

Efficacy of chitosan hydrogel as an Adjunct to Scaling and Root Planning for reducing microbial count and inflammatory levels in gingival crevicular fluid of patients with chronic periodontitis: Randomized Clinical Trial

Bassant Ashraf Badr

bhamed@msa.edu.eg

October University of Modern Sciences and Arts

Ahmed Mohamed Aboulyazeed

October University of Modern Sciences and Arts

Ahmed Mohamed Tawfik

October University of Modern Sciences and Arts

Haidy Ehab Mousa

October University of Modern Sciences and Arts

Tasneem Omar Yousef

October University of Modern Sciences and Arts

Mohamed Ahmed Soliman

October University of Modern Sciences and Arts

Moataz Elkholy

October University of Modern Sciences and Arts

Research Article

Keywords: Chronic periodontitis, chitosan hydrogel, Scaling and root planning, Gingival crevicular fluid, local drug delivery

Posted Date: October 6th, 2026

DOI: <https://doi.org/10.21203/rs.3.rs-11026787/v1>

License: © ⓘ This work is licensed under a Creative Commons Attribution 4.0 International License.

[Read Full License](#)

Additional Declarations: No competing interests reported.

Abstract

Nonsurgical periodontal therapy presented in scaling and root planning (SRP) represents the gold standard management of chronic periodontal disease. On the other hand, inaccessible remaining subgingival biofilm and unmodulated host inflammatory response hinder the effectiveness of SRP. Thus, appropriate adjunct therapy should be considered. The natural polysaccharide chitosan has antimicrobial, anti-inflammatory, and biodegradable characteristics that nominated it as adjunct therapy to be chosen for potential efficacy. This randomized clinical trial aimed to evaluate the adjunctive chitosan hydrogel combined with SRP on subgingival microbial load and gingival crevicular fluid (GCF) inflammatory cytokine level over six weeks. A sample of 126 patients diagnosed with chronic periodontitis were equally randomly allocated to two groups; test group (SRP+ adjunct chitosan hydrogel alone, n = 63), and control group (SRP alone, n = 63). Outcomes were evaluated by collecting GCF samples from all participants at baseline and six weeks post-treatment. Colony-forming units per milliliter were used to quantify bacterial load, and ELISA assay was used to measure concentrations of interleukins 17 (IL-17) and 18 (IL-18) concentrations. At six weeks, the test group exhibited significantly greater reductions in microbial count ($1.45 \pm 0.4 \times 10^3$ vs. $3.12 \pm 0.6 \times 10^5$ CFU/mL, $p < 0.001$) and in GCF concentrations of IL-17 (6.1 ± 1.2 vs. 14.2 ± 2.1 pg/ μ L, $p < 0.001$) and IL-18 (74.5 ± 12.4 vs. 105.8 ± 18.2 pg/ μ L, $p < 0.001$) when compared to SRP alone. Intra-pocket chitosan hydrogel application markedly reduced subgingival bacterial load and pro-inflammatory cytokines, indicating its potential as an effective local adjunctive therapy. The experiment was retrospectively registered with the Thai Clinical Trials Registry under the registration number TCTR20260829007 on August 29, 2026.

BACKGROUND

Chronic periodontitis is a common inflammatory disease that involves the destruction of the periodontal ligaments, the alveolar bone and supporting tissues. It is a result of the interaction between the bacteria and the host's inflammatory response, causing periodontal pockets, clinical attachment loss and advanced tooth mobility or loss. Since it the 11th most common disease it causes a concern especially as it has a long-term progressive nature (1).

The etiology of chronic periodontitis is multifactorial. The bacterial biofilm is considered the number one cause. Different types of pathogens are included such as *Porphyromonas gingivalis*, *Tannerella forsythia*, and *Treponema denticola* which are present in the periodontal pocket and cause tissue destruction (2).

The host immune response also has a role in the progression of the disease. An immune and inflammatory reaction is mediated by neutrophils, macrophages, lymphocytes, B-cells, T-cells and other immune cells. These cells release various inflammatory mediators, such as interleukin-1 β , tumor necrosis factor- α , prostaglandin E₂, and matrix metalloproteinases, which contribute to connective tissue degradation and bone resorption. This response is supposed to help with eliminating the infection; however, it causes an exaggerated host response that increases the tissue destruction (3).

SRP is the gold standard non-surgical therapy for chronic periodontitis. SRP is defined as debridement which includes; instrumentation for disruption and removal of microbial biofilms & mineralized deposits (calculus), and root planning to remove “contaminated” cementum and dentin in order to restore the biologic compatibility of periodontally diseased root surfaces. Although effective in improving clinical parameters, SRP has several limitations, including difficulty accessing deep or complex periodontal pockets, furcation areas, and anatomical variations. Residual bacteria may persist in dentinal tubules or within biofilm structures that resist mechanical disruption. Furthermore, SRP alone may not adequately modulate the host’s inflammatory response, highlighting the need for adjunctive therapies, such as antimicrobials, host modulation agents, or biomaterials, to enhance treatment outcomes (4).

This inadequacy leads to the need for accessory antibacterial therapy. Chitosan has been studied in various forms, a split-mouth trial demonstrated that using 1% chitosan nanoparticle gel adjunct to SRP significantly improved clinical outcomes in chronic periodontitis patients (5). The chitosan gel also demonstrated superior antimicrobial efficacy, significantly reducing the counts of key periodontal pathogens *P. gingivalis* and *T. forsythia*, compared to SRP alone, supporting its efficacy as a local drug delivery agent (6).

Chitosan is a natural polysaccharide found in crustacean shells. Because of its biocompatibility, biodegradability, and mucoadhesive properties, chitosan has become a preferred polymer for dental drug-delivery systems. A wide range of chitosan-based dosage forms—such as gels, films, fibers, microparticles, nanoparticles, and sponges has been developed. It can also enhance the transport of both small and large molecules across the oral mucosa (7).

In addition to its pharmaceutical advantages, chitosan exhibits multiple bioactive effects, including antimicrobial, anti-inflammatory, hemostatic, antiviral, antitumor, wound-healing, and immunomodulatory actions. These properties have led to extensive research on its use in managing various dental conditions. Studies conducted both in vitro and in vivo demonstrate that chitosan can modulate inflammatory responses, either alone or in combination with therapeutic agents, making it promising for periodontal therapy (7).

Subjects and methods

Aim:

To evaluate the adjunctive effect of chitosan combined with SRP on microbial load and inflammatory cytokines in patients with periodontitis, comparing outcomes to SRP alone over a 6-week period.

Ethical approval:

The study was reviewed and approved by the Research Ethical Committee of the Faculty of Dentistry of October University for Modern Sciences and Arts, (approval no.: REC-D 11103-6). All procedures were conducted in accordance with the declaration of Helsinki. Prior to participation, written informed

consents was obtained from all subjects following a detailed explanation of the study objectives. The experiment was retrospectively registered with the Thai Clinical Trials Registry under the registration number TCTR20260829007 on August 29, 2026.

Chitosan hydrogel preparation

The chitosan hydrogel was prepared at the Faculty of Pharmacy pharmaceutical laboratories, MSA university according to the method outlined by (8). 100 mg of Chitosan powder (Sigma Aldrich, USA) with MW 190–310 kDa was first dissolved in 10 mL of 1% acetic acid solution by heating it to 60 °C and stirring at 200 rpm until fully dissolved. The resulting chitosan solution pH was adjusted to 6 using sodium hydroxide. Next, 10 mg polyethylene glycol was added to 1 mL of the chitosan solution in a centrifuge tube, and the mixture was treated with ultrasound at room temperature to form a semi-solid gel in 10 minutes.

Sample size calculation:

The minimum required sample size of 63 per group was determined based on the results and methodology of a similar study conducted by (5). Sample size of 63 per group (n=63 for each treatment arm) with 5% margin of error, 80% statistical power, and a 1:1 allocation ratio yielding a total required sample size of 126 patients to ensure adequate power for detecting meaningful differences in microbial reduction and inflammatory mediator levels at 6 weeks post-treatment.

Study population:

A total of 126 patients diagnosed with chronic moderate periodontitis following the Centers for Disease Control and Prevention in partnership with the American Academy of Periodontology

(CDC/AAP) criteria were enrolled and randomized into two groups.

Eligibility criteria:

Patients in the age group 20-60 years diagnosed with chronic periodontitis having probing depths of > 4 mm and clinical attachment level of 3 mm were included while those with a history of pregnancy, lactation, smoking, use of antibiotics or anti-inflammatory drugs within the last six months were excluded (9).

Study design:

The study was conducted as a randomized clinical trial utilizing a longitudinal follow-up (Fig.1). Patients were evaluated at baseline and at 6-week post-treatment interval, gingival crevicular fluid (GCF) was collected from the deepest periodontal pockets using sterilized filter paper strips according to the Wilson protocol.

Randomization procedure and treatment protocol:

Participants were randomly assigned to the case of control group using computer generated sequence. Allocation concealment was maintained using sequentially numbered, opaque, sealed envelopes (SNOSE), which were opened by the clinician only at the time of intervention. The control group received full-mouth SRP using ultrasonic and hand instruments while the case group received SRP followed by the local application of chitosan hydrogel into the periodontal pockets using a delivery syringe till the entire pocket was loaded with the drug. The pocket entrance was covered with the periodontal pocket to retain the material in the pocket. Subjects were recalled after 6 weeks. GCF samples were transported to MSA research lab in appropriately labelled centrifuge vials at -70 C for bacterial culturing. Samples were subsequently thawed, diluted and cultured on agar media (Fig. 1). Laboratory personnel conducting the microbiological culturing and ELISA cytokine assays were fully blinded to group allocations.

Results

A total of 126 patients (63 in the test group and 63 in the control group) completed the study. There were no statistically significant differences between the two groups at baseline regarding demographic characteristics including age and sex distribution ($p > 0.05$) (Table 1).

Demographic Parameter	Control Group (SRP Alone, n=63)	Test Group (SRP + Chitosan, n=63)
Age (years) (Mean $\pm$ SD)	41.2 $\pm$ 6.8	42.5 $\pm$ 7.1
Sex (Male / Female)	32 / 31	30 / 33

Table 1: Baseline Demographic Characteristics of Study Participants

Statistical Analysis:

Statistical analysis was performed using SPSS. Data were first tested for normality using the Shapiro-Wilk test. The independent t-test was employed for normally distributed data. For non-parametric data (microbial CFU counts), the Mann-Whitney U test was used. Changes from baseline to the 6-week follow-up within each group were analyzed using the paired t-test. For all tests, a p-value of < 0.05 was considered statistically significant. All confidence intervals were set at 95%.

Microbiological evaluation:

A visible reduction in bacterial colonies was observed in the SRP + Chitosan group compared to the SRP alone group at 6-week post-treatment (Fig. 2).

Microbial count was measured in colony forming units (CFU/mL). At baseline there was no statistically significant difference in the mean microbial count between the SRP group and the SRP + Chitosan group where $p > 0.05$. At 6 weeks follow up, the microbial count decreased in both groups, but the reduction was significantly more pronounced in the SRP+ Chitosan group compared to the SRP alone group, with a statistically significant difference of $p < 0.001$ (Table 2).

Time interval	SRP alone (Controls)	SRP + Chitosan (Cases)	P value
Baseline	$6.42 \pm 1.2 \times 10^6$	$6.55 \pm 1.1 \times 10^6$	0.814
6 weeks post-treatment	$3.12 \pm 0.6 \times 10^5$	$1.45 \pm 0.4 \times 10^3$	< 0.001

Table 2: Descriptive statistics and comparison of CFU/mL (log 10) at baseline and after 6 weeks in Control and Case group

Immunohistochemical evaluation:

The concentration of pro-inflammatory cytokine in the gingival crevicular fluid was measured using enzyme-linked immunosorbent assay (ELISA). Both groups showed a reduction in inflammatory markers from baseline to the 6-week follow-up. The case group showed a statistically significant reduction in IL-17 & IL-18 levels (Fig. 3).

GCF levels of IL-17 & IL-18 (pg/μL) at baseline and follow-up is shown in Table 3.

Time interval	Inflammatory market	SRP alone (Controls)	SRP+ Chitosan (Cases)	p-value
Baseline	IL-17	19.8 ± 3.1	20.2 ± 2.8	0.654
6 weeks post-treatment		14.2 ± 2.1	6.1 ± 1.2	p < 0.001
Baseline	IL-18	152.5 ± 25.6	155.1 ± 22.3	0.722
6 weeks post-treatment		105.8 ± 18.2	74.5 ± 12.4	p < 0.001

Table 3: Descriptive statistics and comparison of IL-17 & IL-18 (pg/μL) levels at baseline and after 6 weeks in Control and Case group.

No adverse events, localized allergic reactions, mucosal irritation, or postoperative complications were reported by any participants or observed during clinical evaluations in either treatment group throughout the 6-week follow-up period.

Discussion

The present study evaluated the adjunctive effect of chitosan combined with SRP on microbial load and inflammatory cytokines in patients with periodontitis, comparing outcomes to SRP alone over a 6-week period. The significant reduction in both microbial CFU/mL and pro-inflammatory cytokines IL-17 & IL-18 observed in the SRP + Chitosan group relative to the control group underscores the potential benefits of incorporating chitosan as an adjunct in periodontal therapy.

Microbiological findings indicated a marked decrease in bacterial load post-treatment in both groups, confirming the efficacy of SRP in reducing subgingival biofilm. However, the SRP + Chitosan group demonstrated a significantly greater decline in microbial counts ($p < 0.001$), as evidenced by the plate count agar findings showing less dense bacterial colonies with increased inter-colony gaps (Fig. 1, Table 1). This supports the antibacterial properties attributed to chitosan, which has been reported to disrupt bacterial cell walls and inhibit biofilm formation, thereby enhancing microbial clearance when combined with SRP.

The notable reduction in IL-17 & IL-18 levels in the gingival crevicular fluid of the SRP + Chitosan group compared to SRP alone ($p < 0.001$ for both cytokines; Table 2) further suggest a pronounced reduction of the local inflammatory response. IL-17, a pivotal cytokine in neutrophil recruitment and osteoclastic activity, and IL-18, a key inducer of pro-inflammatory cytokines, are both implicated in periodontal tissue destruction. The decreased levels may reflect reduced the consequent downregulation of inflammatory pathways following adjunctive chitosan use. These findings are consistent with literature demonstrating that periodontal therapy can modulate cytokine profiles favorably, reducing levels of pro-inflammatory mediators such as IL-1 β , TNF- α , and IL-6 (10, 11).

Similar to this study's results, interventions such as diode laser therapy and sub antimicrobial dose doxycycline have shown reduction in inflammatory markers and clinical improvements beyond SRP alone (12-14). Interestingly, while some adjuncts like curcumin-loaded nanoparticles and antimicrobial photodynamic therapy did not confer additional advantages over SRP, the present findings suggest that chitosan's antimicrobial and anti-inflammatory properties may offer meaningful synergistic effects (15, 16)

The combines microbiological and immunohistochemical outcomes support a potential mechanism whereby chitosan enhances periodontal healing by not only directly reducing bacterial load but also mitigating the host inflammatory response, which is central to tissue destruction in periodontitis. The observed cytokine reductions could relate to modulation of Th17 cell activity and inflammatory pathways, which are increasingly recognized in the pathogenesis of periodontal disease (10).

Limitations of the present study include the relatively short follow-up duration and the lack of clinical periodontal parameters, which are important for comprehensive evaluation of therapeutic efficacy. Additionally, the study sample size, though sufficient for detecting biochemical changes, may limit generalizability. Longer-term studies with clinical endpoints are necessary to confirm the sustainability of these effects and to elucidate optimal dosing regimens for chitosan adjuncts.

Conclusion

Given its dual antimicrobial and anti-inflammatory mechanism in the periodontal pocket, the adjunctive intra-pocket application of chitosan hydrogel following SRP resulted in superior reductions in subgingival microbial load and gingival crevicular fluid pro-inflammatory cytokine levels compared to SRP alone. Based on these results, chitosan hydrogel seems to be an efficient local adjuvant for non-surgical

periodontal therapy, both from a biological and a clinical perspective. Nevertheless, there are still drawbacks, such as the short duration of the follow-up and the lack of clinical periodontal markers. Additional prospective multicenter trials with extended follow-up periods and clinical outcomes are recommended including probing depth and clinical attachment level, are necessary to validate these findings.

Abbreviations

SRP: Scaling and root planning

GCF: gingival crevicular fluid

CDC/AAP: Centers for Disease Control and Prevention in partnership with the American Academy of Periodontology

CFU: colony forming units

Declarations

Human Ethics and consent to participate

The study was reviewed and approved by the Research Ethical Committee of the Faculty of Dentistry of October University for Modern Sciences and Arts, (approval no.: REC-D 11103-6). All procedures were conducted in accordance with the declaration of Helsinki. Prior to participation, written informed consents was obtained from all subjects following a detailed explanation of the study objectives.

Consent for publication

Not applicable.

Availability of data and materials

The data generated and analyzed in this study are available upon reasonable request from the corresponding author.

Competing interests

The authors state that they have no apparent financial conflicts or personal ties that possibly seemed to affect the research described in this study.

Funding

This research did not receive any particular support from governmental, corporate, or non-profit agencies.

Authors' contributions

- BA: Study idea, Supervision, Manuscript drafting and guidance in research study
- AA: Data collection, data analysis, data interpretation and reference management
- AT: Data collection, data analysis, data interpretation.
- HE: Data collection, data analysis, data interpretation.
- TO: Data collection, data analysis, data interpretation.
- MS: Data collection, data analysis, data interpretation.
- ME: Supervision, funding and guidance in research study

Acknowledgements

Not applicable

References

1. Nazir M, Al-Ansari A, Al-Khalifa K, Alhareky M, Gaffar B, Almas K. Global Prevalence of Periodontal Disease and Lack of Its Surveillance. *ScientificWorldJournal*. 2020;2020:2146160.
2. Dahlen G, Basic A, Bylund J. Importance of Virulence Factors for the Persistence of Oral Bacteria in the Inflamed Gingival Crevice and in the Pathogenesis of Periodontal Disease. *J Clin Med*. 2019;8(9).
3. Bezerra B, Monajemzadeh S, Silva D, Pirih FQ. Modulating the Immune Response in Periodontitis. *Frontiers in Dental Medicine*. 2022;Volume 3 - 2022.
4. Fabbri C. Clinical effects of adjunctive therapeutic devices in non-surgical treatment of periodontitis patients. 2021.
5. Mallikarjun S. Evaluation of clinical and antimicrobial efficacy of chitosan nanoparticle gel (1%) as a local drug delivery in chronic periodontitis patients- a randomised controlled split-mouth trial. 2025.
6. Jalaluddin M, Kulkarni A, Raj K, Kumari D, Devi KB, Vineeth NS. Assessment of Antimicrobial Efficacy of Chitosan-Based Tetracycline Gel on Periodontal Pathogens: An In vitro Study. *Journal of Pharmacy and Bioallied Sciences*. 2023;15(Suppl 1).
7. Işılay Özdoğan A, Akca G, Şenel S. Development and in vitro evaluation of chitosan based system for local delivery of atorvastatin for treatment of periodontitis. *Eur J Pharm Sci*. 2018;124:208-16.
8. Zhou X, Liu C, Han Y, Li C, Liu S, Li X, et al. An antibacterial chitosan-based hydrogel as a potential degradable bio-scaffold for alveolar ridge preservation. *RSC Advances*. 2022;12(50):32219-29.
9. Wiench R, Fiegler-Rudol J, Latusek K, Brus-Sawczuk K, Fiegler H, Kasperski J, et al. Indocyanine Green as a Photosensitizer in Periodontitis Treatment: A Systematic Review of Randomized

Controlled Trials. Life. 2025;15(7):1015.

10. Duran-Pinedo AE, Solbiati J, Teles F, Frias-Lopez J. Subgingival host–microbiome metatranscriptomic changes following scaling and root planing in grade II/III periodontitis. *Journal of Clinical Periodontology*. 2023;50(3):316-30.
11. de Lima Oliveira AP, de Faveri M, Gursky LC, Mestnik MJ, Feres M, Haffajee AD, et al.
12. Calderín S, García-Núñez JA, Gómez C. Short-term clinical and osteoimmunological effects of scaling and root planing complemented by simple or repeated laser phototherapy in chronic periodontitis. *Lasers in Medical Science*. 2013;28(1):157-66.
13. Matarese G, Ramaglia L, Cicciù M, Cordasco G, Isola G. The Effects of Diode Laser Therapy as an Adjunct to Scaling and Root Planing in the Treatment of Aggressive Periodontitis: A 1-Year Randomized Controlled Clinical Trial. *Photomed Laser Surg*. 2017;35(12):702-9.
14. Caton JG, Ciancio SG, Blieden TM, Bradshaw M, Crout RJ, Hefti AF, et al. Treatment With Subantimicrobial Dose Doxycycline Improves the Efficacy of Scaling and Root Planing in Patients With Adult Periodontitis. *Journal of Periodontology*. 2000;71(4):521-32.
15. Pérez-Pacheco CG, Fernandes NAR, Primo FL, Tedesco AC, Bellile E, Retamal-Valdes B, et al. Local application of curcumin-loaded nanoparticles as an adjunct to scaling and root planing in periodontitis: Randomized, placebo-controlled, double-blind split-mouth clinical trial. *Clinical Oral Investigations*. 2021;25(5):3217-27.
16. de Melo Soares MS, D'Almeida Borges C, de Mendonça Invernici M, Frantz FG, de Figueiredo LC, de Souza SLS, et al. Antimicrobial photodynamic therapy as adjunct to non-surgical periodontal treatment in smokers: a randomized clinical trial. *Clin Oral Investig*. 2019;23(8):3173-82.

Figures

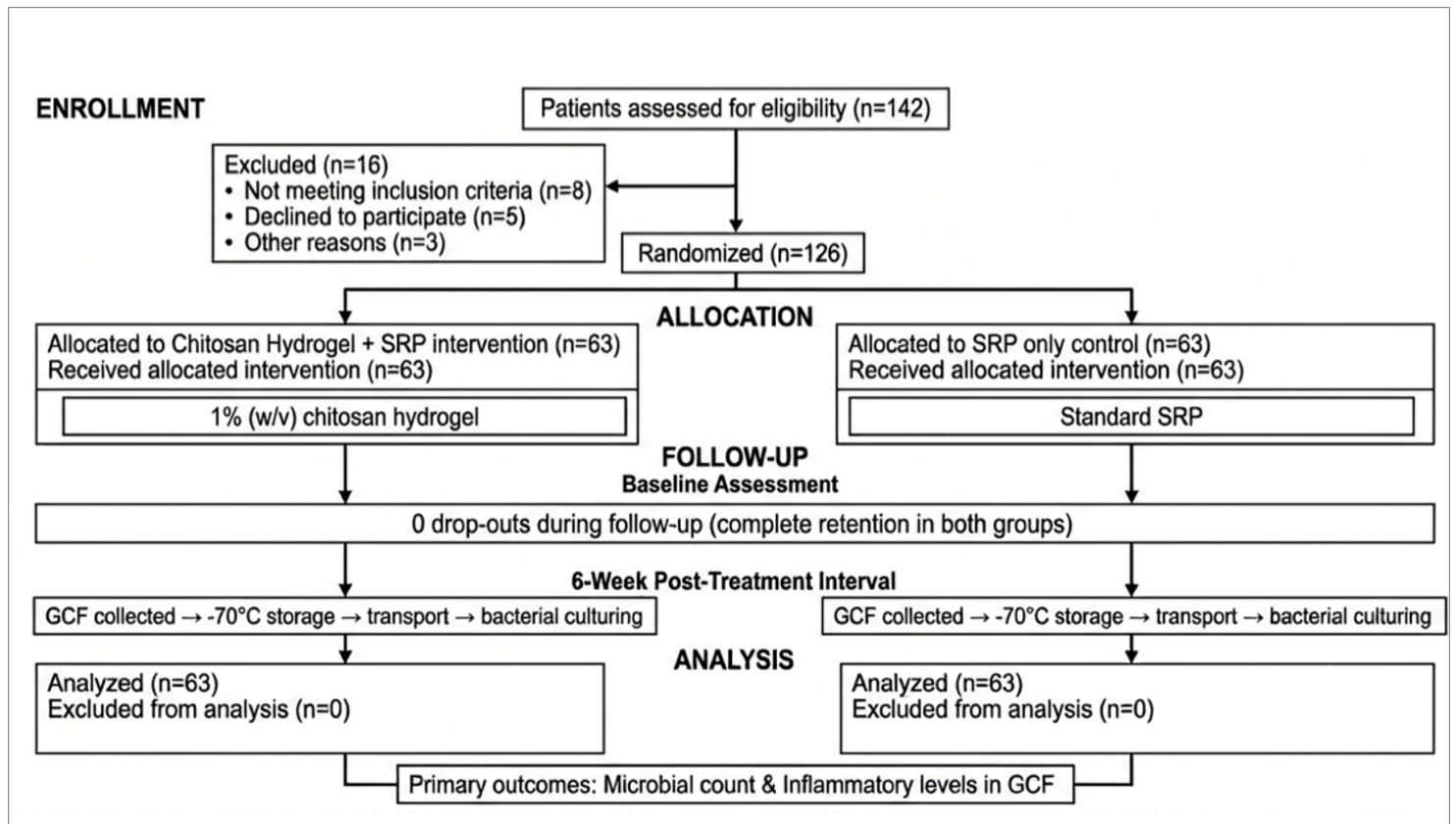


Figure 1

Flowchart for the randomized clinical trial. SRP, scaling & root planning; GCF, gingival crevicular fluid.

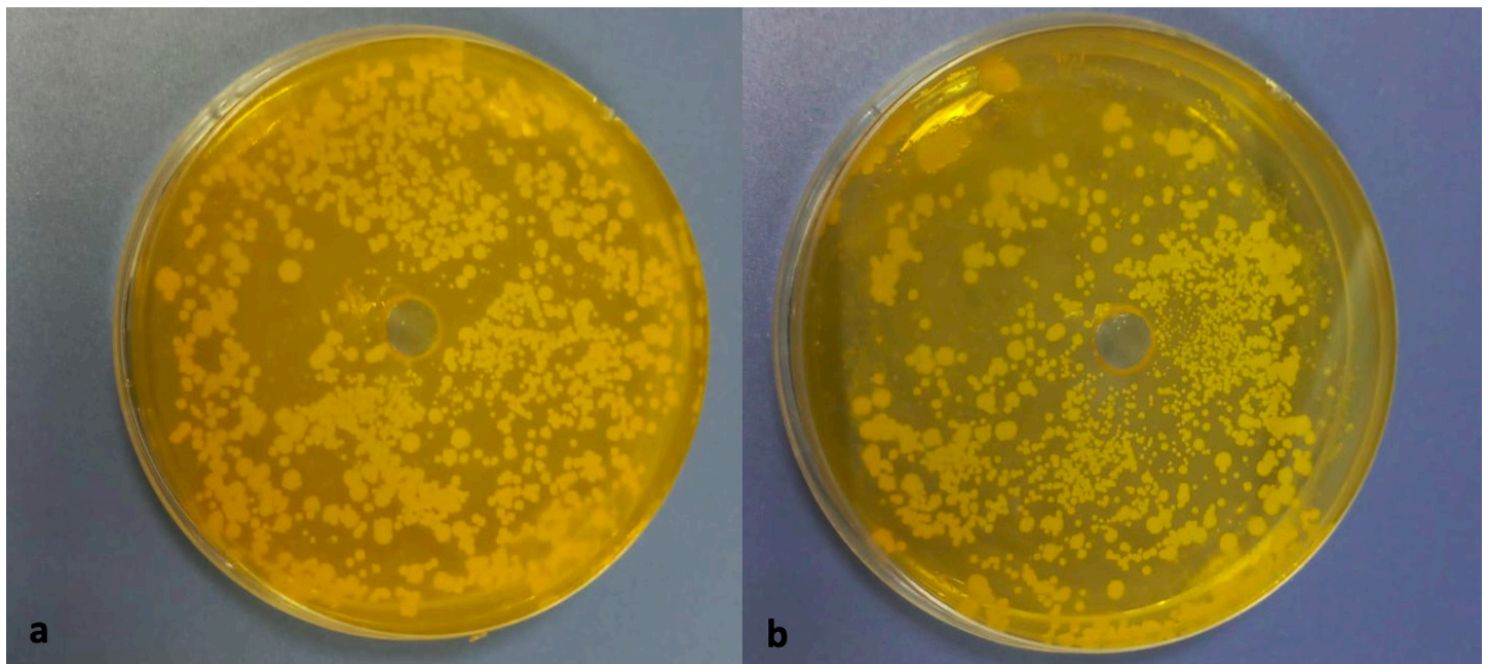


Figure 2

Plate count agar medias showing CFU at 6-week follow-up (a) Control group: displaying high density of bacterial colonies often merged into clusters. (b) Case group: showing a reduction in bacterial colonies with increased gaps between them.

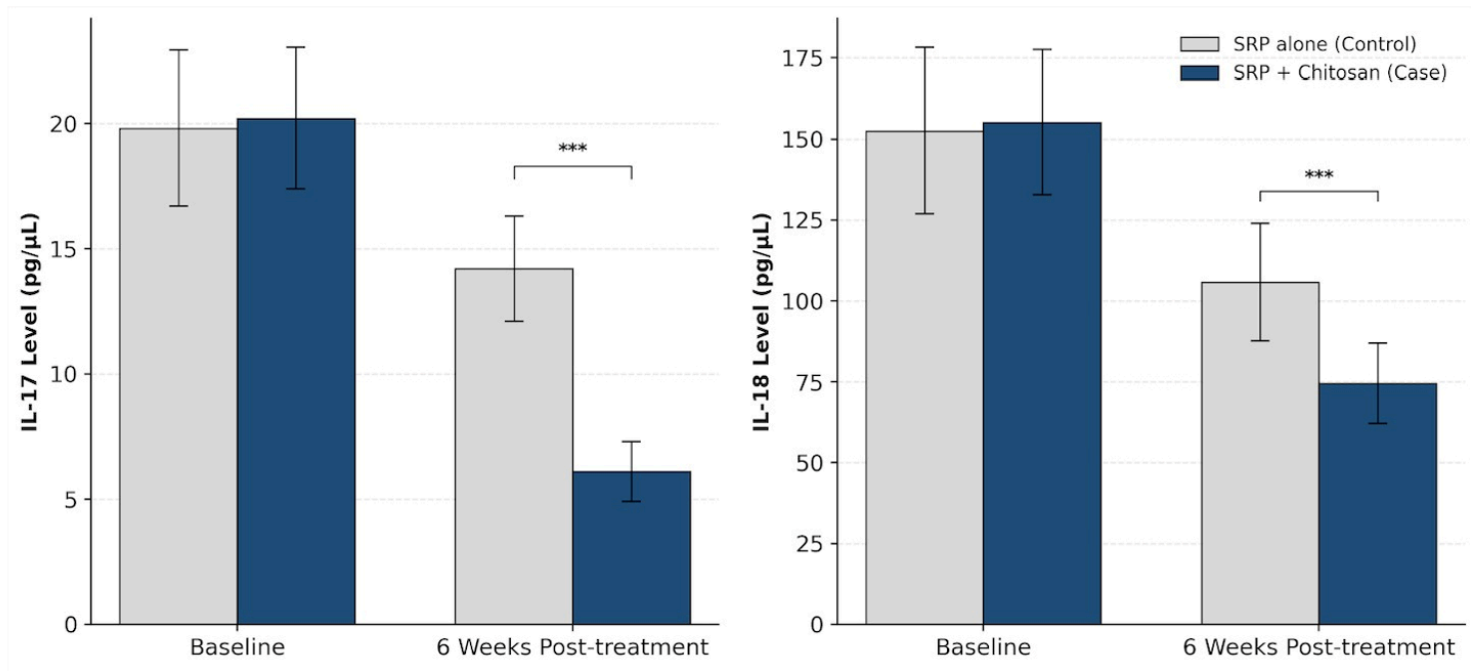


Figure 3

Bar charts comparing mean gingival crevicular fluid (GCF) levels of IL-17 (left) and IL-18 (right) (pg/μL) between the SRP alone (Control) and SRP + Chitosan (Case) groups at baseline and 6 weeks post-treatment. Error bars represent standard deviation; * denotes a statistically significant difference ($p < 0.001$) between groups at 6 weeks.

Supplementary Files

This is a list of supplementary files associated with this preprint. Click to download.

- [CONSORT2010Checklist.docx](#)