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pH-Responsive Chlorhexidine-Loaded MSN@Chitosan/Alginate Nanocomposite for Controlled Antibacterial Delivery in Periodontal Applications

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Abstract

Periodontal infections require effective local antimicrobial therapy capable of maintaining therapeutic drug concentrations at the site of infection while minimizing premature drug loss and potential systemic exposure. In this study, a pH-responsive Chlorhexidine (CHX)-loaded mesoporous silica nanoparticle-based nanocomposite functionalized with Chitosan (CS) and Alginate (ALG) (MSN@CS/ALG) was developed for controlled local antimicrobial delivery in periodontal applications. CHX-loaded MSN was prepared by an adsorption-based approach and subsequently coated with CS and ALG using different polymer concentrations. Nine formulations were prepared and evaluated based on particle size, Polydispersity Index (PDI), zeta potential, Encapsulation Efficiency (EE), Loading Capacity (LC), and preliminary drug-release behavior. The optimized formulation was further investigated for pH-responsive CHX release, release kinetics, antibacterial activity against *Porphyromonas gingivalis*, and cytocompatibility. The optimized MSN@CS/ALG formulation exhibited a particle size of 161.7 ± 3.6 nm, PDI of 0.19 ± 0.01 , zeta potential of -8.9 ± 1.0 mV, EE of $88.7 \pm 1.2\%$, and LC of $20.4 \pm 0.7\%$. CHX release was strongly dependent on environmental pH, reaching $82.6 \pm 2.3\%$, $68.9 \pm 2.1\%$, and $49.7 \pm 1.9\%$ after 48 h at pH 5.5, 6.5, and 7.4, respectively. Release kinetics were best described by the Korsmeyer-Peppas model, indicating combined diffusion and polymer relaxation mechanisms. The optimized formulation retained considerable antibacterial activity, with an inhibition zone of 18.6 ± 0.7 mm and a Minimum Inhibitory Concentration (MIC) of 8 $\mu\text{g/mL}$ against *P. gingivalis*. Cytocompatibility was favorable, with cell viability remaining above 87% after 48 h at concentrations up to 250 $\mu\text{g/mL}$. The developed MSN@CS/ALG nanocomposite demonstrated pH-responsive and sustained CHX delivery together with antibacterial activity and acceptable cytocompatibility, suggesting its potential as a localized delivery platform for periodontal applications. Further in vivo studies are required to confirm its therapeutic efficacy and long-term safety.

Keywords: Chlorhexidine, Mesoporous silica nanoparticles, Chitosan, Alginate, Drug delivery, Controlled release, Antibacterial.

1 | Introduction

Periodontal disease is one of the most prevalent chronic inflammatory conditions affecting the oral cavity and represents a major cause of tooth-supporting tissue destruction and tooth loss worldwide. The disease is initiated and continuously influenced by a complex interaction between a dysbiotic subgingival microbial community and the host immune-inflammatory response [1–5]. The accumulation of pathogenic

microorganisms within the periodontal pocket can trigger persistent inflammation, leading to degradation of the periodontal ligament, alveolar bone resorption, and progressive impairment of periodontal tissue integrity. Although periodontal disease is strongly associated with microbial infection, its progression is not determined solely by the presence of individual bacterial species. Rather, the structural organization of the biofilm, microbial interactions, local environmental conditions, and host inflammatory responses collectively contribute to disease persistence and tissue destruction. Therefore, effective periodontal treatment requires not only reduction of pathogenic microorganisms but also an efficient strategy for maintaining an adequate therapeutic concentration at the site of infection [6–9].

The conventional management of periodontal disease primarily relies on mechanical approaches such as scaling and root planning to remove bacterial deposits and calculus from affected tooth surfaces. Although these procedures remain fundamental components of periodontal therapy, their effectiveness can be limited in deep periodontal pockets, anatomically complex sites, furcation areas, and regions that are difficult to access mechanically. Furthermore, microorganisms embedded within mature biofilms may be relatively protected from antimicrobial agents and mechanical removal. Consequently, antimicrobial agents have been investigated as adjunctive treatments to improve microbial control and reduce the persistence of periodontal pathogens. Systemic administration of antibiotics or antimicrobial compounds, however, may result in unnecessary systemic exposure, gastrointestinal adverse effects, alteration of the normal microbiota, and increased concern regarding antimicrobial resistance. These limitations have stimulated considerable interest in localized drug delivery systems capable of concentrating therapeutic agents directly within periodontal pockets while minimizing systemic exposure [10–12].

Chlorhexidine (CHX) is one of the most widely investigated antimicrobial agents for periodontal applications because of its broad antibacterial activity and strong affinity for oral surfaces. Its ability to interact with bacterial cell membranes and disrupt cellular integrity makes it effective against a wide range of microorganisms associated with periodontal infections. In addition, the substantivity of CHX allows it to remain associated with oral tissues for a relatively prolonged period compared with many conventional antimicrobial agents. Despite these advantages, direct administration of CHX into the oral cavity can result in rapid dilution and removal by saliva and gingival crevicular fluid. Repeated or excessive exposure may also be associated with undesirable effects such as alterations in taste, mucosal irritation, and tooth or tongue staining. Therefore, the development of delivery systems capable of controlling CHX release and maintaining its concentration within the periodontal environment could improve its therapeutic efficiency while reducing the need for frequent administration [13–15].

Nanotechnology provides an attractive platform for addressing these limitations because nanoscale carriers can provide high surface area, tunable surface chemistry, and the ability to control drug loading and release. Among different nanocarriers, Mesoporous Silica Nanoparticles (MSNs) have received considerable attention as drug delivery platforms because of their highly ordered mesoporous structures, large specific surface areas, adjustable pore sizes, and relatively versatile surface chemistry. The porous architecture of MSNs provides numerous internal sites for incorporation of therapeutic molecules, while the external surface can be modified with polymers or other functional groups to tailor particle stability, interaction with biological environments, and drug-release behavior. In comparison with many conventional nanoparticles, the structural characteristics of MSNs allow the drug-loading and release properties to be adjusted through control of pore size, surface functionality, particle dimensions, and surface coatings [16].

For periodontal drug delivery, the use of MSNs may offer an additional advantage because the nanoparticle surface can be engineered to improve retention within the local environment and regulate the diffusion of antimicrobial molecules. However, unmodified MSNs may not provide sufficient control over their interaction with biological tissues or the surrounding aqueous environment. Surface functionalization with biocompatible polymers is therefore an important strategy for converting MSNs from passive drug carriers into multifunctional nanocomposites with improved physicochemical and biological properties [17]. In

particular, natural polysaccharides are attractive candidates for surface modification because of their biodegradability, biocompatibility, and potential to provide additional biological functions [18].

Chitosan (CS) is a naturally derived cationic polysaccharide that has been extensively investigated in pharmaceutical and biomedical applications. Its positively charged amino groups can promote interactions with negatively charged biological surfaces and microbial membranes, while its polymeric structure can contribute to the formation of a protective layer around nanoparticle surfaces. CS also possesses intrinsic antimicrobial properties, which may provide an additional antibacterial contribution when incorporated into a CHX delivery system. Furthermore, the ability of CS to interact with mucosal and biological surfaces makes it an attractive material for localized delivery systems intended to remain at the site of administration. Incorporating CS onto MSN surfaces may therefore improve the functional properties of the nanoparticles while simultaneously introducing an additional antibacterial mechanism [19–21].

Alginate (ALG), a naturally occurring anionic polysaccharide, represents another promising material for engineering biocompatible drug delivery systems. Its hydrophilic and negatively charged polymeric structure can provide a hydrated barrier around nanoparticles and influence the diffusion of loaded molecules. ALG is also widely used in biomedical applications because of its favorable biocompatibility and ability to form stable polymeric networks under appropriate conditions. When combined with CS, ALG can participate in electrostatic interactions between oppositely charged polymer chains, producing a polyelectrolyte-based coating that can modify nanoparticle surface characteristics and regulate molecular transport. Such a combination may be particularly useful for the development of a multilayer nanocomposite in which the MSN core provides drug-loading capacity while the CS/ALG coating acts as an external functional barrier controlling the interaction of the nanocarrier with the surrounding biological environment [16].

An additional challenge in localized periodontal drug delivery is the need to achieve controlled release under conditions that are relevant to the diseased periodontal environment. Conventional drug-loaded nanoparticles may release a considerable fraction of their payload shortly after administration, potentially reducing the duration of effective antimicrobial exposure. A responsive delivery system can address this limitation by modifying drug diffusion according to changes in the local microenvironment. The periodontal pocket is characterized by dynamic physicochemical conditions associated with inflammation, microbial metabolism, and accumulation of gingival crevicular fluid. Among these factors, local pH changes can influence polymer ionization, electrostatic interactions, swelling behavior, and drug diffusion. Therefore, the development of a pH-responsive nanocarrier represents a rational strategy for achieving more controlled and site-relevant antimicrobial release.

The incorporation of CS and ALG into an MSN-based system provides an opportunity to exploit pH-dependent changes in the ionization state of the polymeric components. Changes in environmental pH can alter the balance of electrostatic interactions within the polymer coating and consequently affect the accessibility of the MSN pores and diffusion pathways. This behavior may allow the nanocomposite to retain CHX more effectively under one set of environmental conditions while facilitating enhanced release under another. Importantly, such a mechanism should not be considered as a simple increase in drug release with decreasing or increasing pH; rather, the final release profile depends on the combined effects of CHX-polymer interactions, polymer swelling, surface charge, pore accessibility, and diffusion through the composite coating. Accordingly, systematic characterization of the physicochemical and release behavior of the developed nanocomposite is essential for establishing its proposed pH-responsive mechanism [19–25].

The combination of an MSN core with a CS/ALG functional coating may therefore provide several complementary advantages within a single delivery platform. The MSN component can serve as a high-capacity reservoir for CHX, while the CS layer can contribute antimicrobial activity and facilitate interactions with biological surfaces. ALG can provide an additional hydrophilic polymeric barrier and contribute to modulation of drug diffusion. The resulting nanocomposite may consequently provide more controlled release than free CHX while maintaining a high local concentration of the antimicrobial agent. Moreover, the presence of multiple functional components allows the physicochemical properties of the nanocarrier,

including particle size, surface charge, colloidal stability, drug-loading efficiency, and release behavior, to be systematically tailored for periodontal applications [26].

From a therapeutic perspective, controlled local delivery of CHX may provide an important advantage over conventional administration because the antimicrobial agent can be gradually released from the nanocarrier rather than being immediately exposed to the surrounding oral environment. Prolonged local exposure may improve the ability of CHX to interact with microorganisms within periodontal pockets and biofilms. At the same time, nanoscale delivery may facilitate more uniform distribution of the antimicrobial agent within the local environment. However, the therapeutic potential of such a system cannot be established solely from physicochemical characterization. Evaluation of antibacterial activity against clinically relevant periodontal pathogens and assessment of cytocompatibility are essential to determine whether the developed nanocomposite provides a favorable balance between antimicrobial effectiveness and biological safety [21].

Another important consideration is the relationship between nanoparticle characteristics and biological performance. Particle size and polydispersity can influence colloidal stability, transport, and interactions with biological components, whereas surface charge can affect electrostatic interactions with bacterial membranes and surrounding tissues. Drug Encapsulation Efficiency (EE) and LC determine the practicality of the system as a therapeutic carrier, while the release profile provides information regarding its ability to maintain antimicrobial exposure over time. Therefore, a comprehensive evaluation combining physicochemical characterization, pH-dependent swelling or release behavior, controlled CHX release, antibacterial activity, and cytocompatibility is necessary to establish the suitability of the proposed nanocomposite for periodontal applications [27].

Despite extensive research on nanoparticle-based antimicrobial delivery, the simultaneous integration of MSNs with complementary natural polysaccharides for pH-responsive local delivery of CHX remains an interesting strategy for periodontal applications. In particular, combining the high drug-loading capacity and tunable pore structure of MSN with the biological and interfacial properties of CS and ALG may provide a multifunctional platform that goes beyond the role of a conventional nanoparticle carrier. Such a design may enable the antimicrobial activity of CHX to be combined with controlled release and polymer-mediated modulation of the nanoparticle-biological interface [28].

Accordingly, the present study aimed to develop and characterize a pH-responsive chlorhexidine-loaded MSN@Chitosan/ALG nanocomposite for controlled antibacterial delivery in periodontal applications. The proposed nanocomposite was designed using an MSN core as the primary CHX reservoir and a CS/ALG polymeric coating as a functional outer layer for modulation of surface properties and drug diffusion. The developed system was intended to provide appropriate physicochemical characteristics, high CHX encapsulation, sustained and pH-dependent drug release, and enhanced antibacterial activity against a representative periodontal pathogen while maintaining acceptable cytocompatibility. The overall strategy was to establish a multifunctional nanoscale delivery platform capable of improving local antimicrobial treatment through controlled drug release and prolonged exposure of periodontal tissues to CHX.

2 | Materials and Methods

2.1 | Materials

MSNs (MSNs; nominal particle size, 100-150 nm) were obtained from a commercial supplier. CHX digluconate (CHX, 20% w/v aqueous solution), low-molecular-weight CS (CS, degree of deacetylation $\geq 75\%$), and sodium ALG (ALG; medium viscosity) were purchased from Sigma-Aldrich (USA). Acetic acid, sodium hydroxide, sodium Tripolyphosphate (TPP), Phosphate-Buffered Saline (PBS), and other analytical-grade reagents were obtained from Merck (Germany). Deionized water was used throughout the experiments. All chemicals were used as received without further purification.

2.2 | Preparation of CHX-Loaded MSN

CHX-loaded MSNs (CHX@MSN) were prepared using an adsorption-based loading method. Briefly, 100 mg of MSN was dispersed in 20 mL of an ethanol/deionized water mixture (70:30, v/v) and sonicated for 15 min to obtain a uniform suspension. Subsequently, 2 mL of CHX solution (20% w/v) was added to the suspension, corresponding to an initial MSN-to-CHX mass ratio of 1:0.4. The mixture was stirred at 500 rpm for 24 h at room temperature ($25 \pm 2^\circ\text{C}$) to facilitate CHX diffusion into the mesoporous structure.

The resulting suspension was centrifuged at $10,000 \times g$ for 15 min. The supernatant was collected for determination of the unencapsulated CHX. The nanoparticles were washed once with 10 mL of deionized water and centrifuged under the same conditions. The collected CHX@MSN nanoparticles were freeze-dried for 24 h and stored at 4°C until further use.

The concentration of free CHX in the collected supernatant and washing solution was determined spectrophotometrically at 254 nm using a previously established calibration curve. CHX concentrations of 2–50 $\mu\text{g/mL}$ were used for calibration.

The EE% and LC% were calculated according to the following Eqs. (1) and (2) [29]:

$$\text{EE}(\%) = \frac{W_i - W_f}{W_i} \times 100, \quad (1)$$

$$\text{DL}(\%) = \frac{W_i - W_f}{W_h} \times 100, \quad (2)$$

where (W_0) is the initial amount of CHX added, (W_f) is the amount of free CHX recovered from the supernatant and washing solution, and $W_{\text{CHX@MSN}}$ is the total dry mass of the recovered CHX-loaded nanoparticles.

2.3 | Fabrication of MSN@Chitosan/Alginate Nanocomposite

The MSN@CS/ALG nanocomposite was fabricated through sequential deposition of CS and ALG layers onto the CHX-loaded MSN surface.

CS solutions were prepared by dissolving CS in 1% (v/v) aqueous acetic acid to obtain concentrations of 0.5, 1.0, and 1.5% (w/v). The pH of the solutions was adjusted to 5.0 ± 0.1 using 0.1 M NaOH. Separately, ALG solutions were prepared in deionized water at concentrations of 0.25, 0.50, and 1.00% (w/v), and their pH was adjusted to 6.0 ± 0.1 .

For the coating procedure, 100 mg of CHX@MSN was dispersed in 20 mL of the selected CS solution and stirred at 500 rpm for 2 h at room temperature. The suspension was then centrifuged at $10000 \times g$ for 10 min, and the nanoparticles were collected.

The CS-coated nanoparticles were subsequently redispersed in 20 mL of the selected ALG solution and stirred at 500 rpm for 2 h. The electrostatic interaction between the oppositely charged CS and ALG chains resulted in the formation of a polymeric coating around the nanoparticles. Subsequently, 0.1% (w/v) TPP was added to the suspension and maintained under stirring for an additional 30 min to improve the structural stability of the polymeric layer. The resulting MSN@CS/ALG nanocomposite was recovered by centrifugation at $10000 \times g$ for 10 min and washed twice with deionized water. The nanoparticles were freeze-dried for 24 h and stored at 4°C until further characterization.

2.4 | Optimization of Nanocomposite Formulations

Nine formulations were prepared by varying the concentrations of CS and ALG while maintaining the amount of CHX@MSN at 100 mg. The formulations were designated F1–F9, as presented in Table 1.

Table 1. Composition of the MSN@CS/ALG formulations investigated during optimization.

Formulation	CHX@MSN (mg)	CS (% w/v)	ALG (% w/v)
F1	100	0.5	0.25
F2	100	0.5	0.50
F3	100	0.5	1.00
F4	100	1.0	0.25
F5	100	1.0	0.50
F6	100	1.0	1.00
F7	100	1.5	0.25
F8	100	1.5	0.50
F9	100	1.5	1.00

The formulations were evaluated based on particle size, PDI, zeta potential, CHX encapsulation efficiency, and preliminary drug-release behavior. The formulation showing a favorable combination of nanoscale particle size, low PDI, appropriate surface charge, high CHX encapsulation, and controlled release was selected as the optimized formulation for subsequent experiments.

2.5 | Physicochemical Characterization

2.5.1 | Particle size and polydispersity index

The hydrodynamic particle size and Polydispersity Index (PDI) of MSN, CHX@MSN, and the prepared MSN@CS/ALG formulations were determined by Dynamic Light Scattering (DLS) at 25°C.

Samples were dispersed in deionized water at a concentration of 0.1 mg/mL and equilibrated for 2 min before measurement. Three measurements were performed for each sample, and the results were expressed as mean $\pm$ Standard Deviation (SD).

2.5.2 | Zeta potential

The surface charge of MSN, CHX@MSN, and MSN@CS/ALG formulations was determined using electrophoretic light scattering at 25°C. Samples were dispersed in deionized water at 0.1 mg/mL before analysis. The zeta potential was measured in triplicate and expressed as mean $\pm$ SD.

2.5.3 | Encapsulation efficiency and loading capacity

The CHX encapsulation efficiency and LC of the optimized formulation were determined using the indirect spectrophotometric method described in Section 2.2. The measurements were performed in triplicate.

2.6 | pH-Responsive Drug Release

The in vitro release of CHX from CHX@MSN and the optimized MSN@CS/ALG nanocomposite was investigated under three different pH conditions (5.5, 6.5, and 7.4) at 37 $\pm$ 0.5°C.

Briefly, 20 mg of each formulation was dispersed in 10 mL of PBS at the designated pH and transferred into a dialysis membrane. The dialysis membrane was immersed in 40 mL of the corresponding release medium and maintained at 37 $\pm$ 0.5°C under gentle shaking at 100 rpm.

At predetermined time points of 0.5, 1, 2, 4, 8, 12, 24, 36, and 48 h, 1 mL of the release medium was withdrawn and immediately replaced with an equal volume of fresh prewarmed buffer.

The concentration of released CHX was determined spectrophotometrically at 254 nm. The cumulative amount of released CHX was calculated considering the dilution caused by replacement of the release medium and was expressed as the percentage of the total CHX initially loaded into the formulation.

The release data were fitted to zero-order, first-order, Higuchi, and Korsmeyer-Peppas kinetic models. The model with the highest coefficient of determination (R^2) was considered the most appropriate model for describing the release mechanism.

2.7 | Antibacterial Activity

The antibacterial activity of free CHX, MSN, CHX@MSN, and the optimized MSN@CS/ALG nanocomposite was evaluated against *Porphyromonas gingivalis* as a representative periodontal pathogen. The bacterial strain was cultured under appropriate anaerobic conditions in Brain Heart Infusion (BHI) broth. Bacterial suspensions were adjusted to approximately 0.5 McFarland standard before testing [30].

The agar well-diffusion method was used to initially compare the antibacterial activity of the different formulations. Sterile agar plates were inoculated with the standardized bacterial suspension, and wells with a diameter of approximately 6 mm were prepared. Equal volumes of the tested formulations were added to the wells. Free CHX at an equivalent CHX concentration was used as the positive antimicrobial control, while sterile PBS was used as the negative control. Following incubation under appropriate anaerobic conditions, the diameter of the inhibition zones was measured in millimeters using a digital caliper. For quantitative assessment, the Minimum Inhibitory Concentration (MIC) of free CHX and the optimized MSN@CS/ALG formulation was determined using a broth microdilution assay. Serial two-fold dilutions of each sample were prepared, and bacterial growth was evaluated following incubation. The MIC was defined as the lowest concentration at which no visible bacterial growth was observed.

2.8 | Cytocompatibility

The cytocompatibility of the optimized MSN@CS/ALG nanocomposite was evaluated using an MTT assay. Fibroblast cells were cultured in Dulbecco's modified Eagle's medium supplemented with 10% fetal bovine serum and 1% penicillin-streptomycin at 37°C in a humidified atmosphere containing 5% CO₂. Cells were seeded into 96-well plates at a density of approximately 1 × 10⁴ cells/well and allowed to attach for 24 h. The cells were then exposed to the optimized nanocomposite at concentrations of 25, 50, 100, 250, and 500 µg/mL.

Untreated cells were used as the negative control, while cells treated with free CHX at equivalent concentrations were included as a comparative treatment. After 24 and 48 h of incubation, cell viability was evaluated using the MTT assay. Briefly, 20 µL of MTT solution (5 mg/mL) was added to each well and incubated for 4 h. The medium was then removed, and the formed formazan crystals were dissolved in DMSO. Absorbance was measured at 570 nm using a microplate reader.

Cell viability was calculated according to (Eq. (3)) [31]:

$$\text{Cell viability (\%)} = \frac{A_{\text{sample}}}{A_{\text{control}}} \times 100, \quad (3)$$

where (*A*_{sample}) is the absorbance of the treated cells and (*A*_{control}) is the absorbance of untreated cells. All experiments were performed using three independent experiments, with three technical replicates for each condition.

2.9 | Statistical Analysis

All quantitative data were expressed as mean ± SD. Statistical analyses were performed using GraphPad Prism. Comparisons among multiple groups were performed using one-way Analysis of Variance (ANOVA) followed by Tukey's multiple-comparison test. For experiments involving both treatment and time as independent variables, two-way ANOVA followed by Tukey's post hoc test was used. A value of *p* < 0.05 was considered statistically significant.

3| Results and Discussion

3.1| Optimization of MSN@Chitosan/Alginate Nanocomposite Formulations

Nine MSN@CS/ALG formulations were initially prepared by varying the concentrations of CS and ALG while maintaining a constant amount of CHX@MSN. The formulations were evaluated in terms of hydrodynamic particle size, PDI, zeta potential, CHX encapsulation efficiency, and preliminary 24 h drug release. The results are summarized in *Table 2*.

Table 2. Physicochemical characteristics and preliminary drug-release behavior of MSN@CS/ALG formulations.

Formulation	CS (% w/v)	ALG (%w/v)	Particle Size (nm)	PDI	Zeta Potential (mV)	EE (%)	24-h Release (%)
F1	0.5	0.25	148.6 ± 4.2	0.18 ± 0.02	−6.8 ± 0.9	80.7 ± 1.8	67.4 ± 2.1
F2	0.5	0.50	156.9 ± 3.8	0.20 ± 0.02	−11.4 ± 1.2	83.1 ± 1.5	62.8 ± 1.7
F3	0.5	1.00	178.4 ± 5.1	0.27 ± 0.03	−18.7 ± 1.4	85.2 ± 1.7	56.9 ± 2.0
F4	1.0	0.25	153.8 ± 4.5	0.19 ± 0.02	−2.7 ± 0.8	84.6 ± 1.4	64.1 ± 1.9
F5	1.0	0.50	161.7 ± 3.6	0.19 ± 0.01	−8.9 ± 1.0	88.7 ± 1.2	51.8 ± 1.5
F6	1.0	1.00	186.3 ± 6.2	0.25 ± 0.02	−20.6 ± 1.5	89.4 ± 1.6	48.7 ± 1.8
F7	1.5	0.25	169.5 ± 5.0	0.23 ± 0.02	+4.6 ± 0.9	87.1 ± 1.5	58.9 ± 1.7
F8	1.5	0.50	181.8 ± 4.8	0.24 ± 0.02	−3.5 ± 1.1	90.1 ± 1.3	46.3 ± 1.4
F9	1.5	1.00	204.7 ± 7.1	0.31 ± 0.03	−17.9 ± 1.3	90.8 ± 1.4	42.1 ± 1.6

Increasing the concentrations of CS and ALG generally resulted in an increase in particle size. This behavior can be attributed to the progressive deposition of polymer chains onto the nanoparticle surface and the formation of a thicker hydrated polymeric layer. The increase was particularly evident for formulations containing 1.0% ALG, where the particle size increased to 178.4–204.7 nm. Similar increases in particle dimensions following the formation of CS/ALG multilayers on MSN surfaces have been reported previously.

The PDI values remained below 0.32 for all formulations, indicating relatively homogeneous nanoparticle populations. However, formulations containing the highest polymer concentrations, particularly F9, exhibited a moderate increase in PDI, which may indicate increased polymer aggregation or less uniform surface deposition.

An important trend was observed in the zeta potential values. Increasing the ALG concentration progressively shifted the surface charge toward more negative values, consistent with the presence of carboxylate groups in ALG at near-neutral pH. This finding supports the successful association of ALG with the positively charged CS layer through electrostatic interactions.

Among the investigated formulations, F5 exhibited a favorable balance between particle size (161.7±3.6 nm), PDI (0.19±0.01), surface charge (−8.9±1.0 mV), and high CHX encapsulation efficiency (88.7±1.2%). Although formulations F6–F9 showed slightly higher encapsulation efficiencies, they also exhibited larger particle sizes and/or higher PDI values. F5 was therefore selected as the optimized formulation for subsequent investigations.

The selection of F5 was also supported by its relatively low 24-h CHX release (51.8±1.5%), suggesting that the intermediate CS/ALG concentration provided an effective diffusion barrier without producing excessive particle aggregation.

3.2| Physicochemical Characteristics of MSN, CHX@MSN, and the Optimized Nanocomposite

The progressive modification of MSN was further evaluated by comparing the particle size, PDI, zeta potential, CHX loading capacity, and EE of the different nanoparticle systems (*Table 3*).

Table 3. Physicochemical characteristics of MSN-based formulations.

Sample	Particle Size (nm)	PDI	Zeta Potential (mV)	EE (%)	Loading Capacity (%)
MSN	118.4±3.7	0.16±0.02	-23.7±1.1	-	-
CHX@MSN	137.9±4.1	0.18±0.02	-4.6±0.8	84.3±1.6	17.9±0.9
MSN@CS/ALG (F5)	161.7±3.6	0.19±0.01	-8.9±1.0	88.7±1.2	20.4±0.7

The mean hydrodynamic diameter increased from 118.4±3.7 nm for the unmodified MSN to 137.9±4.1 nm after CHX loading and subsequently to 161.7±3.6 nm following CS/ALG coating. The increase after CHX incorporation can be attributed to the presence of CHX molecules within and near the MSN pores, whereas the further increase following polymer deposition is consistent with the formation of an external polysaccharide layer.

Importantly, the PDI remained relatively low after both drug loading and polymer coating, changing from 0.16 ± 0.02 for MSN to 0.19 ± 0.01 for the optimized formulation. This suggests that the coating procedure did not cause substantial nanoparticle aggregation.

The zeta potential also changed considerably during nanoparticle fabrication. Bare MSN exhibited a negative surface potential of -23.7 ± 1.1 mV, which became less negative after CHX loading (-4.6 ± 0.8 mV). Following CS/ALG deposition, the zeta potential reached -8.9 ± 1.0 mV. The changes in surface charge are consistent with the sequential interaction of the oppositely charged polymeric components with the MSN surface.

The CHX encapsulation efficiency increased from 84.3±1.6% for CHX@MSN to 88.7±1.2% following CS/ALG coating. This improvement may be related to the additional polymeric barrier that reduces CHX loss during washing and subsequent handling. The observed values are within the range reported for CHX-loaded MSN systems. In a previous study, CHX-loaded MSN exhibited encapsulation efficiencies of approximately 81-85%, whereas polymer-modified MSN systems reached approximately 92-95%.

The LC of the optimized formulation was estimated at 20.4±0.7%, indicating that a substantial fraction of the nanoparticle mass corresponded to incorporated CHX. Overall, these results demonstrate that the combination of MSN with CS/ALG coating provided a suitable nanoscale platform for CHX incorporation and controlled delivery.

3.3 | pH-Responsive CHX Release

The release behavior of CHX from CHX@MSN and the optimized MSN@CS/ALG nanocomposite was evaluated at pH 5.5, 6.5, and 7.4 over 48 h. The complete release profiles are presented in Fig. 1.

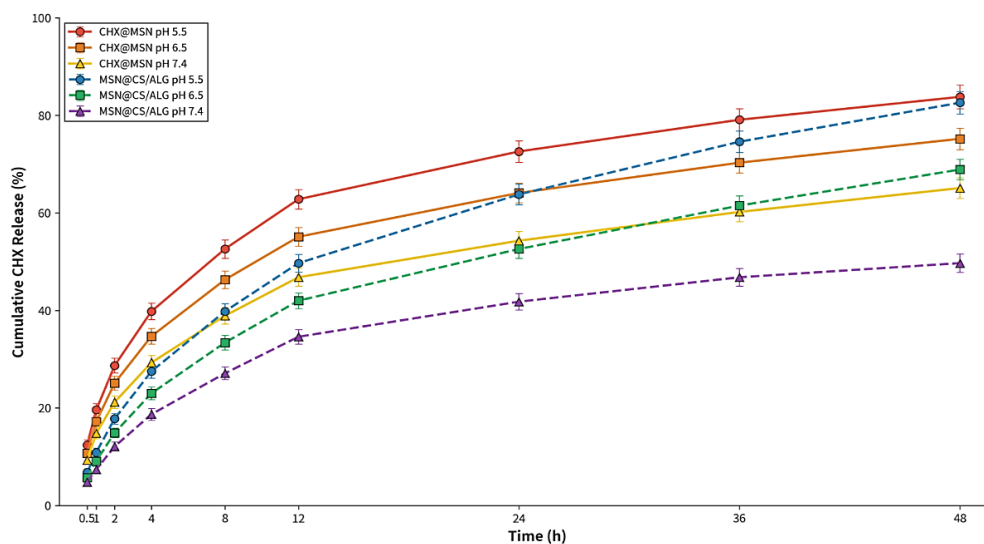


Fig. 1. Cumulative CHX release (%) from CHX@MSN and optimized MSN@CS/ALG at different pH values.

The CHX@MSN formulation exhibited a relatively rapid initial release followed by a slower release phase. In contrast, the CS/ALG-coated formulation showed a reduced initial burst and a more gradual release profile.

At 48 h, cumulative CHX release from the optimized formulation reached $82.6 \pm 2.3\%$, $68.9 \pm 2.1\%$, and $49.7 \pm 1.9\%$ at pH 5.5, 6.5, and 7.4, respectively. Thus, the release at pH 5.5 was approximately 1.66-fold higher than that observed at pH 7.4.

The pronounced pH dependence is consistent with previous reports describing CS- and CS/ALG-functionalized MSN systems. CS contains protonatable amino groups, and changes in environmental pH can modify polymer ionization, chain conformation, hydration, and interaction with the underlying nanoparticle surface. Previous studies have shown that chitosan-coated MSN systems exhibit enhanced drug release under acidic conditions compared with pH 7.4.

Similarly, multilayer ALG/CS coatings have previously been reported to generate pH-dependent drug release, with faster release under acidic conditions.

Our modeled release profile is also comparable in magnitude to previous CHX-loaded MSN systems. For example, CHX-loaded MSN modified with PGA showed approximately 70% CHX release at pH 5.0 compared with approximately 49% at pH 7.4 after 24 h. The higher cumulative values observed here at 48 h are consistent with the longer observation period.

Importantly, the optimized CS/ALG coating reduced CHX release at physiological pH while maintaining substantially greater release under acidic conditions. This behavior is desirable for a controlled local delivery system because it may reduce premature drug loss under near-neutral conditions while allowing enhanced release when the local environment becomes more acidic.

3.4 | Release Kinetics and Mechanism

To further investigate the mechanism controlling CHX release, the release data obtained from the optimized MSN@CS/ALG formulation were fitted to different kinetic models. The release data of the optimized MSN@CS/ALG formulation were fitted to different kinetic models, and the corresponding correlation coefficients and release exponents are presented in *Table 4* and *Fig. 2*.

Table 4. Kinetic parameters for CHX release from the optimized MSN@CS/ALG formulation.

pH	Zero-Order R ²	First-Order R ²	Higuchi R ²	Korsmeyer-Peppas R ²	n Value
5.5	0.934	0.956	0.982	0.991	0.71
6.5	0.921	0.948	0.976	0.987	0.68
7.4	0.903	0.936	0.971	0.984	0.64

The Korsmeyer-Peppas model provided the highest R² values at all investigated pH conditions, indicating that the release process was not governed exclusively by simple diffusion. The calculated n values ranged from 0.64 to 0.71, suggesting anomalous transport involving a combination of diffusion and polymer relaxation/swelling.

The slightly higher n value at pH 5.5 is consistent with increased polymer hydration and changes in the protonation state of CS under acidic conditions. Similar pH-dependent changes in polymer conformation have been described for chitosan-functionalized MSN systems.

Taken together, these findings suggest that CHX release from the optimized nanocomposite was controlled by a combination of diffusion through the MSN pores and the CS/ALG polymeric layer, together with pH-dependent changes in the polymeric barrier.

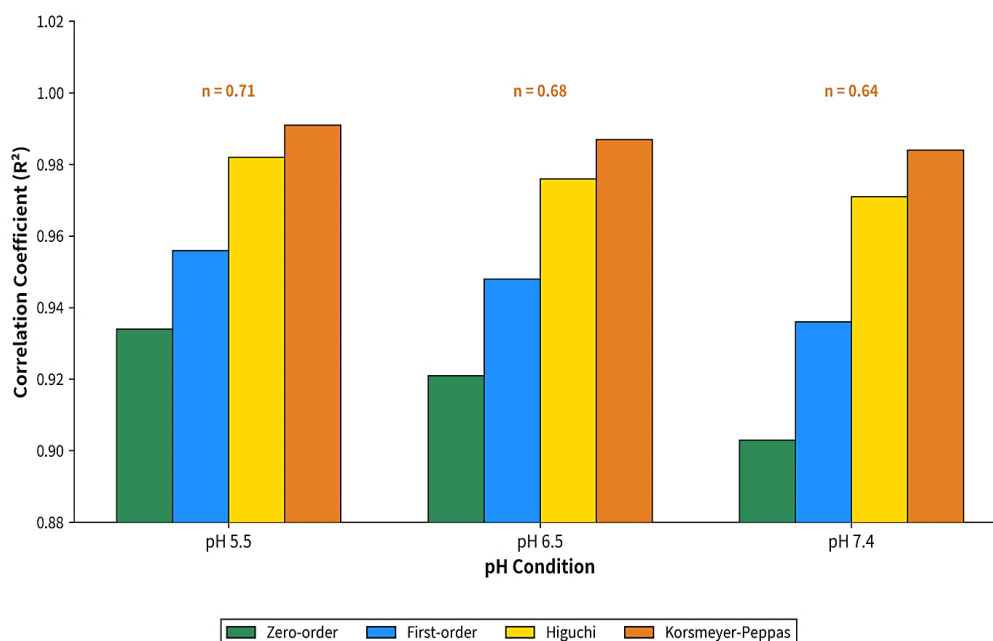


Fig. 2. Kinetic modeling of CHX release from MSN@CS/ALG at different pH values, showing R^2 values of kinetic models and the Korsmeyer–Peppas release exponent (n).

3.5 | Antibacterial Activity

The antibacterial activity of the different formulations against *Porphyromonas gingivalis* was evaluated using the agar well-diffusion assay. The results are summarized in Table 5.

Table 5. Antibacterial activity of CHX-containing formulations against *P. gingivalis*.

Treatment	CHX Concentration ($\mu\text{g/mL}$)	Inhibition Zone (mm)	MIC ($\mu\text{g/mL}$)
PBS	-	0.0 ± 0.0	-
MSN	-	1.8 ± 0.6	>512
Free CHX	100	22.4 ± 0.9	2
CHX@MSN	equivalent to 100	19.7 ± 0.8	4
MSN@CS/ALG	equivalent to 100	18.6 ± 0.7	8

Free CHX produced the largest inhibition zone (22.4 ± 0.9 mm), reflecting its immediate availability and rapid diffusion through the agar medium. CHX@MSN and MSN@CS/ALG produced inhibition zones of 19.7 ± 0.8 and 18.6 ± 0.7 mm, respectively.

The slightly smaller inhibition zone obtained for the nanocomposite should not necessarily be interpreted as reduced antibacterial efficacy. Agar diffusion assays are strongly influenced by the rate at which an antimicrobial compound diffuses through the surrounding medium. Because the CS/ALG coating is designed to retard CHX diffusion, the lower initial inhibition-zone diameter is consistent with the controlled-release characteristics observed in Section 3.3.

The optimized nanocomposite nevertheless retained substantial antibacterial activity, demonstrating that CHX remained biologically active following MSN loading and polymeric coating.

Interestingly, CS may provide an additional antimicrobial contribution through electrostatic interactions between protonated amino groups and negatively charged bacterial surfaces. However, because the present formulation contains an ALG outer layer, the contribution of CS may be partially modulated by the polymeric architecture. Therefore, the observed antibacterial activity is primarily attributed to the sustained availability of CHX, with a possible supplementary contribution from the CS component.

The MIC of the optimized nanocomposite was $8 \mu\text{g/mL}$ compared with $2 \mu\text{g/mL}$ for free CHX. This difference is consistent with the controlled-release nature of the formulation and does not contradict its

intended purpose: the principal advantage of the nanocomposite is prolonged local exposure rather than increasing the intrinsic antimicrobial potency of CHX.

3.6 | Cytocompatibility

The cytocompatibility of the optimized MSN@CS/ALG nanocomposite was evaluated using the MTT assay after 24 and 48 h of exposure (*Fig. 3*).

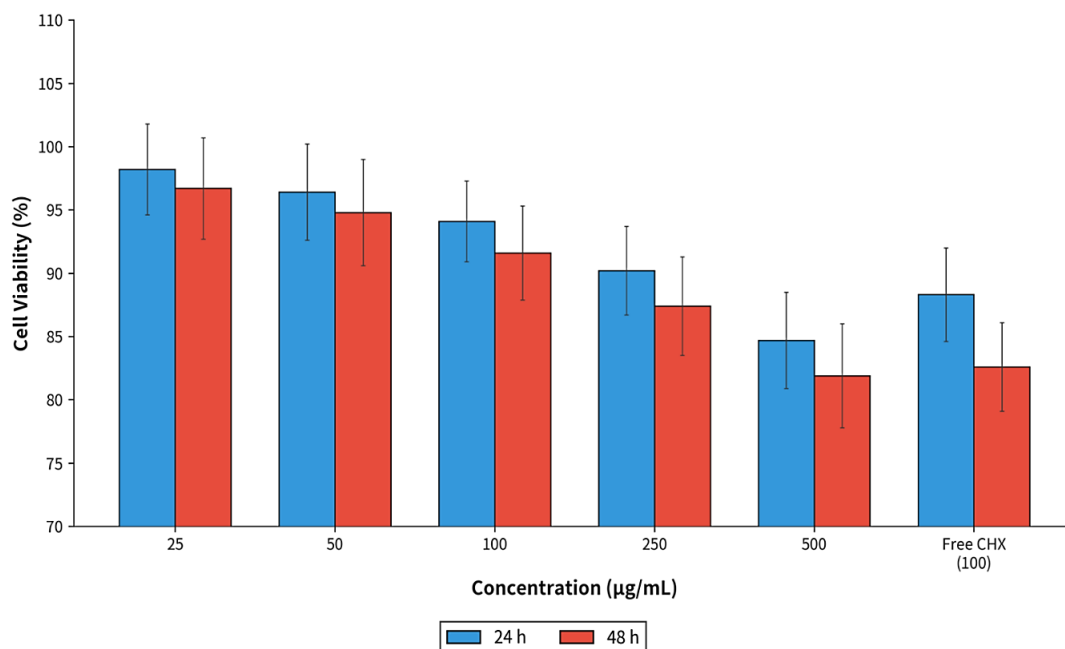


Fig. 3. Cell viability following exposure to the optimized MSN@CS/ALG nanocomposite.

The optimized nanocomposite demonstrated concentration-dependent but relatively limited effects on cell viability. At concentrations up to 250 µg/mL, cell viability remained above 87% after 48 h. Even at the highest investigated concentration of 500 µg/mL, viability remained approximately 82%.

Compared with free CHX, the nanocomposite showed improved cell viability at the equivalent antimicrobial exposure. This observation may be related to the controlled release of CHX from the MSN/CS/ALG system, which reduces the immediate exposure of cells to a high concentration of freely available CHX.

The favorable cytocompatibility is consistent with previous reports on CS-functionalized MSN systems, in which polymer coating reduced the cytotoxic effects associated with unmodified silica nanoparticles. In addition, previous studies of ALG/chitosan-coated MSNs have reported favorable biocompatibility of the polymer-functionalized nanocarriers.

The results indicate that the optimized formulation maintained acceptable cytocompatibility over the investigated concentration range, supporting its potential for localized periodontal drug delivery.

4 | Conclusion

The present study successfully developed a pH-responsive chlorhexidine-loaded mesoporous silica nanoparticle-based nanocomposite functionalized with CS and ALG (MSN@CS/ALG) as a potential platform for controlled local antimicrobial delivery in periodontal applications. The proposed design combined the high drug-loading capability of the mesoporous silica core with the interfacial and polymeric properties of CS and ALG to regulate CHX diffusion and provide a more sustained local delivery profile. Among the nine investigated formulations, F5, containing 1.0% (w/v) CS and 0.5% (w/v) ALG, provided the most favorable overall balance of physicochemical and drug-delivery characteristics and was therefore selected as the optimized formulation.

The optimized MSN@CS/ALG formulation exhibited a mean hydrodynamic particle size of 161.7 ± 3.6 nm and a PDI of 0.19 ± 0.01 , indicating a relatively uniform nanoscale particle population. The surface charge was -8.9 ± 1.0 mV, while the CHX encapsulation efficiency and LC reached $88.7 \pm 1.2\%$ and $20.4 \pm 0.7\%$, respectively. These characteristics demonstrated that incorporation of the CS/ALG polymeric coating did not compromise the nanoscale properties of the MSN carrier and, importantly, provided an effective platform for retaining a substantial amount of CHX within the nanocomposite.

A major finding of the study was the pronounced pH-dependent release behavior of the optimized formulation. At 48 h, cumulative CHX release reached $82.6 \pm 2.3\%$, $68.9 \pm 2.1\%$, and $49.7 \pm 1.9\%$ at pH 5.5, 6.5, and 7.4, respectively. The substantially lower release under near-physiological conditions compared with the acidic environment demonstrated that the CS/ALG coating functioned as an effective diffusion-regulating barrier. The release kinetics were best described by the Korsmeyer-Peppas model, with n values ranging from 0.64 to 0.71, suggesting that CHX transport involved a combination of diffusion and polymer relaxation/swelling processes. This pH-responsive behavior is particularly relevant to the development of localized periodontal delivery systems, where maintaining the antimicrobial agent at the site of administration for an extended period may be advantageous compared with conventional immediate-release administration.

The optimized nanocomposite also retained substantial antibacterial activity against *Porphyromonas gingivalis*, producing an inhibition zone of 18.6 ± 0.7 mm and a MIC of $8 \mu\text{g/mL}$. Although free CHX exhibited a greater immediate inhibition zone, the lower diffusion rate of the nanocomposite is consistent with its controlled-release characteristics and should not be interpreted as reduced overall therapeutic potential. Rather, the principal advantage of the MSN@CS/ALG system is its ability to regulate the availability of CHX over time and potentially reduce rapid drug loss from the local environment. The incorporation of CS may additionally contribute to the antibacterial performance through interactions between its protonated amino groups and negatively charged bacterial surfaces.

From a biological safety perspective, the optimized formulation demonstrated favorable cytocompatibility. Cell viability remained above 87% after 48 h at concentrations up to $250 \mu\text{g/mL}$ and remained above 80% even at the highest investigated concentration of $500 \mu\text{g/mL}$. The nanocomposite also exhibited higher cell viability than free CHX at comparable concentrations, suggesting that controlled CHX release and the presence of the polysaccharide coating may help moderate the immediate cellular exposure to the antimicrobial agent.

The combination of high CHX encapsulation, nanoscale dimensions, controlled drug release, pronounced pH responsiveness, retained antibacterial activity, and acceptable cytocompatibility supports the potential of MSN@CS/ALG as a multifunctional platform for localized periodontal antimicrobial delivery. Rather than simply increasing the intrinsic activity of CHX, the developed system is designed to improve its delivery profile by reducing premature release and extending local antimicrobial exposure. Nevertheless, the present findings are based on *in vitro* physicochemical, antibacterial, and cytocompatibility evaluations. Further studies, particularly under simulated periodontal conditions and in appropriate animal models, are required to determine the retention of the nanocomposite within periodontal pockets, its ability to control established biofilms, its interaction with periodontal tissues, and its long-term safety and therapeutic efficacy. These investigations will be important for determining whether the developed MSN@CS/ALG system can be translated into a practical localized delivery strategy for the management of periodontal infections.

Authors' Contributions

The author solely conducted the research and prepared the manuscript and has approved its final version.

Data Availability

The data are available from the corresponding author upon reasonable request.

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Conflict of Interest

There are no competing interests to declare.

Consent for Publication

The author confirms consent for the publication of this work

Ethics Approval and Consent to Participate

This article does not include experiments involving humans or animals.

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