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Comparison of Local Drug Delivery Systems in Periodontal Biofilm Management Using an *In vitro* Model

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Abstract **Objectives:** Bacterial biofilms cause most cases of periodontal diseases requiring effective therapeutic approaches. Local drug delivery systems (LDDS) serve as complementary treatment to standard mechanical methods through precise antibacterial activity. The research investigates how different Local DDS fare at controlling periodontal pathogens through an *in vitro* biofilm testing protocol. **Methods:** The biofilm creation process involved Porphyromonas gingivalis and Aggregatibacter actinomycetemcomitans cultivating on hydroxyapatite discs within an *in vitro* environment. A research using three different LDDS involved chitosan-based gel as Group A plus doxycycline-loaded microspheres in Group B together with chlorhexidine-based varnish as Group C. During a seven-day period the treated biofilms underwent bacterial viability analysis which included colony-forming unit (CFU) counts accompanied by confocal laser scanning microscopy evaluation. Group A (chitosan gel) reduced CFU by 60.5%, Group B (doxycycline microspheres) by 75.3%, and Group C (chlorhexidine varnish) by 55.8%. Analysis of variance based statistical tests resulted in p values less than 0.05 to determine significance. **Results:** The CFU counts reduced by 60% in Group A and both Group B and Group C showed CFU count reductions of 75% and 55% respectively. Biofilm disruption reached its most effective level in Group B according to confocal microscopy results that were statistically significant compared to the other groups ($p<0.05$). **Conclusion:** The antibacterial performance of periodontal biofilms against tested LDDS reached its maximum with microspheres containing doxycycline. The discovered potential indicates doxycycline-loaded microspheres could function as additional treatment for periodontal diseases. The study's *in vitro* design limits clinical generalizability. Further in vivo and clinical studies are recommended to confirm these findings.

Key Words Local drug delivery, periodontal pathogens, biofilm model, doxycycline, chlorhexidine, chitosan, *in vitro* study

INTRODUCTION

Periodontal diseases form an inflammatory group which attacks the supporting tooth structures because of bacterial biofilms. Disease progression in periodontal infections occurs mainly because of the periodontal pathogens Porphyromonas gingivalis and Aggregatibacter actinomycetemcomitans [1]. Paramount treatment for periodontal conditions such as scaling and root planning exists as the base therapy yet biofilm clearance proves challenging because of deep pockets and unapproachable zones [2]. The need to improve treatment results has motivated researchers to develop local drug delivery systems (LDDS) as an additional antimicrobial approach [3].

LDDS provide targeted antimicrobial treatment which maintains low levels of systemic side effects while enhancing drug availability at infection sites [4]. Different forms of Local Drug Delivery Systems including antibiotic-loaded microspheres, antiseptic gels and bioadhesive varnishes have shown varying effectiveness in reducing bacteria numbers and influencing host immune response [5,6]. The broad-spectrum nature of Doxycycline as a tetracycline antibiotic makes it suitable for clinical therapy involving periodontal treatment [7]. Chlorhexidine-based formulation use has become prevalent because these antimicrobial substances offer effective bactericidal and bacteriostatic properties [8]. Chitosan-based drug delivery systems stand out because they are biocompatible and sustain the release of drugs which extends antimicrobial action [9].

The assessment of various LDDS against periodontal pathogens takes place with the help of controlled *in vitro* biofilm models [10]. Such models effectively reproduce the intricate biofilm systems in periodontal pockets so researchers can study different treatment approaches against these pathogens. The study examines the antimicrobial properties of three separate LDDS using both chitosan gel and doxycycline microspheres alongside chlorhexidine varnish by building biofilms *in vitro*. The study results can help identify optimal methods to use local drug delivery systems for better dual management of periodontal diseases. The research showed that microspheres containing doxycycline would provide better antimicrobial treatment for periodontal pathogens than chitosan gel and chlorhexidine varnish. Standards-based *in vitro* models representing periodontal conditions need expanded comparability studies among different LDDS programs which operate alone. The current study deals with this research void by investigating relative treatment effectiveness. The simulated biofilm models help researchers study drug efficacy *in vitro* although direct clinical examination needs further verification because *in vitro* conditions exclude human host-cell responses.

METHODS

Study Design

Scientists conducted this *in vitro* investigation to evaluate how well three various local drug delivery systems (LDDS) secured against periodontal pathogens when using a biofilm model. *In vitro* testing used a sample size of ten members per research group. The data collection offers restricted foundational information for comparison. The investigators opted against power analysis because this research had an exploratory design. The biofilm procedures took place using sterile methods within laminar airflow cabin areas. The use of sterile tools along with autoclaved media served to stop contamination. Since this research did not engage with human or animal subjects the study excluded the need for ethical approval. Bacterial reduction after LDDS treatment types displayed a strong effect size based on the one-way ANOVA analysis ($\eta^2 = 0.39$). The *in vitro* controlled conditions prevented both missing data and outliers from occurring.

Bacterial Strains and Biofilm Formation

The experimental researchers obtained standard strains ATCC 33277 of Porphyromonas gingivalis and ATCC 29523 of Aggregatibacter actinomycetemcomitans through which they cultivated the microbes anaerobically in brain heart infusion (BHI) broth with the additional supplementation of menadione (1 µg/mL) alongside hemin (5 µg/mL). The research utilized hydroxyapatite (HA) discs as biofilm substrates to establish an imitation of tooth surface structures. The bacterial suspension containing 1×10^8 CFU/mL was applied onto the HA discs that were incubated at 37°C for 72 hours to develop biofilms.

Test Groups and Drug Formulations

The biofilm-coated hydroxyapatite (HA) discs got distributed into four test groups that comprised 10 experimental units per group.

In Group A researchers used a 2% chitosan hydrogel together with antimicrobial agents as the treatment method.

The use of Doxycycline-loaded microspheres consisted of 10% commercial drug-loaded microspheres.

The testing group received chlorhexidine varnish at 1% concentration.

The researchers selected Group D as the control by leaving them without any administration of treatment.

The experiment required all LDDS to follow manufacturer-provided application methods under which samples received seven days of anaerobic incubation for analyzing biofilm survival.

Assessment of Antimicrobial Efficacy

- The procedures for Colony-Forming Unit (CFU) Count included ultrasonic biofilm detachment from HA discs followed by blood agar plating and subsequent serial dilution. The researchers counted CFU to determine bacterial survival after culturing the bacteria anaerobically at 37°C for 48 hours
- The biofilm structure and bacterial viability examinations under CLSM used fluorescent live/dead staining with SYTO 9 and propidium iodide. An image processing software evaluated the proportion of living against dead bacteria

Statistical Analysis

The measurements presented data as mean values along with their standard deviation (SD). The ANOVA one-way analysis enabled group comparisons before applying Tukey's post-hoc tests for specific group pairwise comparisons. Statistical significance arose when the p-value reached below 0.05.

RESULTS

Under CFU count analysis all three local drug delivery systems (LDDS) demonstrated superior bacterial viability reduction levels than the control group. The bacterial inhibition percentage stood at 75.3% for doxycycline-loaded microspheres from Group B while chitosan-based gel from Group A achieved 60.5% bacterial reduction. Group C which used Chlorhexidine-based varnish demonstrated 55.8% as the lowest reduction rate among the available LDDS formulations. The CFU counts from the control group (Group D) remained unchanged which proved that the biofilm continued to survive (Table 1).

CLSM analysis demonstrated the structural condition and viability assessment of bacteria in biofilm formations. Doxycycline microspheres (Group B) caused the most extensive bacterial mortality rate of 74.9% among all treatment groups evaluated. After B. Candida biofilms the

Table 1: Percentage reduction in colony-forming units (CFU) after treatment with various LDDS compared to control

Group	CFU Reduction (%)
Chitosan-based gel (A)	60.5
Doxycycline microspheres (B)	75.3
Chlorhexidine varnish (C)	55.8
Control (D)	0.0

Table 2: Confocal Laser Scanning Microscopy analysis showing live/dead bacterial ratios post-treatment.

Group	Live Bacteria (%)	Dead Bacteria (%)
Chitosan-based gel (A)	40.2	59.8
Doxycycline microspheres (B)	25.1	74.9
Chlorhexidine varnish (C)	45.7	54.3
Control (D)	90.5	9.5

survival rates measured 74.9% for doxycycline, 59.8% for chitosan gel, and 54.3% for chlorhexidine varnish. The control group without treatment kept 90.5% of bacteria alive demonstrating no significant biofilm deterioration in the data (Table 2).

Results from ANOVA showed that significant differences existed among experimental groups with a p-value lesser than 0.05. Doxycycline microspheres proved more effective treatment than chitosan gel and chlorhexidine varnish based on post-hoc analysis ($p<0.05$) yet the difference between chitosan gel and chlorhexidine varnish treatments did not reach statistical significance.

According to earlier research these superior results from doxycycline microspheres are caused by sustained release along with deep biofilm penetration abilities. The adhesive properties of the chitosan gel might have increased drug contact with biofilm but chlorhexidine varnish potentially could not penetrate through deeper biofilm layers effectively.

DISCUSSION

This analysis examined the antimicrobial effectiveness between various local drug delivery systems (LDDS) that treat periodontal biofilms. Doxycycline-loaded microspheres showed the most potent antibacterial effect among the tested LDDS because they achieved maximum bacterial viability reduction compared to the control group. Other studies have already confirmed that local antibiotic delivery proves effective for periodontal therapy [1,2].

Periodontal experts consider scaling and root planing (SRP) as the standard manual treatment for gum infections. Periodontal treatment requires additional therapies other than mechanical methods because the complicated nature of periodontal pockets and biofilm persistence [3,4]. Doxycycline microspheres function as antibiotic-based local drug delivery systems that demonstrate exceptional ability to break down biofilms while suppressing periodontal disease-causing microorganisms [5]. The research demonstrates that doxycycline microspheres reduced CFU counts by 75.3% showing similar performance to earlier findings about their extended release characteristics and strong antimicrobial effects against *P. gingivalis* and *A. actinomycetemcomitans* [6,7].

Scientific interest has been focused on chitosan-based gels because they show both compatibility with biological systems and antimicrobial activity and drug-retention boosting properties at the application point [8]. This current research showed that chitosan-based gel reduced CFU by 60.5% similar to previous assessments of its use as a drug carrier for localized periodontal therapies [9]. The antimicrobial actions of chitosan depend on membrane disruption followed by its ability to sustain the antimicrobial effects through release maintenance [10]. The therapeutic success rate of chitosan-based gel was inferior to doxycycline microspheres thus demonstrating that chitosan offers beneficial periodontal properties yet works better alongside other antimicrobial components.

The clinical application of chlorhexidine-based varnishes remains widespread as an effective periodontal therapy because they demonstrate antimicrobial actions against numerous germs while persisting within treated surfaces [11]. Research findings matched the present study when chlorhexidine varnish reduced bacterial counts by 55.8% as reported in previous studies that documented its effectiveness at lowering both supragingival and subgingival bacteria [12]. The use of chlorhexidine has several constraints because it can cause tooth staining while the persistent presence of the substance may generate microbial resistance [13]. The antimicrobial persistence of chlorhexidine enables it to become a worthwhile supporting measure for periodontal treatment [14].

The evaluation using confocal laser scanning microscopy (CLSM) in this investigation confirmed the effectiveness of the LDDS that was studied. The observed percentage of microbial cell death in this study reached its highest level with doxycycline microspheres at 74.9% compared to chitosan-based gel at 59.8% and chlorhexidine varnish at 54.3%. LASCM results reveal corresponding evidence from past research demonstrating that antibiotic-based LDDS delivers better biofilm penetration than antiseptic-based formulation methods [15].

The *in vitro* setup has limitations because salivary enzymes and immune response and gingival crevicular fluid are not present in the model. Public health implementation of doxycycline microspheres requires consideration of both their price and their availability because of their excellent performance. Doxycycline treatment that lasts for a prolonged period presents a threat to microbial resistance which demands clinical practitioners to administer it with care and responsibility. The biofilm suppression properties of antibiotic-loaded delivery vehicles found by our research agree with the findings of Koo *et al.* (2023) and Sanz *et al.* (2021).

CONCLUSIONS

Doxycycline-loaded microspheres demonstrated the highest antibacterial efficacy, followed by chitosan gel and

chlorhexidine varnish. These LDDS show promise as adjuncts to mechanical periodontal therapy, but further clinical validation is essential before widespread application. The bacterial reduction obtained from all LDDS approaches is considerable yet future users need to weigh long-term effects and methods of action before picking which LDDS to utilize in clinical settings. Widespread animal studies along with randomized clinical tests should be performed to confirm and measure the healing effects of LDDS in periodontal tissues.

Conflicts of Interest

The authors declare no conflicts of interest.

Ethical Statement

Not applicable as this was an *in vitro* study.

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