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Original Article

IN VITRO EVALUATION OF TETRACYCLINE-LOADED CHITOSAN CHIPS AGAINST PERIODONTAL PATHOGENS

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ABSTRACT

Objective: A periodontal pathogen is the main cause of periodontitis. Tetracycline has demonstrated its ability to impede the proliferation of periodontal pathogens. A chip is a device that employs a matrix to distribute drugs evenly into a polymer. The release of the drugs occurs through the process of drug diffusion. It possesses numerous benefits in terms of appealing physical characteristics for use within a pocket. Chitosan is a naturally occurring biopolymer with favorable characteristics for use as a drug delivery system.

Methods: Using solvent casting, chitosan-based periodontal chips containing 0.7% tetracycline were created. Scanning Electron Microscopy (SEM) and Fourier transform infrared (FTIR) were used to characterize the structure and morphology of the chitosan-based periodontal chip. The antibacterial performance was analyzed against *A. actinomycetemcomitans*, *P. gingivalis*, and *F. nucleatum* bacteria grown in an MHA medium.

Results: Based on the results of SEM analysis at 1000x magnification, tetracycline can be seen on the surface of chitosan, while FTIR analysis shows compatibility between chitosan and tetracycline. The average diameter of the inhibition zone of chitosan-based periodontal chips containing 0.7% tetracycline towards *P. gingivalis*, *A. actinomycetemcomitans* and *F. nucleatum* were 14.75±0.75 mm, 25.67±2.082 **m**m, and 35.33±0.764 mm. In tests on *Aa* and *Fn* bacteria, the inhibition zone was larger compared to the PerioChip®. The ANOVA test revealed statistically significant variations in the average diameter of the inhibitory zone across different groups when tested against periodontal infections.

Conclusion: This research has shown potent antimicrobial activity of chitosan-based periodontal chips containing 0.7 % tetracycline against *A. actinomycetemcomitans*, *P. gingivalis*, and *F. nucleatum*. This reseaerch showed more microbial activity than PerioChip® for *A. actinomycetemcomitans* and *F. nucleatum*.

Keywords: Periodontal pathogens, Chip, Tetracycline, Chitosan

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INTRODUCTION

Periodontitis is an inflammatory disorder of the gums caused by bacteria, resulting in the permanent loss of the tissues that hold the teeth in place [1, 2]. It is widely acknowledged that microorganisms significantly contribute to development of periodontal diseases. The three bacteria known to cause periodontal issues are *Fusobacterium nucleatum* (*Fn*), *Aggregatibacter actinomycetemcomitans* (*Aa*), and *Porphyromonas gingivalis* (*Pg*). These bacteria have been found in lesions associated with periodontitis, gingivitis, and healthy periodontal sites [3]. Bacteria, particularly *Pg*, which belongs to the red complex group of bacteria, are the primary factors responsible for periodontal disease. These bacteria are essential in the progression of chronic periodontitis [4, 5]. The primary pathogenic characteristics of *Pg* have been observed and described, specifically, gingipains, which can infiltrate gingival tissue.

Furthermore, *Pg* produces several virulence factors, including a capsule, fimbriae, and lipopolysaccharide, which can cause harm to host cells. This enables *Pg* to infiltrate cells and tissues. All researchers agree that serum antibodies to *Pg* are higher in diagnosed patients with periodontitis [6]. *Aggregatibacter actinomycetemcomitans* is a Gram-negative bacteria found throughout the oral that causes localized aggressive periodontitis (LAP), which often needs additional antibiotic treatment [7]. In addition, *Fn* is an anaerobic Gram-negative bacterium that inhabits the upper digestive system, urogenital tract, mouth cavity, and gut. It serves as a crucial co-aggregation component, as a bridge among early and late colonizers for almost all bacterial species participating in forming oral plaque [4]. Additionally, it generates the adhesion protein FadA, which binds to the endothelial cell's VE-cadherin and the epithelial cell's E-cadherins [8]. *The Fusobacterium nucleatum* strain exhibits robust growth throughout a broad pH spectrum of 5.5–7.0, highlighting the significant pH fluctuations within the oral cavity [9].

The main purpose of ultimate periodontal therapy is to prevent the development of periodontitis and restore lost tissues. There are several ways to achieve it by treating inflammatory tissue, lowering the depth of pockets, lowering dangerous bacterial counts, and stopping more bone loss. The therapy involves mechanical cleanings like scaling and root planing (SRP), and antimicrobial agents administered systemically or locally [1]. Systemic administration of antibiotics is favorable. However, high doses of oral antibiotics are needed to ensure that the gingival crevicular fluid (GCF) carries sufficient levels of the antibiotics. Prolonged antibiotic usage can lead to resistance development [10]. Additionally, local antimicrobials are a supplementary treatment to conventional scaling and root planing for periodontal disease, especially for patients with poor clinical improvement [9].

Chitosan (CS) is a naturally derived biopolymer that has a diverse array of biological applications. Chitosan is a biopolymer that is obtained by the deacetylation process of chitin. Its production involves the use of some multidisciplinary techniques [11]. It is Earth's second-most abundant naturally occurring polymer. It derives from both vegetable and animal sources: non-toxicity, mucoadhesive properties, good film-forming, and broad-spectrum antimicrobial activity. This biopolymer can be enzymatically degraded into a more straightforward form that is not toxic or pollutes the environment. Additionally, CS can be transformed into various derivatives and morphologies, including gels, micro/nanoparticles, fibers, and chips, via chemical or enzymatic processes. Therefore, because of its unique composition and function, chitosan is a potential material for drug carriers [11, 12].

Tetracycline is a broad-spectrum antibiotic that is bacteriostatic and has a dual mechanism of action [13]. Tetracycline is a chemotherapy agent that can reduce collagenase and bone damage through its ability to inhibit the collagenase enzyme. Tetracycline possesses the capacity to accumulate in periodontal tissue and has the potential to

inhibit the growth of periodontal disease [10]. Sachdeva further stated that tetracycline at a concentration of 0.7% is considered to achieve an acceptable level of biocompatibility for tissue use [14]. According to research by Susanto and his colleagues, the average diameter of the inhibition zone produced by the chitosan-based 0.7% tetracycline gel was greater than 27 mm when tested against *Aa*, *Pg*, and *Fn* bacteria. This indicates a highly efficient antibacterial effect [3]. In addition, Andrew *et al.* presented that the chitosan-based hydrogel containing 0.7% tetracycline is nontoxic to fibroblast cells [15]. Local drug delivery systems can mitigate the systemic side effects of antibiotics and enhance local bioactivity during periodontal disease treatments [3].

The periodontal pathogens linked to chronic periodontitis, which are prevalent in the deepest pockets and include *P. gingivalis* and *T. forsythia*, have been significantly reduced by PerioChip®. [16] There are disadvantages of PerioChip®, such as a change in taste, an increase in tartar (calculus) on teeth, expensive and hard to find [17]. The chitosan-based periodontal chips were prepared using a simple dissolution technique without using dangerous organic solvents. The film serves as an indicator of the drug's physicochemical properties and stability. To obtain films of desired quality, one must consider the influence of formula factors [18].

MATERIALS AND METHODS

This experimental study was conducted at the Microbiology Hospital of Universitas Sumatera Utara laboratory and the Oral Biology of Universitas Indonesia. This research was validated by approval of the ethics committee for implementation of health research with the number 417/KEPK/USU/2023 based on the rules of the Nuremberg Code and the Helsinki Declaration. This study did not involve animal experiments; therefore, the ARRIVE guidelines are not applicable.

This research comprised three groups, specifically Group 1: chitosan chip with 0.7% tetracycline; Group 2: Chip made of chitosan; Group 3: PerioChip® (as a positive control). Each group consisted of 9 repetitions in total, comprising 3 Pg samples, 3 Fn samples, and 3 Aa samples.

Preparation and formulation of chips

Periodontal chips were prepared using the solvent-casting method. Precisely, chitosan (2 g) with HM(high molecular weight)-80 and>75 % deacetylated degree (USU) was mixed in a solution of 0.5% acetic acid (50 ml) and stirred for 24 h until completely dissolved. Next, tetracycline (0.35 g) (Sigma Aldrich with assay result HPLC 101.2 %) was slowly introduced to the mixture. The mixture was stirred for six hours. While stirring, 15 ml of NaOH 0.01N was slowly added to adjust the pH to 5.5. Subsequently, 0.005 g of Tripolyphosphate (TPP) was dissolved in deionized water (0.5 ml) as a separate solution. The TPP solution was then incorporated gradually into the CS solution while stirring continuously at room temperature. Then, propylene glycol (1 g) was introduced. The mixture was homogenized at 1000 rpm for 1 min. The process was conducted for 24 h x 3 without disruption to facilitate thorough evaporation. After fully extracting the chips from the glass mold, they were punched out to the appropriate size into 0.4x0.5 sq cm pieces. The chips obtained were sealed using aluminum foil and stored in desiccators until they were required again. As a control, drug-free placebo chips made of just chitosan without tetracycline and plasticizer were made [19, 20].

Characterization

Fourier transform infrared analysis

Fourier transform infrared (FTIR) analysis was conducted utilizing a Shimadzu (model 8400S, Tokyo, Japan) analyzer to analyze the potential interactions between the medicine and excipients. In brief, the samples were crushed using KBr under 300 kg cm^{-2} of pressure. The FTIR spectra of both pure drugs and polymers and a 1:1 ratio physical mixture of drugs and polymers were scanned between the 4000-400 cm^{-1} region [18].

Scanning electron microscopy analysis

Scanning electron microscopy (SEM) was performed on chitosan-based periodontal chips containing 0.7 % tetracycline and chitosan chips. The chip is then coated with gold so that tools can easily detect it. The chip was then placed in the sample holder, and the detected surface with magnifications of 50X, 150X, and 1000X obtained the most apparent appearance of the chip surface [18].

In vitro antibacterial activity

Specimen culture was carried out in anaerobic conditions in a CO_2 incubator. *P. gingivalis* (ATCC 33277), *Aa* (ATCC 33384), and *Fn* (ATCC 25586) were stem-cell specimens of each bacterium cultured purely on Mueller-Hinton Agar (MHA) media prepared in the previous procedure in anaerobic conditions. A total of 1-2 oses of pure cultures of test bacteria that had been cultured and grew well were suspended using 0.9% NaCl solution until turbidity was obtained according to the 0.5 McFarland standard or equivalent to the number of bacteria 1 x 10^6 Colony-Forming Unit (CFU)/ml. Sterile tryptic soy broth (TSB) was taken in the refrigerator and left in the room. Furthermore, using sterile cotton swabs, *Pg*, *Aa,* and *Fn* were taken from *TSB*. After that, they were incubated at 37 C for 24 h [3, 4]. On the MHA media that already contains bacteria, a 0.7% tetracycline chitosan chip is placed with the help of sterile tweezers and then incubated at a temperature of 36-37 °C for 18-24 h in an anaerobic incubator filled with 100% CO_2. The diameter of the inhibition zone is measured every day for three days of observation by looking at the clear zone around the chip. Measurements are made using a caliper without opening the petri dish so that the media is not contaminated [3, 21].

Data analysis

The data obtained from the study were processed using a computerized system using Statistical Package for the Social Sciences (SPSS) V.29 software (IBM SPSS Statistics; IBM Corp; f861ca2610c844047962). Data normality was tested using the Shapiro–Wilk test, and homogeneity of variance was assessed using Levene's test. One-way ANOVA was conducted to compare the inhibition zone diameters among treatment groups, followed by a post-hoc Tukey HSD test for pairwise comparisons. Statistical significance was set at $p<0.05$. Effect sizes (η^2) were calculated to determine the magnitude of group differences.

RESULTS

The main characteristics of chitosan functional groups in the literature are Amide I (due to the high loss coefficient of the C=O group) and NH_2 bands at wave numbers 1647 and 1590 cm^{-1}, respectively. Meanwhile, functional groups in the tetracycline spectrum can be found in the literature, namely: (C=O) at wave number 1675 cm^{-1}; 1650–1600 cm^{-1}, assigned to (C-C) aromatic ring; 1460–1310 cm^{-1} to (OH), (C-C) and (C-C); (N-H) and (C-N) correspond to the band at wave numbers 1250–1200 cm^{-1}.

Based on fig. 1, the FTIR spectra of the chitosan-based periodontal chip containing 0.7 % tetracycline show the same pattern in each variation. It is not much different from the spectrum of the constituent materials. Next, chitosan-based periodontal chips containing 0.7 % tetracycline and periodontal chip chitosan have most of the same functional groups in their structures. The FTIR spectra showed a similar tendency when both were used as chip-building materials. The broadening of the peak intensity is due to overlapping OH, NH_2, and CH groups, where these functional groups dominate in the structure of chitosan and tetracycline compounds, as well as the effect of increasing the radiation dose given to the chip. By adding 0.7% of tetracycline into the chitosan chip material, the resulting FTIR spectra had a similar trend without adding new peaks. Scanning Electron Microscopy analysis was conducted on chitosan-based periodontal containing 0.7% tetracycline, periodontal chip chitosan, and PerioChip®, as shown in the Figures, to analyze the chip morphology. The chip morphology was observed with SEM (JCM 5700) at a voltage of 20 kV. The samples were previously coated with gold in a vacuum and blasted with a Pemtron vacuum apparatus.

Fig. 2 illustrates the chitosan-based periodontal chip containing 0.7 % tetracycline SEM topography at 50x, 150x, and 1000x magnifications. At these magnifications, the chip's images were smooth and even. However, at 1000 magnification, tetracycline

particles are distributed across the surface, indicating the success of chitosan-based periodontal chips containing 0.7 % tetracycline production. The hydrogen bond between CS and tetracycline causes it. Furthermore, fig. 3 shows that the SEM images of periodontal chip chitosan indicate homogeneity in the provision of films. A flat surface is visible with a magnification of 50,150, and 1000 magnification. The chip morphology is visible.

Based on fig. 4, it can be seen that there is an inhibition zone on the periodontal chip containing 0.7% tetracycline in chitosan-based and PerioChip® against the *Pg*, *Aa*, and *Fn*, bacteria, which the periodontal chip chitosan alone does not show the diameter of the inhibition zone against all bacteria with three repetition. The result of assessing the diameter of the inhibitory zone for each chip against *Pg*, *Aa*, and *Fn* bacteria can be seen in table 2.

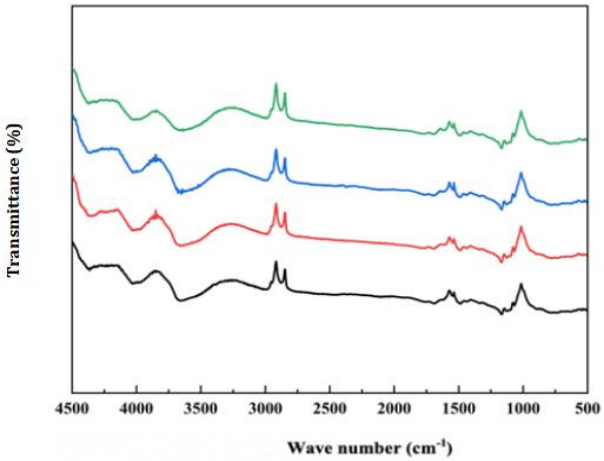

Fig. 1: FTIR spectra of pure chitosan, pure tetracycline, physical mixture, and tetracycline-loaded chitosan chip. The X-axis represents wavenumber (cm^{-1}), and the Y-axis represents transmittance (%). The spectra were recorded in the range of 4000–400 cm^{-1} using FTIR shimadzu 8400S

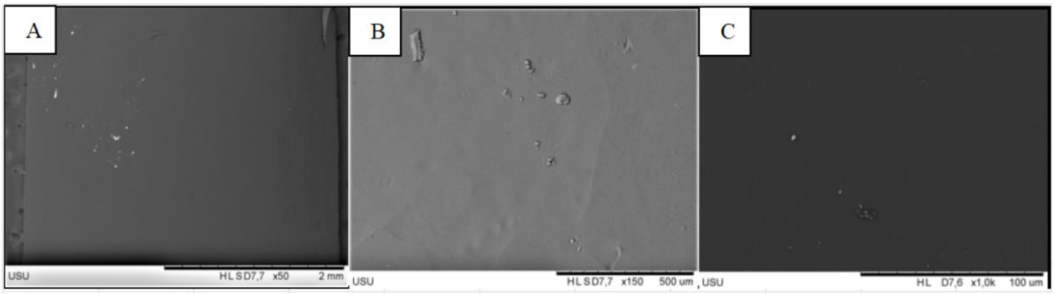

Fig. 2: SEM morphology of chitosan-based periodontal chip with; a) 50x; b)150x; and c)1000 x magnifications

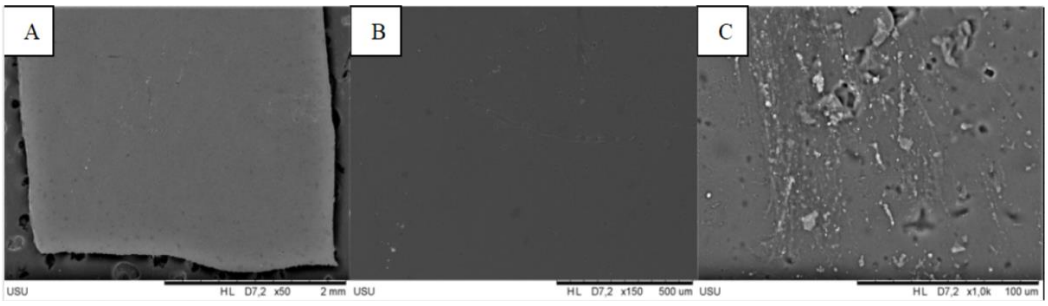

Fig. 3: SEM morphology of chitosan-based periodontal chip containing 0.7 % tetracycline with; a) 50; b) 150x; and c).1000 magnifications

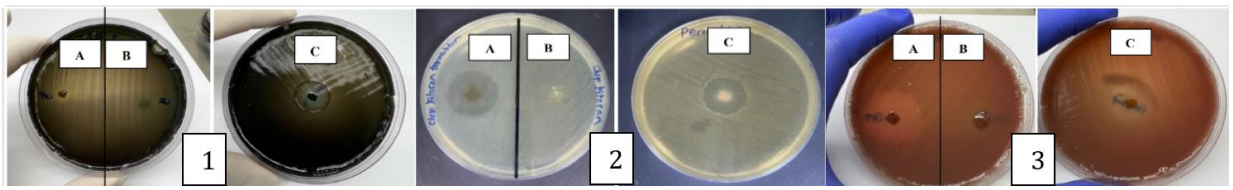

Fig. 4: The inhibition zone formed by each chip against bacteria: 1) Against *P. g*: A). Chitosan-based periodontal chip containing 0.7% tetracycline; B) Periodontal chip chitosan C) PerioChip®. 2) Against *A. a*: A) Chitosan-based periodontal chip containing 0.7% tetracycline B) Periodontal chip chitosan C) PerioChip®. 3) Against *F. n*: A) Chitosan-based periodontal chip containing 0.7% tetracycline B) Periodontal chip chitosan C) PerioChip®

Based on table 1, it is known that the mean value of the diameter of the inhibition zone against *P. gingivalis* bacteria in the chitosan-based periodontal chip containing 0.7 % tetracycline in group and the PerioChip®shows the strength of inhibition in the strong category. In contrast, the periodontal chip chitosan does not show any inhibition zone. This table also shows that the average diameter of the inhibitory zone's diameter in the chitosan-based periodontal chip contains 0.7 % tetracycline and PerioChip®(against *F. nucleatum*). Based on the results of testing using the ANOVA method on three types of bacteria, namely *Pg*, *Aa*, and *Fn* bacteria, with an F value = 9.365 and a significance value of 0.001 for *Pg*, *Aa,* and *Fn* bacteria. Because the p-value<0.05, it can be concluded that there is a significant difference in the diameter of the inhibition zone between the treatment groups. Then, a further test was carried out to determine which groups were significantly different.

The results of the Shapiro–Wilk and Levene's tests confirmed that the data were normally distributed and variances were homogeneous across groups (p>0.05). One-way ANOVA revealed statistically significant differences in the inhibition zone diameters among the treatment groups for all tested bacteria. For *P. gingivalis*, the difference was highly significant (F = 4125.06, η^2 = 0.999); for *A. actinomycetemcomitans*, the result was also significant (F= 377.62, η^2 = 0.992); and for *F. nucleatum*, the difference was most pronounced (F= 34549.00, η^2 = 0.9999). These results indicate a very large effect of the treatment on bacterial growth inhibition.

The low standard deviations observed in the inhibition zone measurements reflect the high consistency of the *in vitro* testing conditions, including the standardized chip formulation, uniform bacterial concentration (0.5 McFarland), and the use of digital calipers for accurate zone measurement.

The Tukey test showed several significant differences between the treatment pairs, including: chitosan periodontal chips were significantly different from chitosan-based periodontal chip containing 0.7 % tetracycline and PerioChip®. Chitosan-based periodontal chip containing 0.7 % tetracycline for *Fn* showed significant differences with almost all other treatments. PerioChip®was significantly different from the chitosan-based periodontal chip containing 0.7 % tetracycline for *Aa* and *Fn*. Thus, the highest antibacterial effectiveness was obtained from Chitosan chips combined with Tetracycline, especially against *Fn*.

Table 1: Inhibition zone of each periodontal chip diameter in *Pg*, *Aa* and *Fn* bacteria

Bacteria	Variable	Repetition			Inhibition zone (mm)	P value
		1	2	3	Mean±SD	
P. gingivalis	Periodontal chip chitosan-tetracycline	14	14.75	15.5	14.75±0.75	0.001*
	Periodontal chip chitosan	0	0	0	0	
	PerioChip®	20.25	-	-	20.25	
A. actinomycetemcomitans	Periodontal chip chitosan-tetracycline	28	24	25	25.67±2.082	0.001*
	Periodontal chip chitosan	0	0	0	0	
	PerioChip®	20	0	0	20	
F. nucleatum	Periodontal chip chitosan-tetracycline	36	35.5	34.5	35.33±0.764	0.001*
	Periodontal chip chitosan	0	0	0	0	
	PerioChip®	22.5	-	-	22.5	

*ANOVA p-value<0.050 = significant between groups

Table 2: Comparison of the inhibition zone diameter of each periodontal chip in *Pg*, *Aa* and *Fn* bacteria

Variable	N	Subset for alpha = 0.05		
		1	2	3
Periodontal chip chitosan-*Pg*	3	.0000		
Periodontal chip chitosan-*Aa*	3	.0000		
Periodontal chip chitosan-*Fn*	3	.0000		
PerioChip®-*Pg*	3	6.6667	6.6667	
PerioChip®-*Aa*	3	6.7500	6.7500	
PerioChip®-*Fn*	3	7.5000	7.5000	
Periodontal Chip chitosan Tetracycline *Pg*	3	14.7500	14.7500	
Periodontal Chip chitosan Tetracycline *Aa*	3		25.6667	25.6667
Periodontal chip chitosan Tetracycline *Fn*	3			35.3333
Sig.		.264	.072	.748

Sig= Significant value
Fn= Fusobacterium nucleatum; Aa= Aggregatibacter actinomycetemcomitans; Pg= Porphyromonas gingivalis

DISCUSSION

The main characteristics of chitosan functional groups can be found in the literature, namely Amide I (due to the high loss coefficient of the C=O group) and NH_2 bands at wave numbers 1647 and 1590 cm^{-1}, respectively; the intense band at wavenumber 3420 cm^{-1} should be assigned to O-H and/or N-H stretching vibrations, as well as intermolecular hydrogen bonds in polysaccharides; absorption bands at wave numbers 1154, 1078 and 1031 cm^{-1}. Meanwhile, functional groups in the tetracycline spectrum can be found in the literature, namely: (C=O) at wave number 1675 cm^{-1}; 1650–1600 cm^{-1}, assigned to (C-C) aromatic ring; 1460–1310 cm^{-1} to (OH), (C-C) and (C-C); (N-H) and (C-N) correspond to the band at wave numbers 1250–1200 cm^{-1} [22].

The FTIR spectra of the chitosan-based periodontal chip containing 0.7 % tetracycline showed the same pattern in each variation. It is not much different from the spectrum of the constituent materials. The chitosan-based periodontal chips containing 0.7 % tetracycline and periodontal chip chitosan have most of the same functional groups in their structures, so their FTIR spectra have some similarities; no new functional groups formed after adding 0.7% tetracycline to the chitosan chips. Khan *et al.*'s research also showed no chemical interactions between the drug and the polymer. This can be seen from the chitosan spectrum, which does not change with tetracycline adsorption, indicating that tetracycline is adsorbed on the surface. This condition also confirms that the absorption of tetracycline mainly occurs on the chitosan surface since the transmission spectrum of chitosan before and after tetracycline adsorption is the same [18].

The chip morphology was examined using SEM. SEM images were taken with 50x, 150x, and 1000x magnifications. The surface of the chitosan-based chip contained tetracycline at 0.7 %, and the periodontal chip chitosan appeared flat, smooth, and compact without visible pores at 50 and 150 magnifications. The SEM image results at 1000 magnification showed that a physical bond occurred between chitosan and tetracycline [18]. The surface of the PerioChip® appears smooth and without pores at 50 and 150 magnification; at a higher magnification, namely 1000 magnification, the morphological surface is flat and smooth [18].

Based on research by Khan *et al.*, the film's surface looks smooth and compact without visible pores at 54 and 56 magnifications. In contrast, at 1000 magnification, visible drug crystals are observed on the film surface. The attachment of the drug to the surface of the film can provide a drug explosion effect at an early stage [18]. This research showed that chitosan-based periodontal chips containing 0.7 % tetracycline and PerioChip® have very strong inhibitory power against the *Aa*, *Pg*, and *Fn* bacteria. The chitosan-based periodontal chip containing 0.7 % tetracycline has a larger zone of inhibition against the *A. a* and *F. n* bacteria compared to the PerioChip®, but against *Pg* bacterium, the PerioChip®shows a larger zone of inhibition than the periodontal chip containing 0.7 % tetracycline in chitosan-based. Pathogen in periodontitis, *P. gingivalis*, has a number of virulence characteristics that might lead to insufficient removal of bacterial infections. In order to modify the host immunological response, *P. gingivalis* secretes outer membrane vesicles (OMVs), which introduce virulence factors into host cells [23].

Chips containing chitosan alone did not show an inhibition zone for these three bacteria. Research by Susanto *et al.* also indicates that chitosan gel containing 0.7% tetracycline has a very strong inhibition zone against these three bacteria [3]. Jalaluddin *et al.*'s research evaluated the antimicrobial effectiveness of chitosan gel containing 0.7% tetracycline against *A. actinomycetemcomitans* and *P. gingivalis*. Chitosan gel containing 0.7% tetracycline showed a strong inhibition zone against the bacteria *A. actinomycetemcomitans* and *P. gingivalis*. This indicates that chitosan gel containing 0.7% tetracycline effectively inhibits the growth of *A. actinomycetemcomitans* and *P. gingivalis* [24]. In this study, periodontal chip chitosan with a concentration of 4% was ineffective in inhibiting the growth of *P. gingivalis*, *A. actinomycetemcomitans,* and *F. nucleatum,* Gram-negative anaerobic bacteria. These results follow Susanto and Adha's research that 4% and 3% chitosan gel is ineffective in inhibiting the growth of *Pg*, *Aa,* and *Fn* bacteria [3, 4]. Following the administration of chitosan nanoparticles containing 0.7% tetracyclines, there was a significant rise in FGF-2 expression on days 3 and 8, along with a decrease in the proportion of BOP and PD in all groups [25]. The low standard deviations observed in the inhibition zone measurements reflect the high consistency of the *in vitro* testing conditions, including the standardized chip formulation, uniform bacterial concentration (0.5 McFarland), and the use of digital calipers for accurate zone measurement.

Scaling-root planing is the gold standard in treating periodontal disease. Still, it often fails to eliminate pathogenic bacteria within the gingival tissue or other areas that periodontal instruments cannot reach. Local antimicrobial application is indicated in localized periodontitis and areas that frequently recur and do not respond well to scaling root-planing therapy. Local use of antibacterial agents administered directly into the periodontal pocket can provide higher drug concentrations to the infected area and reduce possible systemic side effects compared to systemic use of the drug [1]. Several researchers have observed that local administration of drugs can reach 100 times higher concentrations in the area where the drug is administered compared to systemic administration. This will reduce the total dose given to the patient by more than 400 times, thereby avoiding drug resistance. Long-term use of systemic antibiotics can disrupt the body's typical systems and cause bacterial resistance [26].

Tetracycline is a broad-spectrum antibiotic that inhibits prokaryotic protein synthesis by ribosomes [27]. The antibiotics with the highest twenty-four-hour drug release rate are tetracycline [27]. Besides that, it also has anti-inflammatory and immunosuppressant effects. Tetracycline is known to specifically inhibit cellular growth by binding to the 30S ribosomal subunit of microbes, changing the integrity of the cell membrane and causing macromolecular dysfunction, cellular lysis, and cellular death. Tetracycline also inhibits the activity of matrix metalloproteinases (MMP-8 and MMP-9) produced by PMN by binding Ca2+and Zn2+, which are at the active site preparation on its surface, contain drug particles or chlorhexidine, which are distributed bound in an orderly manner [27].

The strong antimicrobial activity of the 0.7% tetracycline-loaded chitosan chip is attributed to its controlled release profile and synergistic interaction with bacterial membranes. Chitosan's cationic nature disrupts the negatively charged cell walls of Gram-negative pathogens, enhancing membrane permeability and promoting tetracycline uptake [11]. The polymer matrix enables sustained drug release, maintaining therapeutic concentrations locally while minimizing systemic exposure [17]. This dual mechanism contributes to stronger inhibition zones observed in vitro. Clinically, such localized delivery systems may offer superior outcomes as adjuncts to scaling and root planing by improving compliance, reducing systemic side effects, and minimizing antibiotic resistance [25].

CONCLUSION

This study concluded that FTIR analysis showed no shift or disappearance of peaks, and no new functional groups appeared on the periodontal chip containing 0.7 % tetracycline in chitosan-based. The results of SEM analysis of the periodontal chip containing 0.7 % tetracycline chitosan-based showed a smooth chip topography and the presence of tetracycline on the surface of the chitosan base. Periodontal chips containing 0.7 % tetracycline in chitosan-based were proven to be effective in inhibiting the growth of periodontal pathogenic bacteria *P. gingivalis* with strong zone diameter and *A. actinomycetemcomitans* and *F. nucleatum* with powerful diameter zone.

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AUTHORS CONTRIBUTIONS

Savana Ersa-Conception, prepared the first draft and revised the manuscript, designed and analyzed the data, and carried out the experiments; Irma Ervina: Data design, experimentation, manuscript review, supervision, and visualization; Armia Syahputra: Manuscript review, supervision, and visualization; Harry Agusnar—supervision and visualization, manuscript revision; Aini Hariyani—supervision and visualization, manuscript revision.

CONFLICT OF INTERESTS

Declare that there is no conflict of interest regarding the publications of this paper. The data supporting the findings of this study are available from the corresponding author upon reasonable request.

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