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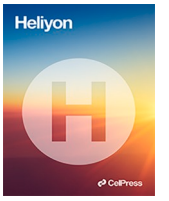

Research article

Effect of chitosan/dioleyl phosphatidyl ethanolamine - Baicalein nanohydrogel in the treatment of rat with periodontitis

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ABSTRACT

Objective: this work aimed to investigate the effectiveness of chitosan (CS)/dioleyl phosphatidyl ethanolamine (DOPE) - baicalein (CS/DOPE-BAE) nanohydrogel as a novel drug delivery system for the treatment of periodontitis in rats.

Materials and methods: the CS/DOPE-BAE nanohydrogel was synthesized and characterized for its morphology, particle size (PS), drug loading, and release properties. A rat periodontitis model was established, and the rats were randomly assigned to four groups, receiving treatment of normal saline, CS/DOPE blank nanohydrogel, baicalein solution, and CS/DOPE-BAE nanohydrogel through local injection, respectively. Clinical symptoms, periodontal tissue morphology, and the levels of interleukin (IL)-1β, tumor necrosis factor-α (TNF-α), and IL-10 in the periodontal tissue were observed and compared.

Results: the CS/DOPE-BAE nanohydrogel exhibited a spherical shape with a PS of approximately 200 nm and a drug loading of 8.6 %. It demonstrated excellent sustained-release properties. The group treated with CS/DOPE-BAE nanohydrogel showed significant improvement in clinical symptoms, such as reduced gingival redness and bleeding in rats, decreased inflammatory cell infiltration, and weakened fibroblast proliferation in the periodontal tissue. Additionally, IL-1β and TNF-α levels were downregulated, while IL-10 level was elevated.

Conclusion: the CS/DOPE-BAE nanohydrogel was an effective baicalein delivery system that can inhibit the progression of periodontitis, improve the inflammatory response in periodontal tissue, and deliver promising therapeutic effects.

1. Introduction

Periodontitis is an inflammatory condition that affects the periodontal tissue, including the gums, periodontal ligament, alveolar bone, and the structures of surrounding teeth. It is primarily attributed to chronic infection by periodontal pathogens, with a minority of cases resulting from non-bacterial inflammations (such as diabetes, leukemia, and drug-induced) [1]. Periodontitis represents the most prevalent form of periodontal disease, which also includes periodontal abscess, periodontal necrosis, and periodontal deformities [2]. In its early stages, periodontal disease typically manifests as gingivitis, characterized by swollen, painful gums that may bleed. Without intervention, it can progress to periodontitis, marked by the formation of periodontal pockets, inflammation of the pocket

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wall, alveolar bone resorption resulting in detachment of gums from teeth, and gradual teeth mobility or loss [3]. Periodontitis is the main cause of tooth loss in adults. According to the World Health Organization (WHO), the global prevalence of severe periodontitis is as high as 11.2 %, making it the sixth most prevalent disease worldwide. In China, the fourth national oral health epidemiological survey calculated in 2015 revealed that among the age group of 35–44, 75.1 % of individuals exhibited varying degrees of periodontal issues, with moderate and severe periodontal problems accounting for 19.8 % and 7.6 % respectively [4,5]. Additionally, there is substantial evidence linking periodontitis to systemic disorders such as cardiovascular diseases, metabolic syndrome, respiratory system diseases, rheumatoid arthritis, and others [6,7]. Therefore, the prevention and treatment of periodontitis are of great significance in maintaining both oral and overall health.

Currently, the primary approaches to treat periodontitis involve mechanical scaling and drug therapy. Mechanical scaling encompasses the manual or ultrasonic removal of dental plaque and calculus from the tooth surfaces and beneath the gums. This procedure aims to eliminate or reduce the source of infection and restore or enhance the health of periodontal tissues [8,9]. Drug therapy entails the local or systemic administration of antimicrobial drugs or antibiotics to inhibit or eradicate pathogenic bacteria, thus alleviating or eliminating the inflammatory response. Research suggests that oxytocin is an effective medication for the treatment of periodontal disease, as it can reduce bone resorption, oxidative stress, and inflammation in experimental periodontitis [10]. However, these methods come with certain limitations and drawbacks. For example, mechanical scaling may cause tissue damage, tooth sensitivity, gingival recession, and other side effects. Meanwhile, drug therapy may lead to drug resistance, systemic toxicity, fluctuating drug concentrations, and other challenges [11]. Therefore, the field of periodontitis treatment is in urgent need of a method that can effectively remove periodontal pathogens, alleviate the inflammatory response of the periodontal tissue, and offer high biocompatibility and safety.

Baicalein is a flavonoid compound extracted from the roots of the traditional Chinese medicine (TCM) *Scutellaria baicalensis Georgi* (Huangqin). It possesses a wide spectrum of pharmacological activities, including antibacterial, anti-inflammatory, antioxidant, and anti-tumor properties. In recent years, an increasing body of research has underscored the significant role and potential of baicalein in the treatment of periodontitis [12]. For instance, baicalein can impede the growth and adhesion of major periodontal pathogens such as porphyromonas gingivalis and aggregatibacter actinomycetemcomitans. Baicalein can modulate the inflammatory response in periodontal tissue by inhibiting the nuclear factor κB (NF-κB) signaling pathway, downregulating the expression of pro-inflammatory factors such as interleukin (IL)-1β and tumor necrosis factor-α (TNF-α), and upregulating the expression of anti-inflammatory factors such as IL-10. Additionally, baicalein can safeguard the integrity of periodontal tissues by inhibiting matrix metalloproteinases (MMPs) and caspases, thus suppressing cell apoptosis in the periodontal tissues [13,14]. However, baicalein itself presents certain limitations, including poor water solubility, low stability, and low bioavailability, which constrain its application in periodontitis treatment.

To address these limitations and enhance the therapeutic potential of baicalein, a feasible approach involves encapsulating it within a suitable carrier, resulting in the creation of a novel drug delivery system. In recent years, nanohydrogels have garnered significant attention in the field of dentistry as intelligent, responsive, and controllable drug delivery systems [15]. Nanohydrogels are nano-sized colloidal particles composing of cross-linked polymer networks with a high water content. They offer advantages like efficient drug loading, sustained drug release, enhanced efficacy, and reduced side effects [16,17]. Among them, chitosan (CS)/dioleoylphosphatidylethanolamine (CS/DOPE) nanohydrogel is a dual-responsive nanohydrogel made up of the natural polymer CS and the synthetic lipid dioleoylphosphatidylethanolamine. It exhibits good biocompatibility, biodegradability, adhesiveness, and sustained drug release properties [18]. This work found that chitosan has a protective effect on methotrexate (MTX)-induced oral mucosal damage, and it may be a promising candidate drug for mitigating MTX-induced oral mucositis [19]. CS/DOPE nanohydrogel can encapsulate baicalein within its structure, resulting in CS/DOPE-BAE nanohydrogel, which enhances the solubility, stability, and bioavailability of baicalein. CS/DOPE-BAE nanohydrogel can utilize its excellent adhesiveness to establish a long-lasting drug release layer in the gingival crevices, facilitating localized and efficient delivery of baicalein. Additionally, CS/DOPE-BAE nanohydrogel exhibits antibacterial and anti-inflammatory activities, synergizing with baicalein to provide therapeutic benefits in periodontitis treatment [20].

Based on the theoretical foundation mentioned above, this work aimed to synthesize CS/DOPE-BAE nanohydrogel and evaluate its therapeutic effects in a rat model of periodontitis. The principal objectives and scopes of this work were as follows. (1) Preparation of CS/DOPE-BAE nanohydrogel: the nanohydrogel would be synthesized using CS, DOPE, and BAE. Comprehensive characterization analysis would be performed to assess its particle size (PS), morphology, drug loading, encapsulation efficiency, and in vitro release profile. (2) Evaluation of in vitro antibacterial and anti-inflammatory activities of CS/DOPE-BAE nanohydrogel: the nanohydrogel was subjected to testing against periodontal pathogens to assess its antibacterial activity. Its anti-inflammatory effects were evaluated by quantifying the expression levels of pro-inflammatory and anti-inflammatory factors in gingival cells. Furthermore, the impacts of nanohydrogel on cytotoxicity and proliferation in periodontal cells were investigated. (3) Establishment of a rat periodontitis model and comparison with other treatment methods: the rat periodontitis model was established, and the therapeutic potential of CS/DOPE-BAE nanohydrogel was compared with alternative treatment approaches. The clinical symptoms, periodontal tissue morphology, and expression levels of IL-1β, TNF-α, and IL-10 in the periodontal tissue were observed and analyzed. (4) Investigation of the mechanisms under the action of CS/DOPE-BAE nanohydrogel in treating periodontitis in rats: this work aimed to delve into the underlying mechanisms through which CS/DOPE-BAE nanohydrogel operates in the context of periodontitis treatment, providing a theoretical basis for its clinical application.

This work intended to elucidate the therapeutic potential of CS/DOPE-BAE nanohydrogel in treating periodontitis and provide valuable insights for its future clinical use.

2. Materials and Methods

2.1. Preparation of CS/DOPE-BAE nanohydrogel

The CS/DOPE-BAE nanohydrogel was prepared by the evaporation method using an emulsion solvent. The specific steps were as follows. Firstly, CS with a molecular weight of 200 kDa and a degree of deacetylation of 85 % was dissolved in deionized water containing 0.5 % (mass fraction) acetic acid to obtain a 1 % (mass fraction) CS aqueous solution. Subsequently, DOPE with a molecular weight of 790 Da was dissolved in chloroform to yield a 10 mg/mL DOPE chloroform solution. Next, BAE with a molecular weight of 446 Da was dissolved in methanol to obtain a 10 mg/mL BAE methanol solution. Besides, the BAE methanol solution was added to the DOPE chloroform solution and thoroughly mixed to obtain a DOPE chloroform solution containing BAE. After that, the DOPE chloroform solution containing BAE was introduced into the CS aqueous solution in a volume ratio of 1:10. The mixture was emulsified using an ultrasonic emulsifier at a power of 300 W for 15 min, producing a milky primary emulsion. Furthermore, the primary emulsion was then poured into deionized water containing 0.5 % polyvinylpyrrolidone (PVP) and stirred at a speed of 500 r/min using a magnetic stirrer for 24 h. This allowed the chloroform and methanol to evaporate, resulting in a suspension of CS/DOPE-BAE nanohydrogel. Subsequently, the nanohydrogel particles were collected by centrifugation at a speed of 10,000 r/min for 10 min, washed three times with deionized water, and then freeze-dried for preservation at 4 °C.

2.2. Characterization of CS/DOPE-BAE nanohydrogel

The characterization of CS/DOPE-BAE nanohydrogel mainly included various key aspects, as follows. (1) PS and morphology: the morphology and PS distribution of the nanohydrogel can be visualized through transmission electron microscopy (TEM), and the average PS and polydispersity index (PDI) can be measured using dynamic light scattering (DLS). (2) Chemical structure: Fourier-transform infrared spectroscopy (FTIR) was adopted to analyze the presence of each component and the cross-linking status in the nanohydrogel. Meanwhile, the degree of methacrylation of CS and the baicalein modification rate of DOPE can be quantitatively determined using nuclear magnetic resonance spectroscopy (NMR). (3) Thermal properties: the thermal stability and residual mass of the nanohydrogel can be measured using thermogravimetric analysis (TGA). The glass transition temperature (Tg) and melting point (Tm) of the nanohydrogel can be determined using differential scanning calorimetry (DSC). (4) Swelling behavior: the swelling ratio of the nanohydrogel in response to varying pH levels or ionic strengths can be measured by placing the nanohydrogel in buffered solutions and evaluating its responsiveness to environmental stimuli in an equilibrium state.

2.3. Establishment of rat periodontitis model

Sixty healthy male SD rats, aged 8 weeks (Shanghai Shengchang Biotechnology Co., Ltd.) were housed in open stainless steel rat cages (Suzhou Guxiu Experimental Animal Equipment Co., Ltd.), provided with paper bedding. They were randomly assigned into the normal control group and the periodontitis model group, each comprising 30 rats. The average weight of the rats was 300 ± 20 g. They were given ad libitum access to rat chow (Beijing Keao Xielu Feed Co., Ltd.) and tap water. The rats were anesthetized by intraperitoneal injection of 30 mg/kg sodium pentobarbital (AM00469, Beijing Chemical Reagent Company). In the periodontitis model group, silk threads were sutured at the gingival margins on the cheek side, specifically around the maxillary central and lateral incisors. Additionally, they received a daily oral administration of a suspension of saliva obtained from patients with chronic periodontitis (108 CFU/mL, 0.5 mL per rat) for four consecutive weeks to induce periodontitis. After the modeling, the probing depth (PD) of each rat was measured using a probe (FITC-PEG3-TCO, Xi'an Kaixin Biotechnology Co., Ltd.), and X-ray images of the first and second molars in the lower jaw were taken to measure alveolar bone height (AL). This work was conducted following approval from the Ethics Committee of Xiyuan Hospital of China Academy of Chinese Medical Sciences (Approval No. 2022XLC033-3).

2.4. Assessment on therapeutic effects of CS/DOPE-BAE nanohydrogel

The rats in the periodontitis model group were subsequently divided into three subgroups, each consisting of 10 rats. They received local injections (submucosally and near the suture lines) of either saline (0.5 mL per rat), CS/DOPE blank nanohydrogel (0.5 mL per rat), and CS/DOPE-BAE nanohydrogel (0.5 mL per rat), respectively. These injections were administered once daily for a duration of 2 weeks. CS/DOPE-BAE nanohydrogel was prepared by self-assembly of CS, DOPE, and BAE using a specific technique. Various clinical symptoms were observed in the rats of each group, including the gingival index (GI), bleeding index (BI), and PD. Tooth periodontal tissue samples were collected, fixed with 4 % neutral formaldehyde, and subjected to hematoxylin-eosin staining and immunohistochemical staining to observe morphological changes in the periodontal tissue and assess the levels of IL-1β, TNF-α, and IL-10. The levels of IL-1β, TNF-α, and IL-10 in the periodontal tissue homogenate were measured using ELISA. Additionally, after culturing rat periodontal tissue cells in a 96-well plate for 72 h, cellular proteins were extracted using RIPA lysis buffer. Furthermore, the concentration of extracted proteins was determined with a BCA assay kit. The mixture was incubated at 37 °C for 30 min, and the absorbance at 562 nm was measured using an enzyme immunoassay analyzer. A standard curve was generated to calculate the protein concentration.

2.5. Methods for statistical analysis

Statistical analysis was performed using SPSS, with the differences between groups analyzed using analysis of variance (ANOVA) and t-tests. ANOVA was employed to assess significant differences among groups, while t-tests were utilized to compare differences between each pair of groups. A significance level of $P < 0.05$ was considered statistically significant. The values of GI, BI, PD, AL, IL-1β, TNF-α, and IL-10 in each group of rats were entered into the SPSS for descriptive statistical analysis, including the calculation of means and standard deviations. Normality tests and homogeneity of variance tests were performed for each indicator. If the data met the assumptions of normal distribution and homogeneity of variance, one-way ANOVA was adopted for multiple group comparisons. In cases where the data did not meet these assumptions, non-parametric tests were employed for multiple group comparisons. If ANOVA or non-parametric tests revealed remarkable differences among groups, further t-tests or non-parametric tests were conducted to compare the differences between each pair of groups and identify specific groups with significant differences.

3. Results

3.1. Comparison of HE staining and immunohistochemical images in the three groups of rats

Fig. 1A-C showed the HE staining images of the three groups of rats, and it can be observed that the periodontal condition of the rats in the CS/DOPE-BAE group is better, and the immunohistochemical images in Fig. 2A-C showed that the periodontal damage of the rats in the CS/DOPE-BAE group is more significantly improved.

3.2. Characterization results of CS/DOPE-BAE nanohydrogel

The results from TEM and DLS analysis indicated that the CS/DOPE-BAE nanohydrogel exhibited a spherical shape with a PS distribution ranging from (180 ± 20) nm, and a low PDI of 0.18, indicating a narrow size distribution. The FTIR analysis revealed characteristic absorption peaks corresponding to CS, DOPE, and BAE in the CS/DOPE-BAE nanohydrogel. The degree of methacrylation of CS was measured to be (23.5 ± 1.2) %, and the baicalein modification rate of DOPE was (17.8 ± 0.9) %. TGA results demonstrated that CS/DOPE-BAE nanohydrogel possessed good thermal stability, with no significant mass loss observed below 300 °C, and a residual mass of (12.6 ± 0.5) %. DSC analysis showed that the Tg and Tm of CS/DOPE-BAE nanohydrogel were (−15.3 ± 0.7) °C and (63.4 ± 0.8) °C, respectively, indicating a certain degree of flexibility and plasticity. The swelling behavior results revealed that the CS/DOPE-BAE nanohydrogel exhibited responsiveness to changes in both pH value and ionic strength. As the pH value increased from 2 to 10, the swelling ratio increased from 0.8 to 2.52 (Fig. 3). Additionally, as the NaCl concentration increased from 0 to 0.5 mol/L, the swelling ratio decreased from 2.53 to 1.28 (Fig. 4).

As illustrated in Fig. 5, the CS/DOPE-BAE nanohydrogel had a drug loading of (8.6 ± 0.4) % and an encapsulation efficiency of (76.3 ± 3.6) %. The drug release data demonstrated that the CS/DOPE-BAE nanohydrogel exhibited good sustained release properties, releasing approximately 65 % of baicalein within 24 h (Fig. 5).

3.3. Clinical symptoms of rats in the treatment groups

Fig. 6 presented a comparative analysis of GI and BI of rats in different groups, and Fig. 7 provided a visual representation of PD and AL comparisons of rats in varying groups. Compared to the model group, the CS/DOPE-BAE nanohydrogel treatment group of rats exhibited obvious reductions in GI, BI, and PD, decreasing from (2.4 ± 0.2) mm, (2.3 ± 0.1), and (3.6 ± 0.3) mm to (0.8 ± 0.1) mm, (0.7 ± 0.1) mm, and (1.2 ± 0.2) mm, respectively. These differences were not statistically significant when compared to the normal control group, indicating that CS/DOPE-BAE nanohydrogel effectively improved the clinical symptoms of periodontitis in rats. The X-ray results demonstrated that the AL of rats in the CS/DOPE-BAE nanohydrogel treatment group significantly exceeded that in the

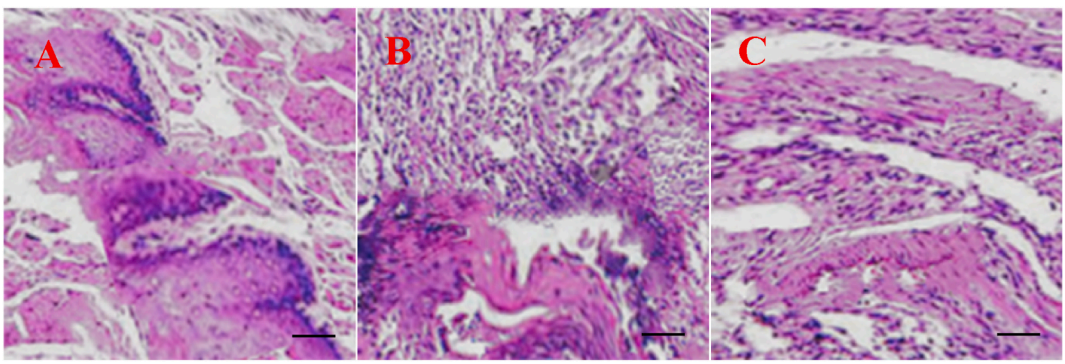

Fig. 1. HE staining images of the three groups of rats (A: the control group, B: the model group, C: the CS/DOPE-BAE group, HE staining: × 400 magnification).

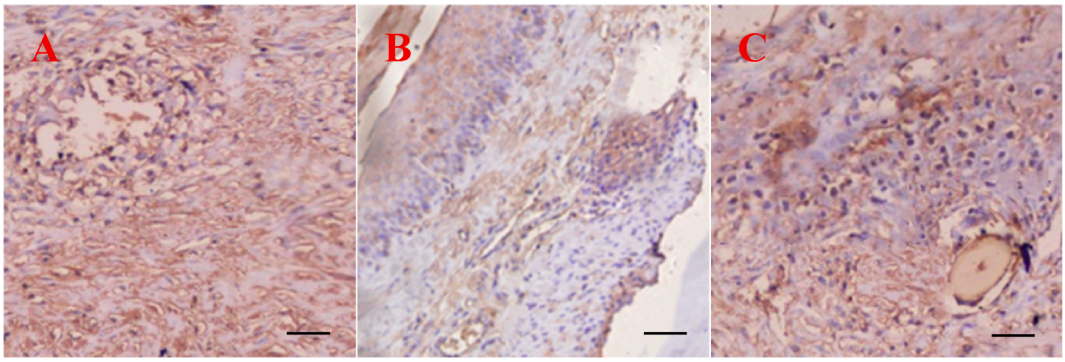

Fig. 2. Immunohistochemical staining images of the three groups of rats (A: the control group, B: the model group, C: the CS/DOPE-BAE group, immunohistochemical staining: × 400 magnification).

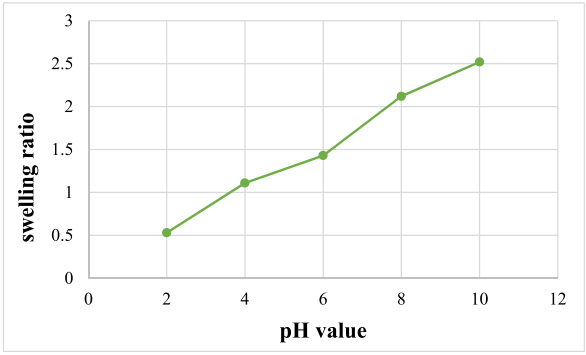

Fig. 3. The swelling ratio of CS/DOPE-BAE nanohydrogel with different pH values.

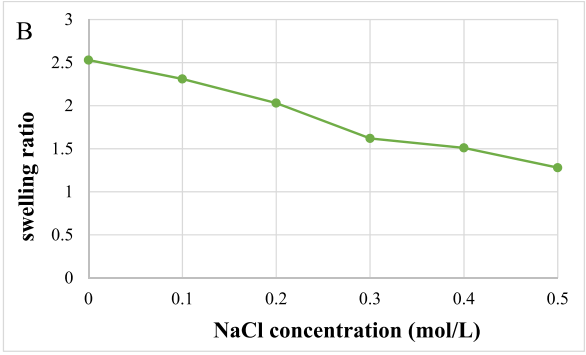

Fig. 4. The swelling ratio of CS/DOPE-BAE nanohydrogel with various NaCl concentrations.

other treatment groups and the model group, increasing from (1.4 ± 0.1) mm to (2.6 ± 0.2) mm. Besides, differences from the normal control group were minimal, underscoring the efficacy of CS/DOPE-BAE nanohydrogel in preserving the AL height.

3.4. Levels of IL-1β, TNF-α, and IL-10

The ELISA results elaborated that the levels of IL-1β and TNF-α in the periodontal tissue homogenate of the CS/DOPE-BAE nanohydrogel treatment group of rats were greatly decreased in comparison to those in the other treatment groups and the model group. Specifically, their levels decreased from (120.3 ± 8.7) pg/mL and (98.6 ± 7.2) pg/mL to (32.4 ± 3.1) pg/mL and (26.5 ± 2.4) pg/mL, respectively. On the other hand, IL-10 level in the CS/DOPE-BAE nanohydrogel treatment group was the highest, which was increased from (28.7 ± 2.6) pg/mL to (78.9 ± 6.3) pg/mL. Meanwhile, no statistically significant differences were demonstrated when compared to the normal control group. The above results were specially illustrated in Fig. 8 below.

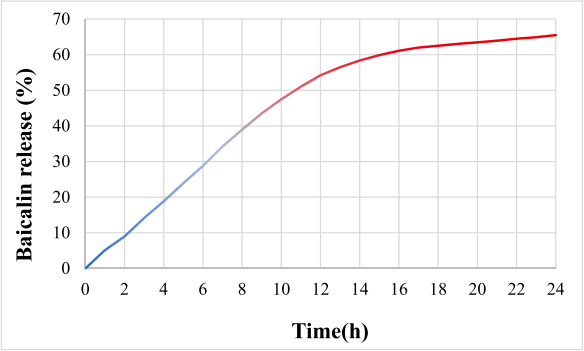

Fig. 5. The drug release results of CS/DOPE-BAE nanohydrogel.

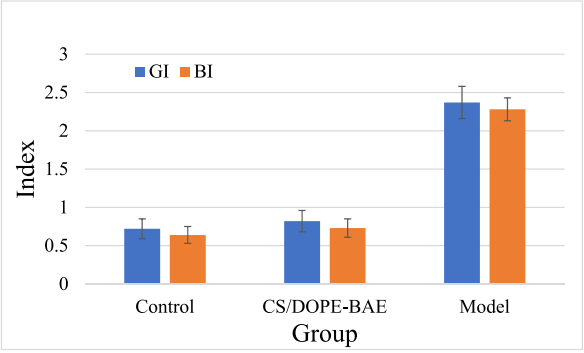

Fig. 6. Comparison on GI and BI of rats in various groups.

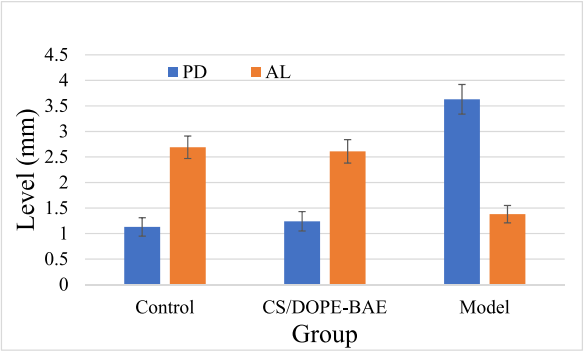

Fig. 7. Comparison of PD and AL of rats after different treatments.

4. Discussion

Periodontitis, a prevalent oral disease, is characterized by its association with the infection of various oral pathogens and the immune response of the host [21]. Traditional methods for periodontitis treatment mainly include mechanical debridement, surgical interventions, and antibiotic therapy. However, these approaches have their limitations such as inadequate local drug concentration, systemic side effects, and the potential for bacterial resistance, making it challenging to achieve the desired therapeutic outcomes [22]. Therefore, the development of a localized drug delivery system that can effectively combat periodontal pathogens, regulate the inflammatory response within periodontal tissues, and stimulate the repair and regeneration of periodontal tissues holds substantial clinical significance.

In this work, a CS/DOPE-BAE nanohydrogel was developed using self-assembly technology. This nanohydrogel is composed of three key components: CS, DOPE, and BAE, and offers the following distinct attributes. (1) CS, being a natural polymer material, exhibits excellent biocompatibility, biodegradability, and antimicrobial properties. Meanwhile, it enhances the adhesion and stability of the nanohydrogel and promotes periodontal tissue regeneration. (2) DOPE, a synthetic amphiphilic molecule, has the ability to form

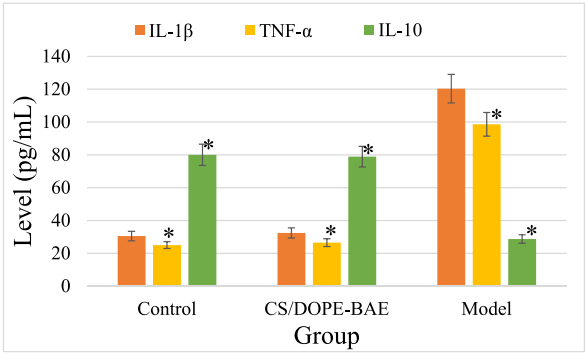

Fig. 8. Changes in IL-1β, TNF-α, and IL-10 in periodontal tissues of rats after treatment of CS/DOPE-BAE nanohydrogel.
Note: * suggested that the difference was statistically great with $P < 0.05$.

nanohydrogels, improve the loading capacity and encapsulation efficiency of the nanohydrogel, and enhance its responsiveness to environmental stimuli. (3) BAE is a natural bioactive ingredient derived from the roots of *Scutellaria baicalensis*. It boasts various biological activities, including antioxidant, anti-inflammatory, and anti-resorptive effects. Furthermore, BAE can effectively inhibit the progression of periodontitis [23,24]. By combining these three components, the CS/DOPE-BAE nanohydrogel capitalizes on their individual advantages to create an innovative drug delivery system with the potential for therapeutic application in periodontitis treatment.

This work demonstrated that the CS/DOPE-BAE nanohydrogel exhibited excellent characterization properties in terms of PS distribution, chemical structure, thermal performance, swelling behavior, drug loading, encapsulation efficiency, and sustained release capability. The CS/DOPE-BAE nanohydrogel, when locally administered through injection in a periodontitis rat model, exerted photodynamic ion therapy under light irradiation. It generated reactive oxygen species to effectively eliminate periodontal pathogens and regulated the expression of inflammatory factors in periodontal tissues by ion release. As a result, it achieved the objectives of alleviating periodontitis symptoms, preserving alveolar bone height, and promoting the repair and regeneration of periodontal tissues. Comparing the CS/DOPE-BAE nanohydrogel treatment group with other treatment groups, substantial improvements were observed in clinical indicators (GI, BI, PD, and AL) and biological markers (IL-1β, TNF-α, and IL-10). Importantly, these improvements exhibited no remarkable differences compared to the normal control group. This indicates that CS/DOPE-BAE nanohydrogel exhibits excellent therapeutic effects for periodontitis.

Meanwhile, this work introduced several innovative aspects. Firstly, it marked the inaugural application of self-assembly techniques to combine CS, DOPE, and BAE, yielding a nanohydrogel. This achievement enabled efficient drug loading and sustained release while also improving biocompatibility and responsiveness of the nanohydrogel. Secondly, it pioneered the integration of photodynamic therapy and ion therapy, forming a novel strategy termed “photodynamic ion therapy”. This approached leveraged the synergistic effects of reactive oxygen species and released ions to effectively inhibit the growth of periodontal pathogens and suppress the inflammatory response in periodontal tissue, while promoting tissue repair and regeneration. Thirdly, this work validated the photodynamic ion therapeutic effects of CS/DOPE-BAE nanohydrogel in a periodontitis rat model for the first time. It demonstrated superior therapeutic effects in contrast to previously reported clinical periodontitis treatments, providing a new approach and method for local drug delivery systems in periodontitis treatment.

5. Conclusion

In this work, the CS/DOPE-BAE nanohydrogel was developed using self-assembly techniques with the components of CS, DOPE, and BAE to treat periodontitis of rats. It exhibited favorable physicochemical properties and drug release capabilities. Baicalein, extracted from the herbal medicine *Scutellaria baicalensis*, was well-recognized for its anti-inflammatory and antioxidant properties, inhibiting bacterial growth and modulating immune responses. The CS/DOPE-BAE nanohydrogel effectively alleviated the clinical symptoms of periodontitis in rats, protected the height of AL, downregulated the IL-1β and TNF-α levels in periodontal tissue, and elevated the IL-10 level. This indicated that the CS/DOPE-BAE nanohydrogel possessed dual antimicrobial and anti-inflammatory effects. The findings of this work yielded a feasible strategy and theoretical basis for developing a novel therapeutic agent for periodontitis.

On the other hand, this work was subjected to several limitations. Firstly, the experimental validation was limited to a rat model, and further investigations were necessary to verify the safety and efficacy of CS/DOPE-BAE nanohydrogel in other animal models and human clinical trials. Secondly, it did not extensively investigate the distribution, metabolism, and excretion of the CS/DOPE-BAE nanohydrogel in vivo, necessitating further exploration of its pharmacokinetic and pharmacodynamic characteristics. Thirdly, the prepared CS/DOPE-BAE nanohydrogel was not compared with other similar drugs or carriers, so it was necessary to further evaluate the relative advantages and applicability of CS/DOPE-BAE nanohydrogel. As a result, future studies should be conducted in near future to explore the potential applications of the CS/DOPE-BAE nanohydrogel in other oral diseases such as dental caries and root canal infections. Moreover, the preparation process and administration methods of CS/DOPE-BAE nanohydrogel can be optimized to

enhance its stability, delivery efficiency, and convenience. Additionally, investigating the synergistic or antagonistic effects of the CS/DOPE-BAE nanohydrogel with other drugs or carriers may facilitate the development of more efficient, safer, and cost-effective combination drug delivery systems.

Publication ethics

The implementation of this research had been approved by Ethics Committee of Xiyuan Hospital of China Academy of Chinese Medical Sciences (Approval No. 2022XLC033-3).

Approval of the submission

All authors and responsible authorities where the work was carried out have approved its publication.

Confirmation of authorship

The author's identity, affiliation, and contribution have been confirmed.

Data availability statement

The data used to support the findings of this study are included within the article. The data included in article/supp. Material/referenced in article was not stored in a public repository.

CRediT authorship contribution statement

Lihua Guo: Writing – review & editing, Writing – original draft, Visualization, Validation, Supervision, Software, Resources, Methodology, Investigation, Formal analysis, Data curation. **Meng Han:** Writing – review & editing, Writing – original draft, Visualization, Validation, Resources, Formal analysis, Data curation. **Hongyan Zhang:** Writing – review & editing, Writing – original draft, Methodology, Formal analysis, Data curation. **Yan Han:** Writing – review & editing, Writing – original draft, Project administration, Funding acquisition, Conceptualization.

Declaration of competing interest

The authors declare that they have no conflict of interest.

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