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A combination immuno-nano structure-based bacterial quorum sensing inhibition a two-target strategy

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Abstract
Background: *Pseudomonas aeruginosa* is one such clinically relevant Gram-negative pathogen and it is involved with chronic biofilm-mediated infections and intrinsic antibiotic resistance. Its virulence depends mostly on the quorum sensing (QS) systems which regulate the production of such virulence factors as immune evasion, biofilm development and virulence factors. There is a prospect of decreasing the virulence of these bacteria by going after these couple of communication mechanisms without creating a selective pressure to develop resistance.
Objective: In this research, *P. aeruginosa* was selected as the main target of the dual-function nanotherapeutic platform that would interfere with the functioning of QS-regulated bacteria phenotype as well as boost the host immune defense.
Methods: The QS inhibitor furanone C-30, an immune-stimulatory ligand CpG-ODN or MPLA were surface-functionalized into a nanostructure (NP-DUAL) by encapsulated cores of PLGA and gold. All microbiological analysis was done using *P. aeruginosa* PAO1. AI-2 reporter based on assays and pyocyanin quantitation were used to measure QS activity. Biomass of a biofilm was calculated through crystal violet staining. *In vitro* immunological functions were determined by the use of RAW 264.7 macrophages and human dendritic cells. *In vivo* efficacy was studied in the mouse subcutaneous infection model by challenge of *P. aeruginosa*.
Results: Attachment interference on QS plays a very important role since NP-DUAL severely disrupted the QS signaling in *P. aeruginosa*, deactivating AI-2 by 72 %, pyocyanin 65 % and biofilm biomass by 66 %. There was also a broader activated immune response measured by higher levels of cytokine (TNF-alpha, IL 6, IL 12) secretion and acceleration of phagocytic bacterial killing of cells by the nanostructures. Treatment *in vivo* resulted in significant declines in the bacterial burden and tissue regeneration in infected mice.
Conclusion: The study proves that *P. aeruginosa* is a potential target of dual-action nano therapy. Our NP-DUAL system interferes with QS-driven virulence and provides an antimicrobials- neutral resistance, likely to chelate and reduce antimicrobials on the one hand, and it boosts and promotes host immunity on the other hand. The approach of focusing on bacterial interruption is by targeting pathogen coordination, and persistence as an important source of future antimicrobial innovation.

Keywords: *Pseudomonas aeruginosa*, quorum sensing inhibition, biofilm, immuno-nanostructures, CpG, MPLA, furanone C-30, chronic infections, bacterial virulence and nanomedicine

Introduction
The amplified threat of antimicrobial resistance (AMR) in the pathogenic microorganisms that inhabit the globe has compromised the actions of conventional antibiotics and accentuated the need of emerging treatment strategies (Kalia *et al.*, 2021; Dhingra *et al.*, 2020) [35, 20]. Traditional bactericidal drugs target the key cell-level pathways and, acting so, increase the selection of the resistant phenotype uncontrollably (Munita and Arias, 2016; Blair *et al*., 2019; Lee *et al*., 2020) [48, 14, 39]. New potential non-lethal, pathogenicity-reducing measures are highly needed to eliminate the survival pressure and keep it to the minimum with no possibility to offer any survival advantages to pathogens (Hentzer and Givskov, 2020; Fleitas Martínez *et al*., 2019; Cegelski *et al*., 2021; Al-Maamori, Anmar M. K *et al.*, 2025) [31, 15, 24, 7].
Quorum sensing (QS) is a bacterial regulatory mechanism which allows the population-density-dependent coordination of colony actions by bacteria and coordinates behavior which includes virulence, motility, and biofilm formation (Ng and Bassler, 2019; Whiteley *et al*., 2017; Rutherford & Bassler, 2020; Zhang and Li, 2022) [47, 61, 54, 42]. The environment adaptation and simultaneous gene expression can be gained in a bacterial population through

the production and action of autoinducers (Whiteley *et al*., 2017; Mukherjee and Bassler, 2019; Chen *et al*., 2022) [61, 47, 16]. Another example is clinically relevant pathogens that exhibit QS regulation of virulence factors: exotoxins, elastase, extracellular polymeric substances produced by *Pseudomonas aeruginosa*, *Vibrio harveyi*, and *Staphylococcus aureus* (Papenfort and Bassler, 2016; Skindersoe *et al*., 2021) [51, 58]. At that, the urge to create quorum sensing inhibitors (QSIs) that would not be bactericidal lies strong in this context because it would inhibit bacterial virulence (Maura and Rahme, 2017; Defoirdt, 2018; Kalia *et al*., 2022; Gerdt and Blackwell, 2018) [46, 19, 39, 28].

Even though the QSIs hold a high potency, most of them do not interfere with the host immune responses which form one of the crucial elements of eliminating an infectious pathogen (Roux *et al*., 2020; Bjarnsholt *et al*., 2021; Jiang *et al*., 2019) [53, 13, 34]. During chronic or biofilm-related diseases, the infection of bacteria will be elusive or even depressive in immune signaling (Roux *et al*., 2020; Otto, 2020) [53, 50]. One can enhance innate immune stimulation, in particular, via Toll-like receptors (TLRs), and boost antimicrobial responses, including the phagocytosis process and the production of pro-inflammatory cytokines (Chen *et al*., 2023; Li *et al*., 2021; Zhang *et al*., 2024) [17, 41, 9]. The modulation of immunity can also be very helpful when responding to infections that are moderately or insufficiently immune coordinated (Zhou *et al*., 2022) [34].

Advancing nanotechnology introduces new technologies to take quorum sensing quenching along with stimulation of immune responses. The derivation of NPs can be completed to conduct a reduced and focused licensing of QSIs along with immune ligands (Zhang *et al*., 2021; Wang *et al*., 2022; Ahmad *et al*., 2023; Mohammed, Khaldoon Jasim *et al*., 2024) [62, 59, 37, 46]. These immuno-nanostructures can interact with bacterial and host immune system and can have the potential to block the chat between these two immune systems and at the same time activate antimicrobial host immunity (Wang *et al*., 2023; Liu *et al*., 2023) [60, 18]. It was established that functionalized nanostructures may interfere with QS-regulated phenotypes and reduce the biofilm mass in the cultures *in vitro* and *in vivo* (Ali *et al*., 2022; Khan *et al*., 2021; Ren *et al*., 2019; Basak *et al*., 2024) [5, 37, 52, 12]. In addition to that, nanoparticles that are conjugated with TLR agonist- and/or cytokine inductive molecules have shown potency levels in regard to reprogramming macrophage and dendritic cell activity at high levels (Huang *et al*., 2020; Liu *et al*., 2022; Abdul-Ameer, A. H *et al*., 2024) [32, 42, 1].

Putting the quorum quenching and immune activation trigger into the same system of nanoparticle size would be a powerful dual-target treatment. These systems help overcome bacterial resistances systems, boost the host defense, and suppress pathology with greater efficacy as compared to the interventions of any specific mode (Dutta *et al*., 2023) [22]. Markedly, the immuno-nanostructures could be specific on particular bacteria, and spare the surrounding host tissues (Gao *et al*., 2019; Alwan, Afrah Mehdi *et al*., 2024) [25, 9].

The present research is aimed at the creation of immuno-functionalized nanostructures that not only subvert bacterial QS mechanisms, but also induce innate immune responses. The combination of the two mechanisms is aimed at creating a synergistic, non-antibiotic solution towards control of multidrug-resistant bacterial infection with the help of this strategy.

Objective of the Study

This research undertaking will seek to design and test immuno-functionalized nanostructures that have the capacity to inhibit bacterial quorum sensing signaling routes, as well as boost innate immune response concurrently. The aim is to develop a dual- target therapeutic approach that lowers bacterial virulence, interferes with biofilm- formation and enhances effective host-mediated clearance of the pathogen, especially in the cases of multidrug resistant pathogen infections.

Methods and Materials Study design

The present study was aimed at the development and assessment of immuno- nanostructures specifically directed against bacteria quorum sensing and evasion of the immune system with the primary focus on *Pseudomonas aeruginosa* - a pathogen with clinically relevant opportunistic behavior and quorum-sensing (QS)-regulated virulence and native antibiotic resistance (Gellatly and Hancock, 2019; Azimzadeh *et al*., 2025; Harmine *et al*., 2024; Chen *et al*., 2023) [27, 11, 10, 17].

Materials

Since *P. aeruginosa* played a role in this study, all the experimental/reagent components were chosen with all the considerations of relevance to this organism. *P. aeruginosa* PAO1 (ATCC 15692) was the bacterial strain used where PAO1 was a reference strain commonly used in the study of quorum sensing and biofilm (García-Contreras *et al*., 2021) [26]. Moreover, universal autoinducer-2 activity (AI-2) was determined using *Vibrio harveyi* BB170, a strain of bioluminescent biosensor, as was previously confirmed in the interference of quorum sensing (Papenfort and Bassler, 2016) [51]. Murine RAW 264.7 macrophages and human dendritic cells were added to test the activation of the host immune system against the *P. aeruginosa* because they were conventional models of innate immune system evaluation (Wang *et al*., 2022) [59]. And furanone C-30, gold (III) chloride, PLGA, CpG-ODN, MPLA were all the chemical reagents that were acquired at Sigma-Aldrich and *In vivo* Gen. TNF-9, IL-6 and IL-12 cytokine detection kits were purchased as a kit form BioLegend. The supplied media and all culture materials were devoid of endotoxins to prevent any perturbance of an immune assay, which was among the processes in nanoparticle-based immunology studies (Santosaningsih *et al*., 2024) [57].

Nanostructures synthesis

Owing to the strong biofilm formation and resistance capabilities of *P. aeruginosa* that were made possible due to quorum sensing, nanoparticle-based therapy that enables direct delivery of quorum sensing-inhibitors to the bacterial microenvironment was developed. The synthesis process of gold nanoparticles through the citrate reduction method and the preparation process of PLGA nanoparticles through nanoprecipitation method were both the commonly used synthesis routes of biofunctional nanocarriers, which were standard and repeatable (Mahmoudi *et al*., 2021; Gong *et al*., 2024) [44, 29]. To restrict the quorum sensing circuits of *P. aeruginosa*, the nanoparticles were surface conjugated with furanone C-30 (Saleh *et al*., 2019) [56], and with CpG or MPLA ligands to activate innate immune cells

(macrophages and dendritic cell) (Huang *et al*., 2020; Li *et al*., 2021) [32, 41]. The resulting nanostructures were grouped as NP-QSI (quorum sensing inhibitor only), NP-IMM (immune stimulant only) and NP-DUAL (both agents), based of frameworks employed within dual-function therapeutic systems (Chen *et al*., 2023) [17].

Nanostructures characterization

The biofilms formed by *P. aeruginosa* were extremely dense and have a high level of extracellular polymeric substance (EPS) making accurate nanoparticle size and surface characteristics necessary. The measurement of hydrodynamic diameter and zeta potential was carried out using dynamic light scattering (DLS), which allowed to achieve the best advancement into *P. aeruginosa* clusters (Donkor *et al*., 2023) [21]. Morphology of the core was confirmed by transmission electron microscopy (TEM). The success of conjugation of the ligands was confirmed in FTIR and XPS, which was advisable in the process of nanoparticle surface validation (Xu *et al*., 2021) [62]. The amount of drug that could be loaded on it and the subsequent release rate was designed to fit the slow growth and acidic microenvironment conditions installed by *P. aeruginosa* biofilms (Saba *et al*., 2025) [55].

Inhibition of quorum sensing assessment

Either to directly assess interference with *P. aeruginosa* quorum sensing, several assays were used. To screen general AI-2 inhibition, *V. harveyi* BB170 was the first to be screened because it can produce interspecies quorum-sensing signals (Whiteley *et al*., 2017) [61]. Second, the production of such an essential QS-regulated virulence factor of *P. aeruginosa* as pyocyanin spectrophotometrically was measured 24 hours following NP formulations treatment, in accordance with the validated *P. aeruginosa* virulence assays (Harmine *et al*., 2024; Alyousef *et al*., 2024) [30, 10]. In the end, the biofilm colony forming of *P. aeruginosa* was determined by tagging it with crystal violet stains and the resultant quantification as an index of the QS-based community structure and durability (Azimzadeh *et al*., 2025; Abdel-Fatah *et al*., 2024) [11, 2].

Immunomodulatory assessment

Since *P. aeruginosa* was known to elude the phagocytic clearance by QS-mediated processes, the outcome concerning the effect on the innate immune activation by immuno-nanostructures was established *in vitro*. The nanoparticle formulations were exposed to RAW 264.7 and dendritic cells. Culture supernatants were used to test ELISA in order to determine the concentration of TNF-alpha, IL-6, and IL-12, the most significant macrophage activation markers (Wang *et al*., 2022; Chen *et al*., 2023) [59, 17]. To see whether the immune ligands could endow and boost the bactericidal effects of macrophages, phagocytosis of FITC-labeled *P. aeruginosa* by macrophages was examined by the help of flow cytometry, a well-utilized strategy in immuno-nanoparticle research (Gong *et al*., 2024) [29]. Moreover, via RT-qPCR, the mRNA levels of pro- inflammatory genes (Il6, Tnf, Nos2) have been determined as a certain indication of the activation at the transcriptional level, according to the existing immunogenomic protocols (Li *et al*., 2023).

***In vivo* model of infection**

The study used 24 mice of the same BALB/c strainer (6 mice each in the control, NP-QSI, NP-IMM and NP-DUAL). The sample size was calculated on the basis of the past similar studies and a priori power analysis with use of G*Power computer program (effect size = 0.9, alpha = 0.05, power = 0.8), and was assumed to confer adequate statistical power to identify the difference between expected bacterial burden and inflammatory response.

A murine subcutaneous infection model was used as a model to demonstrate *P. aeruginosa* skin infection to test the efficacy of therapy. *P. aeruginosa* inoculation of mice was carried out by injection of 1 10 7 CFU of *P. aeruginosa* in PBS. The rationale behind this model selection was based on the known ability to form biofilm-like abscesses on the soft tissue of the bacterium and its clinical significance relevance on wound-related infections (Fazal *et al*., 2022) [23]. Once the infection was established, the three days of treatment with the nanoparticle formulation were conducted intradermally at one node next to the lesion. The medicinal effect on *P. aeruginosa* colonization and host response was evaluated by quantifying bacterial load within infected tissues, systemic cytokine and immune cell tissues infiltration, and histopathological observation of lesion and host immune response structure, which aligns with the prior analysis of insufficient therapeutic nanoparticle systems (Donkor *et al*., 2023; Santosaningsih *et al*., 2024) [21, 57].

Ethical statement

Any animal experiments were carried out in accordance with the internationally recognized ethical guidelines and administrative ethics of those who use laboratory animals. Study followed the ARRIVE (Animal Research: Reporting of *In Vivo* Experiments) guidelines. Registration of specific procedures through formal protocol was not numbered, and all the processes were examined internally and performed under ethical supervision to reduce the number of uncomfortable animals.

Statistical analysis

All quantitative experiments have been made with biological and technical replications. The outcome was presented as mean values and standard deviation. One-way ANOVA and Tukey post hoc test were used to analyze the differences between the intergroups. Therefore, p-value of less than 0.05 was regarded significant. Statistical analysis of data was performed in GraphPad Prism (v9.0): a widely used statistical software in the field of biomedical nanotechnology (Mahmoudi *et al*., 2021; Alrubaie, M. F. K., & Khafaji, S. S 2025) [44, 6].

Results

A physical description of the nanoparticles synthesized indicated that all the formulations fell in the nanoscale measurements. The average diameter of NP-QSI formulation was

85.4 nm and of NP-IMM was a bit bigger and was 90.2 nm. The dimension of DUAL- functionalized NP-DUAL was 92.8 nm indicating that both quorum sensing inhibitors and immune ligands were on its surface. Nanoparticles had negative surface charge with zeta potential of −19.8 into -22.5, according to chemical formula NP-IMM and NP-QSI, respectively, contributing to colloidal stability and compatibility in biology. Table 1.

Table 1: Size of nanoparticles and zeta potential

Formulation	Size (nm)	Zeta Potential (mV)
NP-QSI	85.4	-22.5
NP-IMM	90.2	-19.8
NP-DUAL	92.8	-21.3

According to the formulation analysis, there was good encapsulation of active agents. In the case of quorum sensing inhibitor (QSI) loading, NP-QSI reached loading percentages of 68.1 but NP-IMM only reached 59.5 percent loading of the immune ligand. NP-DUAL had equal loading efficiencies between the agents with 65.3 percent and 56.2 percent in the QSI and immune ligand respectively. The release kinetics *in vitro* of 72 hours proved to be sustained and controlled releasing with cumulative release of 78.6, 74.4, and 81.1 percent in NP-QSI, NP-IMM, and NP-DUAL, respectively. Such regulated profile confirms their appropriateness in treating bacterial infections that persist. Table 2

Table 2: Drug loading and release efficiency

Formulation	QSI Loading (%)	Immune Ligand Loading (%)	Cumulative Release (72h, %)
NP-QSI	68.1	0.0	78.6
NP-IMM	0.0	59.5	74.4
NP-DUAL	65.3	56.2	81.1

Bacterial quorum sensing inhibitory activity of the formulations was tested by the *Vibrio harveyi* BB170 bioluminescence assay. The inhibition caused by NP-QSI on the signal activity of AI-2 was found to be 61.7 percent with NP-DUAL having the maximum inhibition of 73.4 percent. NP-IMM that did not contain QSI activity reduced slightly (5.2%) with no significant change compared with the untreated control. Such findings validate the activity of the functional nanostructures in quorum quench and illustrate that dual formulation does not lose and improves this ability. Table 3.

Table 3: AI-2 Quorum sensing inhibition

Formulation	AI-2 Activity Reduction (%)
Control	0.0
NP-QSI	61.7
NP-IMM	5.2
NP-DUAL	73.4

Pyocyanin production was determined after the treatment as it was one of the central virulence factors of *P. aeruginosa*. The control untreated gave a pyocyanin level of 21.8 0g/mL. It was lowered to 9.4 ug/mL with an NP-QSI, which means that it was lowered by 56.9 percent. The effect of NP-IMM was insignificant (20.5 20.5 -19.4), and NP- DUAL recorded the greatest inhibition and brought down the pyocyanin to 6.8 6.8 6.8 3.7, which translated to 68.8% of inhibition. Such data strengthens the anti-virulence position of the quorum sensing inhibition and the better performance of the two-pronged technique. Table 4.

Table 4: Suppression of pyocyanin produce

Formulation	Pyocyanin Level (µg/mL)	% Reduction
Control	21.8	0.0
NP-QSI	9.4	56.9
NP-IMM	20.5	5.9
NP-DUAL	6.8	68.8

The growth of biofilm by *P. aeruginosa* was quantified through staining with crystal violet staining. Optical density of the control group reading at 570 nm was deemed at a mean of 1.25. The NP-QSI also reduced biofilm formation to a great extent where biomass was 0.61 (51.2 % reduction). Np - immm inhibited it in poor measure (6.4 percent), whereas the np-dual inhibited the formation of biofilm to 0.42 OD, which was a 66.4 percent decrease. The statistics indicate that the disruption of quorum sensing has a direct connection to biofilm inhibition, and this influence was greatest in the context of immune activation. Table 5.

Table 5: Knowledge biofilm biomass quantification

Formulation	OD_{570}	% Inhibition
Control	1.25	0.0
NP-QSI	0.61	51.2
NP-IMM	1.17	6.4
NP-DUAL	0.42	66.4

RAW264.7 macrophages incubated with NP formulations were evaluated in cytokine release. Immunological reaction to NP-IMM and NP-DUAL was strong compared do NP-QSI and control. The treatment of NP-IMM produced elevated concentrations of TNF-alpha (148 pg/mL), IL-6 (122 pg/mL) and IL-12 (97 pg/mL). NP-DUAL subsequently raised the levels to 165, 139 and 108 pg/ml respectively. By contrast, NP- QSI caused only modest elevation of cytokines, and the control indicated a baseline level. Such data proves the immune stimulatory potential of the ligand-decorated nanostructures, particularly in the dual-target format. Table 6.

Table 6: Macrophage cytokine levels (pg / mL)

Formulation	TNF-α	IL-6	IL-12
Control	45	37	18
NP-QSI	58	41	20
NP-IMM	148	122	97
NP-DUAL	165	139	108

Post-treatment phagocytic capacity of macrophage with FITC-labeled *P. aeruginosa* was observed. The phagocytic index of the control group was noted to be 34.5% where NP- QSI had minimal effect (36.1%). Conversely, NP-IMM increased phagocytosis by a significant amount to 72.4%. The greatest index was recorded with NP-DUAL at 81.3, which means that the combination of immune stimulation and QS inhibition not only suppress bacterial communication but also induces active immune clearance to take place. Table 7.

Table 7: Phagocytic Index of RAW264.7 macrophages

Formulation	Phagocytic Index (%)
Control	34.5
NP-QSI	36.1
NP-IMM	72.4
NP-DUAL	81.3

Bacterial burden in infected murine skin tissues were examined after being treated. The control group had a mean level of bacteria at 8.7 10 7 CFU/m. This was minimized by 60.9 percent in NP-QSI to 3.4 10 7 CFU/g. NP-IMM recorded a humbler decrease
(25.3%), decreasing the numbers to 6.5 10 7 CFU/g. A 78.2% decrease in bacterial load to 1.9x10 7 CFU/g was found with the highest therapeutic effect given by NP-DUAL. This data confirms the synergistic approach of quorum-sensing interference in combination with immune modulation in the treatment of *in vivo* infections. Table 8.

Table 8: *In Vivo* bacterial levels

Formulation	Mean CFU/g Tissue	% Reduction
Control	8.7×10^7	0.0
NP-QSI	3.4×10^7	60.9
NP-IMM	6.5×10^7	25.3
NP-DUAL	1.9×10^7	78.2

To determine inflammation, necrosis and neutrophil infiltration histological analysis of sections of infected skin was done on a 0-5 severity-based scale. The inflammation figure in the control group was 4.5, necrosis 3.8 and neutrophil infiltration 4.2. The most moderate improvement was demonstrated by NP-QSI (2.7, 2.3, and 2.4). The impact of NP- IMM was moderate (3.9, 3.5, 3.7), and NP-DUAL proved to be most effective with regard to the histological improvement (1.6 on the scale of inflammation, 1.2 on scale of necrosis, and 1.4 on infiltration). These findings show that dual-targeted therapy not only has an effect of decreasing the bacteria load, but also restricting tissue damage and the pathology of the immune system. Table 9.

Table 9: Histopathological Scoring (0 to 5 scores)

Formulation	Inflammation	Necrosis	Neutrophil Infiltration
Control	4.5	3.8	4.2
NP-QSI	2.7	2.3	2.4
NP-IMM	3.9	3.5	3.7
NP-DUAL	1.6	1.2	1.4

Discussion

This study reveals how the novelty dual-functionalized immuno-nanostructures (NP- DUAL), which already withstand quorum sensing (QS) and biofilm development enormously in *Pseudomonas aeruginosa*, also stimulate host immune systems. Such results could be aligned with the current work on multifunctional nano therapies and could be fitted into the developing series of non-antibiotic antimicrobial strategies. The resultant of the synthesized nanoparticles was 85-93 nm and had rather negative zeta potentials. These physicochemicalities were vital in biofilm penetration and cellular entering. Similar particle dimensions and surface potentials have been linked to an increased contact with the bacterial membrane and biofilm surfaces (Saba *et al.*, 2025) [55]. Compared to silver nanoparticles synthesized with *P. aeruginosa* enzymes, those produced greenly demonstrated the same features and were demonstrated to be stable, non-toxic, and bacterially targetable (Gong *et al*., 2024) [29]. Similarly, glucosamine-coated gold nanoparticles were reported as very stable and good biofilm penetrants in ventilator- associated pneumonia models (Santosaningsih *et al*., 2024) [57]. A similar study done on biogenic silver nanoparticles in another study showed size-dependent antimicrobial activity in both Gram-positive and Gram-negative organisms (Al-Momani *et al*., 2023) [8] demonstrating the consistency in our findings with nanotherapeutic design principles.

One of the most important mechanisms that were targeted in the study was QS inhibition. NP-DUAL inhibited AI-2 activity by more than 70 per cent and greatly inhibited pyocyanin production, a recognized QS-controlled virulence factor. Anti-QS properties similar to those reported by plants have also been relayed with the use of plant-based molecules such as harmine, which reduced the virulence and motility of *P. aeruginosa* (Alyousef *et al*., 2024) [10]. Moreover, zinc oxide nanoparticles that were synthesized by biomass had significant effect to inhibit QS-mediated phenotypes such as elastase and pyocyanin production (Saleh *et al*., 2019) [56]. Sub-MIC levels of Ag nano have been reported as suppressing QS genes (lasI rhlI) and also to decrease the release of toxin. The results concur with these findings and lead to the thought that QS-targeting nanostructures present a suitable solution to deactivating bacterial virulence without facilitating resistance.

The NP-DUAL formulation attained more than 66 per cent inhibition of the biofilm mass of *P. aeruginosa*. That is in line with the findings of Abdel-Fatah *et al*., (2024) [2] where the funicle-produced silver-Carthamus nanoparticles inhibited the formation of biofilm and virulence gene expression. On the same note, another study conducted by Azimzadeh *et al*., (2025) [11] found out that the combination of silver nanoparticles and colistin destroyed the integrity of biofilms in multidrug-resistant *P. aeruginosa*. A high level of biofilm mass inhibition (approximately 60%) following interactions with the surface charge mediated by chitosan-based nanoparticles was also reported in another study (Mahmoudi *et al*., 2021) [44]. The improved antibiofilm activity observed with NP-DUAL could be explained by the fact that, by acting synergistically, the destabilization of the quorum sensing signals in addition to the activation of the immune system can promote the faster elimination of biofilms *in vivo*.

A profile of immune response indicated that NP-DUAL greatly boosted the levels of TNF-alpha, IL-6 and IL-12 and improved the phagocytic rate of macrophage. These findings agree with nanoparticles-based immunotherapies involving the activation of the innate immune system against persistent bacterial infections (Chen *et al*., 2023) [17]. Recently, TLR ligand-functionalized nanocarriers comprising lipids in a form of lipid nanocarriers stimulated the macrophage response and enhanced bacterial clearance (Wang *et al*., 2022) [59]. Gong *et al*., (2024) [29] proved the possibility of repolarizing tumor-associated macrophages with the help of engineered nanoparticles and increasing

their phagocytosis, which could also be applied to antibacterial domains. Moreover, nanogels of CpG oligodeoxynucleotides polymer conjugates were demonstrated to elicit the synthesis of pro-inflammatory cytokines and the resolution of infections (Huang *et al*., 2020) [32]. The overall results validated the immunostimulatory activity undertaken by NP-DUAL and confirmed the advantage of integrating immune-directed components into antimicrobial nanomaterials.

In the murine infection model with a subcutaneous injection with infectious dose, NP- DUAL reduced bacterial burden and increased histopathological scores, compiled of inflammation, necrosis, and neutrophil infiltration significantly. These phenomena were in line with previous observations that nanoparticle-based therapies have more success combating *P. aeruginosa* infection than antibiotics alone (Donkor *et al*., 2023) [21]. As an example, silver nanoparticle synthesized *in vivo* increased the healing of the wound and decreased pro-inflammatory cytokine levels in wounds infected with *P. aeruginosa* (Fazal *et al*., 2022) [23]. Another experiment with polyphenol-coated nanoparticles demonstrated the enhancement of tissue integrity and lowered infiltration of leukocytes into bacterial abscesses, the immune-enhancing effects of NP-DUAL would have the ability to induce histological improvement, which was substantiated by evidence that pro-inflammatory immune activation plays a critical role in recovery of chronic infection.

Conclusion

This study establishes a two-in-one nanotherapeutic approach which was effective to interfere with quorum sensing by bacteria, and promotes the acquisition of cellular innate immunity to the host. The NP-DUAL nanostructures synthesized had a strong attenuation effect on QS- mediated virulence factors i.e., AI-2 signaling and pyocyanin production, and a strong reduction in biofilm biomass. In addition to this they induced a strong activation of macrophages, increased the level of pro-inflammatory cytokines, and enhanced phagocytic clearance. Treatment with NP-DUAL reduced bacterial burden and rescue tissue pathology in a mouse model of infection *in vivo* to a substantial degree. These discoveries support the promise of quorum sensing blockade in tandem with immune-enhancing activity as a non-resistance-inducing intervention with great strength alongside conventional antibiotics. Today, this immuno-nanoplatform is one of the perspectives in the development of the fight against resistant and chronic infections.

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Conflicts of interest

There are no conflicts of interest.

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