the PVA solution.

Keywords: *Pseudomonas aeruginosa*, Biofilm, PVA, nitric oxide.

أغشية مُستَحدَثة من عديد فينيل الكحول مُطلِقة لأكسيد النيتريك لِتشتيت الأغشية الحياتية للزوائف الزنجارية

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الخلاصة

تم تحضير أغشية جديدة من عديد فينيل الكحول (PVA) المُطلِقة لأكسيد النيتريك عن طريق مزج نتروبروسيد الصوديوم (SNP) ، والذي يعمل كمانح لاكسيد النتريك (NO) في محلول PVA المتشابك مع انهيدريد الماليك. تم استعمال طريقة طبق المعايرة الدقيقة لقياس قدرة ثماني عزلات من الزوائف الزنجارية على تكوين الاغشية الحياتية على طبق المعايرة الدقيقة مطلية ب PVA طبق المعايرة الدقيقة مطلية بأغشية PVA المُطلِقة لأكسيد النيتريك وقسطرة مطلية بأغشية PVA المُطلِقة لأكسيد النيتريك. كما تم استعمال تفاعل البلمرة

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الكمي المتسلسل لتحديد تاثير PVA واكسيد النيتريك والخليط بينهما في التعبير الجيني للجينات *pslA* و *pela* و*algD* و*nirS* و *bdlA* . أظهرت النتائج ان جميع العزلات الثمانية كانت منتجة للاغشية الحياتية القوية على طبق المعايرة الدقيقة غير المطلية ، بينما لوحظ انخفاض كبير في تكوين الأغشية الحياتية عند استعمال طبق المعايرة الدقيقة مطلية بفيلم PVA المُطلِق لأكسيد النيتريك، وكذلك عند استعمال القسطرة المطلية بنفس الغشاء، مقارنةً طبق المعايرة الدقيقة المطلية بـ PVA فقط و طبق المعايرة الدقيقة غير المطلية. أما على المستوى الجيني، فقد تم تسجيل انخفاض معنوي في التعبير الجيني لجيني *pelA* و *pslA* بعد تعريض البكتيريا لمزيج SNP–PVAولـ SNP وحده. ومع ذلك، لم يظهر الجين *algD* انخفاضًا معنويًا عند التعرض لنفس العوامل مقارنةً بمحلول PVA .وعلى العكس من ذلك، لوحظت زيادة غير معنوية في التعبير الجيني لجيني *nirS* و *bdlA* بعد التعرض لمزيج SNP–PVA ولـ SNP وحده مقارنةً بمحلول PVA

1. Introduction

Pseudomonas aeruginosa is a prevalent nosocomial pathogen in Iraqi hospitals that can lead to life-threatening infections characterized by robust biofilm formation [1]. Alginate, Psl, and Pel are the three different exopolysaccharides that make up the biofilm components of this pathogen [2]. One of the most prevalent infections linked to healthcare is catheter-associated urinary tract infections (CAUTI), even with strict guidelines for prevention in place. When silicone catheters were coated with a biofilm-preventative agent that included benzalkonium chloride, polyacrylic acid, and glutaraldehyde, they completely prevented all uropathogens from forming biofilms, with the exception of *P. aeruginosa*, which did not exhibit any decrease in biofilm biomass as compared to uncoated silicone catheters [3]. Several strategies are used to reduce the prevalence of infections associated with biofilms, including the use of novel materials for catheter manufacture and the functionalizing of their surface with antibacterial surfaces [4]. Catheters coated with a hydrogel are designed to alter the surrounding microenvironment, thereby inhibiting the growth of biofilm. Moreover, 20% of hospitalised patients, particularly those in intensive care units, have a urinary catheter, and 70% of UTIs are associated with urinary catheters [5].

For the past fifty years, nitric oxide (NO) has been utilised as an antibacterial agent and has consistently shown promise for usage in biomedicine. Although NO-donors have historically been applied to polymer surfaces to prevent bacterial attachment, limited research has examined their effectiveness when coating urinary catheters [6].

Polyvinyl alcohol (PVA) is a synthetic polymer that is safe, non-toxic and has good biocompatibility. It is beneficial in various applications, including packaging, polymer recycling, and controlled drug administration systems, due to its high hydrophilicity and exceptional chemical stability [7]. It is generally known that PVA is one of the few vinyl polymers that can show swelling behaviour while degrading more quickly. This is possible due to the hydroxyl groups that affect the hydrophilic nature of the substance. PVA films and hydrogels have been employed in medical applications [8]. This study aimed to provide the first evidence of incorporating SNP into a PVA/maleic anhydride (MA) film and to evaluate the mixture's effect on *P. aeruginosa* biofilm formation, with potential applications in medical devices such as urinary catheters to prevent biofilm development.

2. Materials and Methods

2.1 Isolation and Identification of P. aeruginosa

A total of eight *P. aeruginosa* isolates were obtained from the Microbiology laboratory, Department of Biology, College of Science, University of Baghdad. All of these were originally derived from patients admitted to Ghazi Alhariri Hospital in Baghdad Medical City. The Vitek 2 compact system was applied to confirm the identification.

2.2 Exposure of Pseudomonas aeruginosa to nitric oxide

2.2.1 Preparation of the stock solution of sodium nitroprusside

An 80 mg/ml solution of sodium nitroprusside (SNP) was used to prepare a stock solution. This concentration was obtained by dissolving 0.8 g of SNP in 10 ml of sterile distilled water. The resulting SNP solution was then kept in the dark.

2.2.2 Estimation of Minimum Inhibitory Concentration of SNP

Teh *et al*., provided a procedure for the Resazurin-based turbidometric test, which was used to determine the minimal inhibitory concentration (MIC) of SNP [9]. To summarise, double serial dilutions of SNP (40, 20, 10, 5, 2.5, 1.250, 0.625, 0.3126, 0.156, and 0.0781 mg/ml) were prepared in Mueller-Hinton broth using a 96-well microtiter plate. Then, each well received 20 µl of bacterial suspension, which had been adjusted to a MacFarland 0.5 standard with a turbidity of $1.5\times10^{\wedge 8}$ CFU/ml and was thoroughly mixed. Following an overnight incubation period at 37°C, each well received 5 µl of resazurin (6.75 mg/ml), which was then added and incubated for an additional 4 hours at the same temperature. The lowest concentration before the observed colour change, from blue to pink, was determined to be the MIC.

2.2.3 Preparation of polyvinyl alcohol / maleic anhydrate film

The polyvinyl alcohol solution (16 mg/ml) was prepared by dissolving 0.16 g of PVA (CDH, Indian) in 10 ml of distilled water using a hot plate with a magnetic stirrer at 85-90°C for 1hr until completely dissolved [10]. Then, 0.5 g of maleic anhydride was added to the PVA solution with the help of a magnetic stirrer, and a crosslinking reaction occurred at 120°C for 30 min. The obtained clear solution was sterilized by filtration through a 0.22 mm membrane filter and transferred to 96-well polystyrene microtiter plates by spraying it onto the surface of the microtiter plate to form a soft layer of film. It was then left to dry for 2 days at 45°C. To prepare a mixture of SNP with PVA film, the MIC of SNP was added to the clear solution of PVA /maleic anhydrate, then it was sprayed onto a polystyrene microtiter plate and left to dry for 2 days at 45°C to form a film.

2.2.4 Effect of sodium nitroprusside mixed with PVA film on biofilm formation

In this study, a microtiter plate technique has been used to investigate how NO affected the production of biofilms on PVA film surfaces [11]. Three freshly cultured isolates from agar plates were inoculated in 10 ml of trypticase soy broth (TSB) with 1% glucose and compared at a 0.5 McFarland scale. The inoculated broth was then incubated at 37 °C for 18 hrs. The overnight- incubated bacterial suspension was then diluted 1:100 with fresh TSB by adding 100 µl of bacterial suspension to 9900 µl of TSB.

Each well of a sterile polystyrene tissue culture plate coated with a mixture of PVA and SNP was filled with 200 µl of diluted prepared bacterial suspension. Each isolate was inoculated in triplicate. The microliter plates were incubated for 24 hrs at 37°C. Each well's content was carefully removed by tapping the plates after incubation. To eliminate free-floating planktonic bacteria, the wells were twice cleaned with 200 µl of phosphate buffer saline. Adherent bacterial biofilms on the plate were fixed by incubating for 30 minutes at 37°C. A volume of 200 µl of 1% crystal violet was then used to stain the wells for 15 minutes. Following three rinses with sterile distilled water to remove excess dye, the microtiter plates were allowed to dry for 45 minutes at room temperature. Thereafter, 200 µl of ethanol solution was used to dissolve the dye linked to the adhering biofilm. The optical densities of the stained bacterial biofilm were measured using a micro-ELISA reader (BioTek, USA) at 630 nm wavelength after adding ethanol for 15 minutes. The same procedure was used to measure the biofilm formation capacity of bacterial isolates on microtiter plates coated with SNP-free PVA film.

2.2.5 Effect of nitric oxide on biofilm formation associated with PVA-coated catheter

Silicon foley catheter (100%) was cut into 1 cm-long pieces. After preparation of PVA / Maleic anhydrate solution and PVA/ Maleic anhydrate solution mixed with SNP. Afterwards, these solutions were poured into a Petri dish; each dish was used to impregnate 24 pieces in one day. Then, all pieces were dried and sterilized under a UV lamp overnight to be ready for use in determining the biofilm formation capacity on catheters coated with PVA film and a mixture of PVA film with SNP. Furthermore, twenty-four uncoated pieces were used to determine the capacity of biofilm formation on the uncoated catheter by placing them in a 96-well polystyrene microtiter plate, while nine pieces served as a negative control and were processed as previously mentioned [11]. To distinguish between the isolates for adhesion values, the optical density cutoff (ODc) was applied (ODc = average OD of negative control + 3× standard deviation (SD) of negative control) [12]. Weak adherence is indicated by optical density OD < 2*ODc, but strong biofilm and moderate adherence between them are indicated by 4*ODc ≤ OD [13].

2.3 Expression of pelA, pslA, algD, nirS, and bdlA under nitric oxide stress

2.3.1 Primer selection

The primers grouped in Table 1 were utilized in this work.

Table 1: Primers utilized in this work

Genes	Sequence 5' → 3'	Product size (bp)	Reference
fbp	F: CCTACCTGTTGGTCTTCGACCCG R: GCTGATGTTGTCGTGGGTGAGG	284	[14]
algD	F-GCTCAACCTGTCGCGCTACT R-GAACTCGCCACCACTTCGTC	423	[15]
pelA	F-CCTTCAGCCATCCGTTCTTCT R-TCGCGTACGAAGTCGACCTT	118	[16]
pslA	F-ATAAGATCAAGAAACGCGTGGA R-TGTAGAGGTCGAACCACACCG	146	[16]
nirS	F: CGGCCGAAGAAACAGCTCAACGAC R: AGTAGGCGCCGGCGATGGTGTAG	328	[17]
bdlA	F: TCAAGGTGGTCAAGTACGCC R: ATGGTGGCGAGGAAGTTGTC	154	[18]

2.3.2 RNA Extraction of Pseudomonas aeruginosa

RNA was recovered from the *P. aeruginosa* biofilm using the *TransZol* Up Plus RNA Kit (Transgen/China). The Nanodrop instrument was utilised to measure the concentration and purity of the extracted RNA. After that, RNA was converted to cDNA using EasyScript® First-Strand cDNA synthesis SuperMix (TransGen Biotech, China).

2.3.3 Gene Expression

The levels of gene expression for genes under investigation were measured, and the results were normalized using *fbp.* Once the reaction mixture preparation was complete (Table 2), the tubes were placed into the thermal cycler, which had been set up as shown in Table 3.

Table 2: Quantitative RT-PCR (qPCR)

Reagent	Volume (µl)
Master Mix	10
Forward Primer (20X)	0.5
Reverse Primer (20X)	0.5
cDNA (100 ng)	8
Nuclease-Free Water	1

Table 3: The protocol of qPCR

Step	Temperature (°C)	Time (seconds)	Cycles
Initial Denaturation	94	30	1
Denaturation	94	5	45
Extension	60	35	
Melt Curve	90	15	100

2.4 Statistical Analysis

Each experiment was conducted with three iterative trials, and the findings were analysed using GraphPad Prism 9.5.1. Kolmogorov-Smirnov and Shapiro-Wilk tests were performed to test the normal distribution of data. To determine the mean and standard deviation, the research parameters were assessed for their influence. The T-test was utilised to assess the impact of nitric oxide on biofilm. P-values less than 0.05 were considered significant. A fold change of <2 was considered insignificant based on the criterion of Rasigade *et al.,* [19].

3. Results and discussion

3.1 Biofilm formation

All eight isolates displayed strong biofilm, as depicted in Figure 1. According to Shatti, *et al* ., [20], 100% of the isolates under study have the ability to form biofilms. However, 19, 12, and 9 isolates out of 40 developed strong, moderate and weak biofilms, respectively. In a study conducted by Al-Maeni [21], 20 isolates of *P. aeruginosa* were classified into three groups based on their biofilm-forming abilities: weak, moderate, and strong biofilm formers. As well as in research by Nader *et al*., [22], which revealed that 31/37 (83.78%) isolates produced biofilm, and the remaining 6 isolates were non-biofilm producers when assayed by microtiter plate assay.

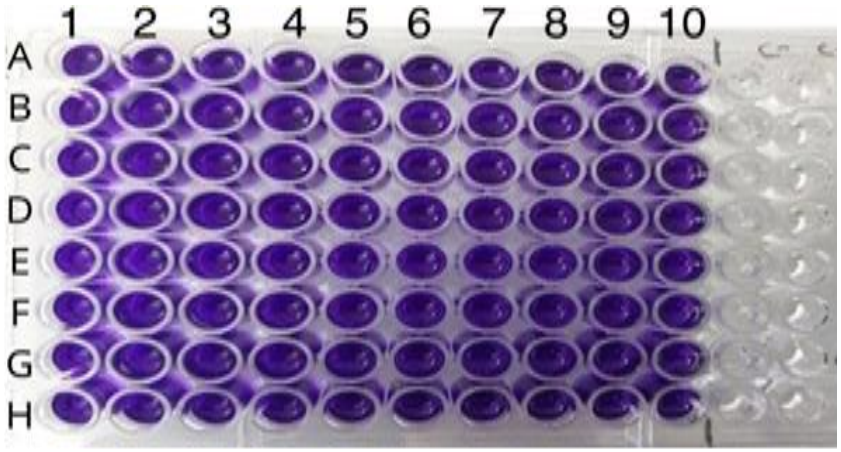

Figure 1: Microtiter plate revealing biofilm of *Pseudomonas aeruginosa* isolates; PA1=(A1-A10), PA2 =(B1-B10), PA3= (C1-C10), PA4=(D1-D10), PA5=(E1-E10), PA6=(F1-F10), PA6=(F1-F10), PA7=(G1-G10), PA8=(H1-H10), controls represented in wells (A11-H11) and (A12-H12).

3.2 Estimation of minimum inhibitory concentrations of sodium nitroprusside

The MICs of SNP were demonstrated in a range of 1.25-0.312 mg/ml, whereas the mixing of PVA with SNP led to a decrease in the MICs of SNP in a range of 0.625-0.156 mg/ml. Note that the PVA alone has no effect against isolates of *P. aeruginosa* (Figure 2). Nitric oxide is bonded to a transition metal to form metal nitrosyl complexes. SNP, a representative NO donor of this class of compound, has been used clinically for a long time to treat cardiovascular and high blood pressure conditions. Recent findings by Al-Qaissy and Al-Khafaji [23] highlighted that most of the *P. aeruginosa* isolates demonstrated resistance to the commonly used

antibiotics ceftazidime and ceftriaxone. Their study further indicated that bacterial horizontal gene transfer plays a key role in the emergence of antibiotic resistance in *P. aeruginosa*, which is responsible for respiratory infections. In another study investigating plasmid-associated fluoroquinolone resistance in nosocomial *P. aeruginosa* isolated from burn wound infections, the findings suggested that the plasmid content of *P. aeruginosa* could be a preliminary predictor for fluoroquinolone-based treatment protocols of nosocomial infections [24].

Using SNPs has been demonstrated to enhance antibiotic or antimicrobial treatment in Gram-negative bacteria, such as *P. aeruginosa*, and to prevent biofilm formation and biofilm dispersal [25]. Several single-species biofilms and multispecies biofilms, including those found in cystic fibrosis sputum, have been demonstrated to be efficiently dispersed by SNP at 500 nM [26].

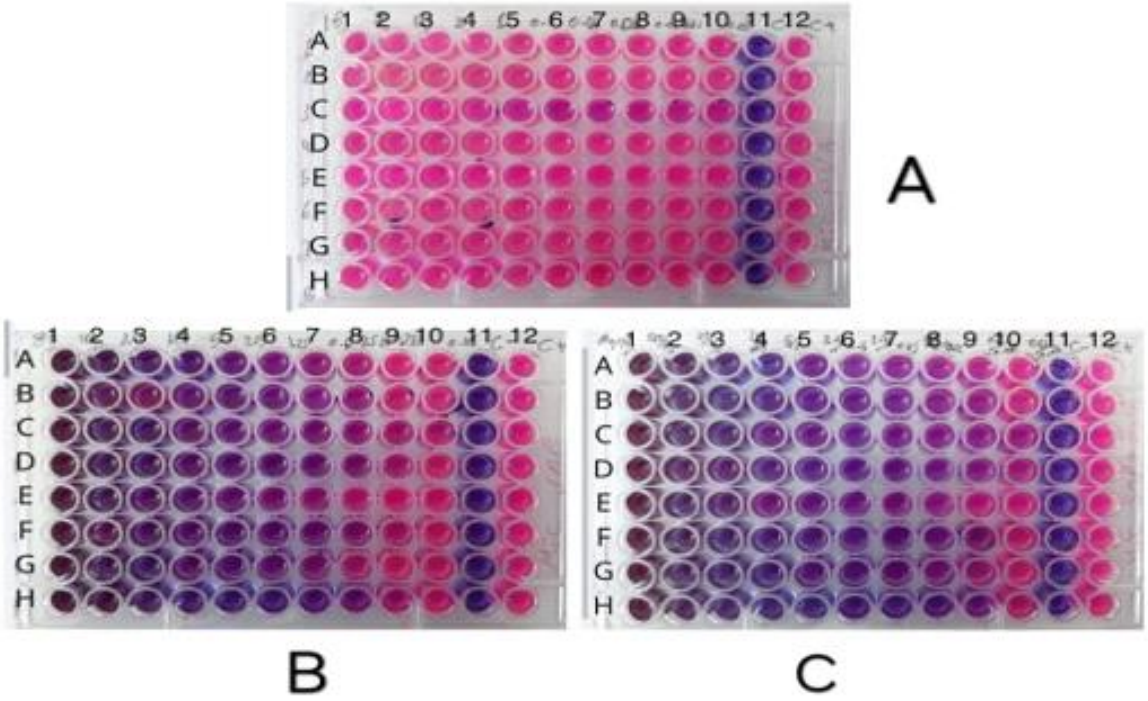

Figure 2: (A) Serial double concentrations of polyvinyl alcohol against eight isolates of *P. aeruginosa* (16-0.0156 mg/ml), there is no effect on these isolates, (B) serial dilution of SNP concentrations against 8 isolates of *P. aeruginosa*, the MIC was represented at range of1.25-0.312 mg/ml, (C) mixing of polyvinyl alcohol with sodium nitroprusside results in decreasing the MICs of sodium nitroprusside in a range of 0.625-0.156 mg/ml. In all these figures (A, B, and C), bacterial isolate PA1 is represented in wells (A1-A10), PA2 in (B1-B10), PA3 in (C1-C10), PA4 in (D1-D10), PA5 in (E1-E10), PA6 in (F1-F10), PA7 in (G1-G10), and PA8 in (H1-H10). Wells (A11-H11) represent the negative controls, while wells (A12-H12) indicate the positive controls.

3.3 Effect of ½MIC of sodium nitroprusside mixed with PVA /MA film on biofilm formation

This study may be considered the first to demonstrate the incorporation of SNPs with PVA/MA film and evaluate the effect of the mixture on biofilm establishment by *P. aeruginosa*. The results displayed a highly significant effect of the mixture in inhibiting biofilm formation compared to the blank (PVA/ MA) film and uncoated polystyrene, as shown in Figures 3 and 4.

Luo *et al*., [27] highlighted that NO-releasing self-healing hydrogels of PVA/ polyethyleneimine (PEI) have a significant effect in inhibiting the development of *P. aeruginosa* biofilm, making them a potentially effective treatment option for wounds afflicted

by bacteria or biofilm. The NO-releasing self-healing hydrogels were prepared by embedding PEI into PVA hydrogels. As well as the incorporation of nitric oxide donor into the self-healing hydrogel dressing consisting of PVA/borax (PVA/B) and carboxymethyl chitosan (cmCHI), exhibited a threefold increase in antibiofilm activity, greater than that of the blank self-healing hydrogel (PVA-B-cmCHI) by inhibiting 80% of the biofilm formation [28]. PVA and chitosan crosslinked blends loaded with silver nanoparticles produced hydrogels that have greatly increased the antibacterial and inhibitory action against PVA biofilms [29].

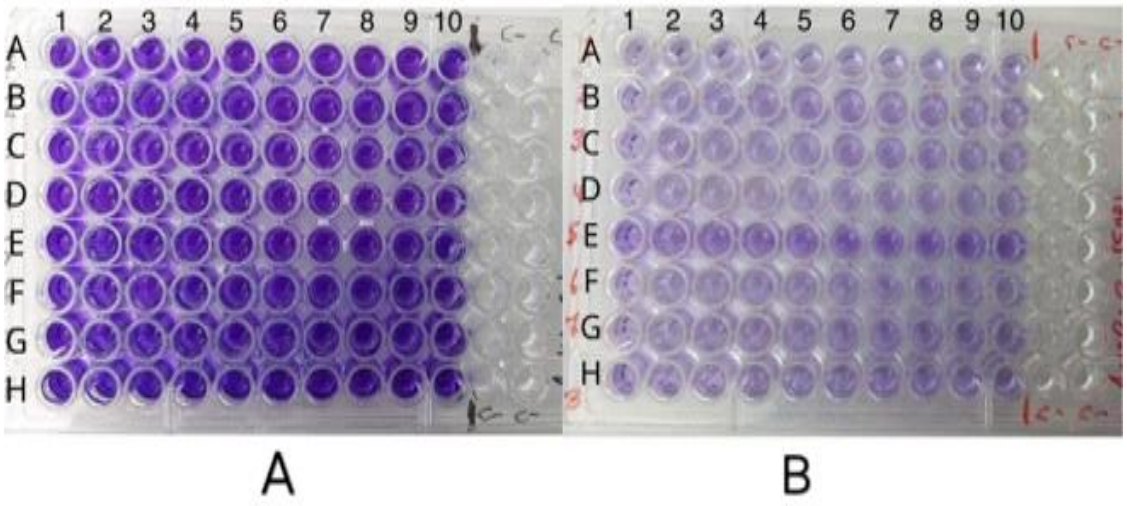

Figure 3: Biofilm formation capacity of eight isolates of *P. aeruginosa* on a microtiter plate coated with (A) Polyvinyl alcohol film and (B) the effect of sodium nitroprusside on biofilm formation of *P. aeruginosa* isolates on Polyvinyl alcohol film-coated microtiter plate.

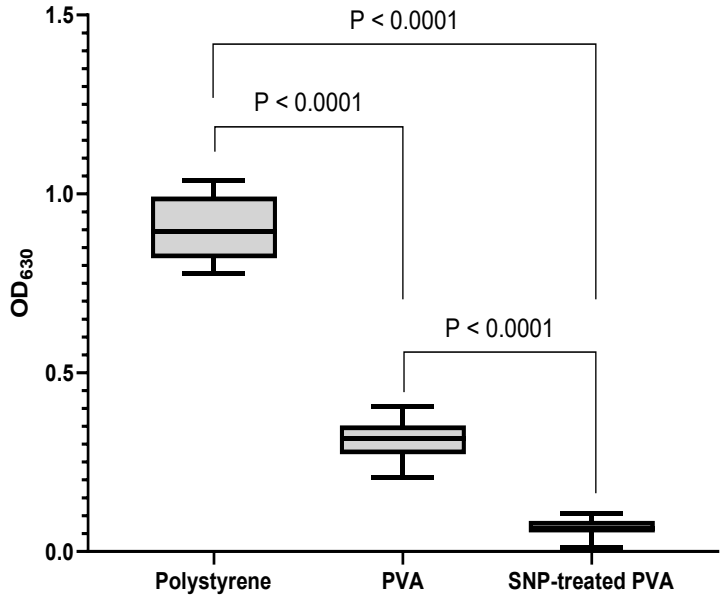

Figure 4: A highly significant effect of the mixture (Polyvinyl alcohol; PVA and sodium nitroprusside; SNP) against biofilm formation of *Pseudomonas aeruginosa* in comparison to the blank PVA film-coated polystyrene microtiter plate and uncoated microtiter plate. OD_{630} refers to the optical density measured at 630 nm.

3.4 Effect of nitric oxide on biofilm formation associated with PVA/MA film-coated catheter

One of the medical applications of using PVA/MA film was considered in coating urinary catheters. In this research, the SNP was used as an NO donor. As a result, the NO-releasing

PVA/MA film-coated catheter has a highly significant effect on preventing biofilm development, as shown in Figure 5. It has been developed to use polymer coatings that enable the manipulation of NO release, controlled by metal nitrosyls [26].

According to a study by Ran *et al*., [30], PEG (polyethylene glycol)/ PVA-coated catheters have the potential to regulate drug release rates for urinary catheterization to a desired level, with the expansion of research on the use of dispersion agents to eliminate medical biofilms. In another study, the role of nitric oxide in reducing biofilm was assessed using a combination of electrochemical NO release technology with antimicrobial medicines to treat hospital biofilm-associated infection situations, particularly infections resulting from intravascular catheters [31]. Additionally, the 3D-printed seconidazole catheter disrupted biofilms and inhibited all virulence components mediated by quorum sensing of two significant keystone pathogens that cause UTIs [32].

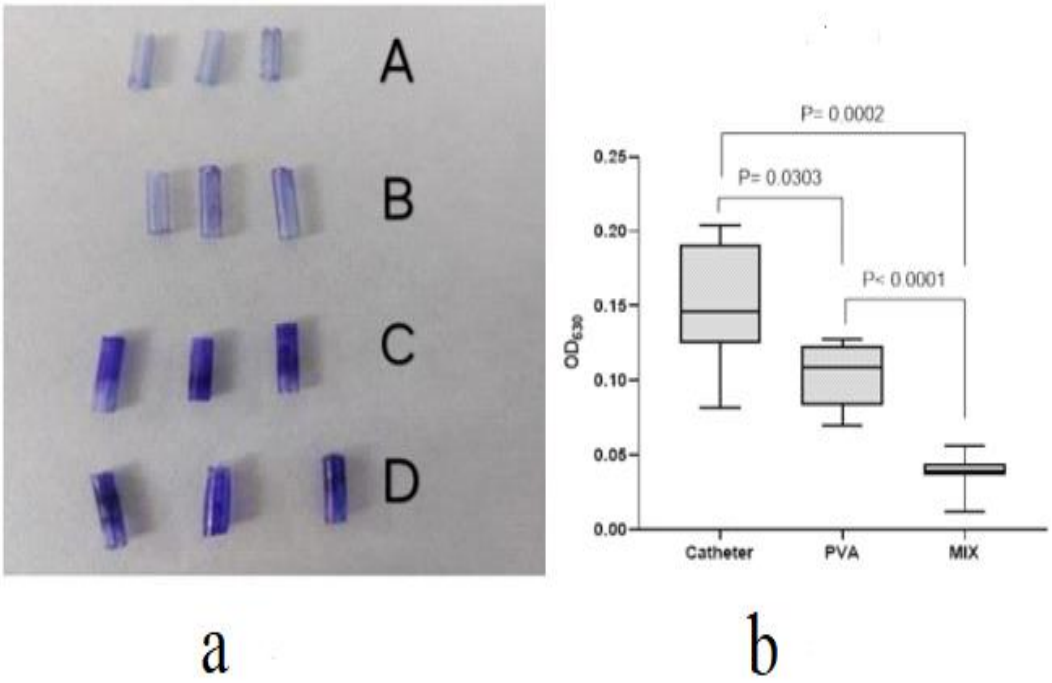

Figure 5: *Pseudomonas aeruginosa* biofilm formed on catheter pieces. (a) Photograph showing stained biofilm on catheter pieces in four different groups: A, negative control of biofilm formation of *P. aeruginosa* on the catheter; B, NO-releasing-PVA/MA film-coated catheter; C, biofilm formed on PVA/MA film-coated catheter; and D, biofilm formed on the uncoated catheter. (b) Box plot demonstrating the biofilm intensity across these groups, with data presented as the median and interquartile ranges.

3.5 Effect of SNP mixed with PVA on the expression of pelA, pslA, algD, nirS and bdlA genes.

This study, for the first time, has revealed the effect of PVA and the mixture of SNP with PVA on the expression of *P. aeruginosa* biofilm-forming genes and genes that are involved in the regulation of biofilm formation, given that these genes are the most responsible genes in the biofilm of *P. aeruginosa* [33].

The results shown in Figure 6 are as follows: *pelA* demonstrated a significant downregulation after exposure to ½MIC of SNP, while it revealed a highly significant downregulation after exposure to a mixture of SNP with PVA compared to PVA alone.

The *pslA* gene showed significant downregulation after exposure to ½MIC of SNP and downregulation when exposed to a mixture of SNP with PVA compared to PVA. While the *algD* gene is represented as non-significant downregulation after exposure to SNP mixed with PVA in comparison to PVA only. The mean of gene expression of the *algD* gene was 0.230 ± 0.172 after exposure to SNP; furthermore, it reached 0.380± 0.166 when exposed to a mixture of PVA and SNP, while the mean of gene expression when exposed to PVA alone was 0.415 ± 0.241. *nirS* gene expression has displayed non-significant upregulation after exposure to SNP and the mixture of SNP with PVA, as represented by the means (1.972 ± 0.7359) and (1.667 ± 0.7960), respectively, compared to the PVA solution, which represented 1.510 ± 0.815. In the presence of SNP, a *P. aeruginosa* strain deficient in NIR does not disperse, whereas a NO reductase mutant displays a hyperdispersal phenotype. Through the induction or suppression of the expression of the complete denitrification pathway, NO itself may exert feedback regulation in *P. aeruginosa*.
Involving the genes *nirS* and *norCB*, through direct interactions with the regulators ANR (repression) [34] and DNR (induction) [35].
Markedly, the results of gene expression for *bdlA* demonstrated non-significant upregulation after exposure to SNP (fold change = 2.435 ± 1.666) and the SNP/PVA mixture (fold change = 2.176 ±1.802). In comparison, the PVA alone scored a fold change of 1.282 ± 0.869.

It has been demonstrated that the *bdlA* chemotaxis regulator plays a role in the nitric oxide-mediated dispersal of biofilms [17]. The concentration of ci-di-GMP decreases as a result of nitric oxide activating phosphodiesterase enzymes (PDEs). Numerous studies have been conducted on the ci-di-GMP signaling pathway in *P. aeruginosa*. The chemotaxis sensor BdlA detects NO in this organism, activating PDEs, DipA and RbdA. Degrading matrix enzymes like the endonuclease EndA are also produced in greater quantities as the concentration of c-di-GMP decreases. The matrix components are consumed by the secreted matrix-degrading enzymes, leading to the dispersion of cell from the biofilm [36]. Another family of heme-based NO-binding proteins, known as NO-sensing proteins (NosP), has been identified in *P. aeruginosa*. According to experiments, NosP binds to NO, which regulates the phosphorylation of a phosphotransfer domain containing histidines, causing biofilm dispersal [37]. It is important to acknowledge certain limitations that may influence the broader interpretation and applicability of these findings. Firstly, only eight clinical isolates of *P. aeruginosa* were used, which might limit the generalizability of the findings to all strains of the bacteria. Secondly, the experiments were conducted under laboratory conditions (using microtiter plates and catheter pieces), which do not fully mimic the complex *in vivo* environment within a living organism, including immune responses and nutrient availability.

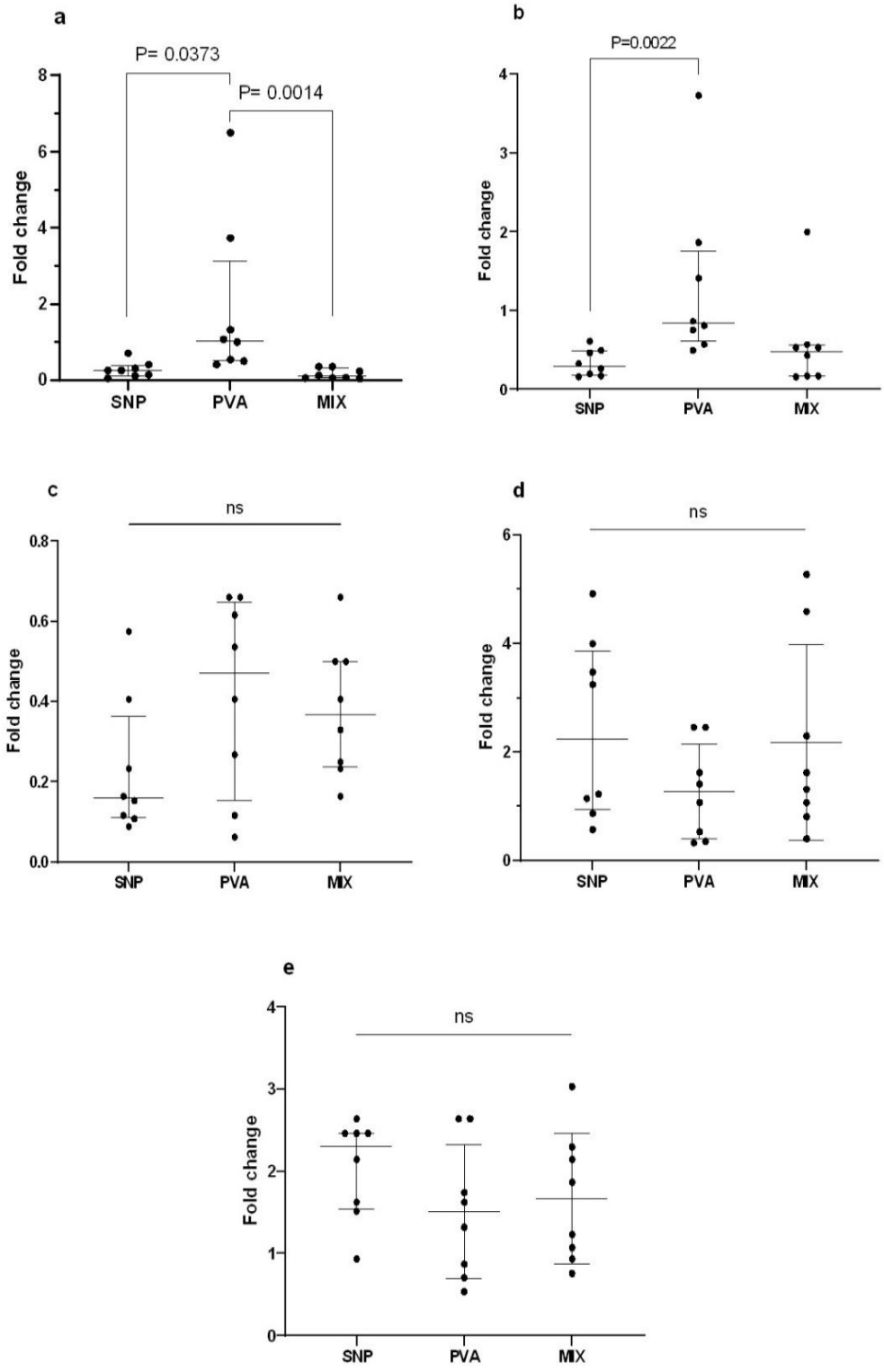

Figure 6: Fold change in gene expression of (a) *pelA*, (b) *pslA*, (c) *algD*, (d) *bdlA*, and (e) *nirS* after exposure to Polyvinyl alcohol, sodium nitroprusside and a mixture of Polyvinyl alcohol and sodium nitroprusside.

4. Conclusion

The key findings demonstrated that these NO-PVA films significantly reduced biofilm formation on both microtiter plates and catheter pieces, surpassing the effects of PVA alone or uncoated surfaces. Furthermore, the incorporation of SNP into PVA films decreased the minimum inhibitory concentrations (MICs) of SNP. When exposed to NO and NO-PVA combinations, the study found that the genes *pslA* and *pelA*, which are essential for the development of biofilm matrix, were significantly downregulated. Although *bdlA* did not exhibit a significant increase, its biological significance is suggested by its recognized function as a chemotaxis regulator in NO-mediated biofilm dispersal. Overall, the NO-releasing PVA films effectively inhibit *P. aeruginosa* biofilm formation, providing promising evidence for their potential use in various medical applications, particularly for preventing medical device-associated infections.

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Conflict of interest

The authors declare no conflict of interest

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