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In vitro evaluation of antimicrobial effectiveness chitosan based tetracycline gel on some pathogenic periodontal bacteria

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Abstract
Plaque bacteria are the main cause of periodontal disease such as periodontitis. Generally, most of the identified bacteria are anaerobic negative-gram as *Porphyromonas gingivalis, Agregatibacter actinomycetemcomitans, Fusobacterium nucleatum*. Tetracycline is a broad spectrum antibiotics that can inhibit the growth of pathogenic bacteria of periodontal. The objective of this study was to analyze the effect of chitosan based tetracycline gel in inhibiting periodontal pathogenic bacteria growth with *in vitro* evaluation. gel made by mixing the tetracycline each concentration with 0,4 g chitosan were dissolved using 1% citric acid and added with aquabidest up to 100 ml. The bacteria used were pure culture of *Aggregatibacter actinomycetemcomitans* (ATCC 29522), *Porphyromonas gingivalis* (ATCC 33277) and *Fusobacterium nucleatum* (ATCC 25586) and cultured in *Muller Hinton Agar* (MHA) media. Bacteria test done by placing the chitosan based gel into *Muller Hinton Agar* (MHA) media that have been made wells by 5 mm, after incubation for 1 day, inhibition zone was measured using caliper. The average diameter of inhibition zone of each tetracycline gel 0,5%, 0,7% and 1% chitosan based againts *A. actinomycetemcomitans, P. gingivalis* dan *F. nucleatum* up to 27 mm. 1% tetracycline gel based chitosan showed the greatest inhibition zone diameter and visible presence of significant differences in concentrations 0,7%, 0,5%. while 0.5% tetracycline gel based chitosan showed the smallest diameter. The result suggested that 0,5%, 0,7% and 1% chitosan based tetracycline gel has strong influence on *A. actinomycetemcomitans, P. gingivalis* and *F. nucleatum* bacteria.

Keywords: tetracycline gel, chitosan, periodontal pathogen

1. Introduction
Plaque bacteria are the main cause of inflammatory gingiva [1]. The ability of bacteria to attach to the host can cause infectious diseases such as gingivitis and periodontitis. Bacteria in the oral, especially bacterial pathogens such as *Porphyromonas gingivalis* (*P. gingivalis*), *Aggregatibacter actinomycetemcomitans* (*A. actinomycetemcomitans*) has a very high virulence trait [2].
Aggregatibacter actinomycetemcomitans is a gram-negative bacteria found in the oral cavity and one of the etiology of agressive periodontitis [3] These bacteria have the ability to produce leukotoksin which can lead damage to periodontal tissues and one type of bacteria that is considered as periodontal pathogenic bacteria and become as etiologic factor in periodontitis [4, 5] *Porphyromonas gingivalis* is black pigmented gram-negative bacteria and become one of the bacteria that causing periodontitis. These bacteria are able to secrete a protease enzyme that can cause damage to the host tissues [6]. *Fusobacterium nucleatum* (*F. nucleatum*) is a gram-negative anaerobic bacteria and very dominant in oral enviroment [7]. *Fusobacterium nucleatum* plaque known as the bridge coaggregation and become most instrumental in helping aggregation *Streptococci* and obligate anaerob [8]. *Fusobacterium nucleatum* can secrete peptides that affect the cells and host immune response [7].
Conventional treatment of periodontal disease can be done with the removal of plaque and calculus mechanically namely scaling and root planing but in some cases it did required additional antimicrobial [9]. Additional treatments such as the use of antibacterials may provide better treatment outcomes, especially in lesions of the tooth with anatomical more complex shape [10]. Antimicrobial administered either systemically or locally can help reduce the number of pathogenic bacteria in periodontal pockets and oral cavities [11].

Treatment of periodontal disease with chemical additives intended to better control the infection by using antibiotics and antiseptic [9].

Antibiotics can be done locally and systemically [12]. systemic administration of antibiotics has been widely reported to generate resistance [13]. Antibiotics locally is a new approach to the management of localized periodontal infection. The main advantage of usage locally antibiotics is can be incorporated directly into the pocket, avoiding side effects, increasing the exposure to microorganisms targeted by higher concentration so that the treatment becomes more terapeutic [14].

Several types of antibiotics are often used locally such as Tetracycline and derivatives that minocycline and doxycycline and metronidazole. Tetracycline is the most often used antibiotics in the study. Tetracycline is an antibiotic widely spectrum which may affect anaerobic bacteria and anaerobic fakultative [15] Tetracycline can also directly inhibit MMPs (Matrix Metalloproteinase) and collagenase issued by host immune cells in response to microorganisms [16]. Tetracycline usual dose given to the patient as much as 250 mg 4 times daily so that makes patient compliance in taking the drug more decrease [17].

Biocompatibility acceptable use of Tetracycline is at a concentration of 0.7%. [7]. Sachdeva in clinical research states that the use of tetracyclines locally combined with scaling and root planing on chronic periodontitis may reduce pocket depth, gingival index and plaque index [18]. Mohammed M in the research states that doxycycline gel is effective against pathogenic bacteria periodontal [8].

Local administration of drugs is a means to application certain drugs on the side, allowing concentrations reached 100 times higher than the systemically administration [19]. Preparations that have been used for locally administration to the periodontal is a paste, ointment, gel, fiber, strip, and chips. Goodson *et al* study on the fiber blank made of ethyl vinyl acetate as a carrier Tetracycline explained that the ethyl vinyl acetate fiber can not be resorbed and should be discarded after 10 days. Drug release system in this way is more difficult to be applied into the periodontal pocket [20]. Preparations semi-solid (gel) has several advantages, among others: the drug release is faster, easier manufacture, providing an easier, more biocompatible and more mucoadhesive thus allowing the dosage is attached to the pocket, elimination through the normal catabolic pathway more quickly, reducing the risk of irritation or allergic reaction in placing side [19].

Chitosan-based hydrogels as a conducting material (drug vehicle) has been widely described in the literature. Chitosan is a substance that is not toxic, stable, can be sterilized and become as polymer that is biocompatible with a unique look and has antimicrobial properties, stimulate the immune system, hemostatic and wound healing, because of its characteristics, the chitosan can be considered to be the formulation of a conductor of antibiotics into the pocket periodontal and very favorable locally for periodontitis [21]

Popa L, Ghica M, Elena C in research on the use of chitosan as a raw material gel stating that the chitosan gel is a system that is adequate in the release of the medication locally in periodontal pockets, this material can stay in the pocket and the release of antimicrobial agents in sulcus liquid can be controlled. The release of Tetracycline gel with a concentration of 1% and 3% based kitosan has been test by L Popa [21]

Material And Methods

The type of this study is laboratory experimental study. This study was conducted at oral biology laboratory, Faculty of Dentistry, Airlangga University Surabaya. Samples of this study were pure culture of *A. actinomycetemcomitans* (ATCC 29 522), *P. gingivalis* (ATCC 33 277) and *F. nucleatum* (ATCC 25 586) were cultured with Mueller Hinton Agar (MHA).

The number of repetition in this study were 4 times. Tetracycline Gel 0.5%, 0.7%, 1% chitosan-based made with the preparation method reported by Popa L *et al* in 2013. Each material for the manufacture of tetracycline gel based chitosan weighed. Chitosan was added as much as 0.2 g, Citric acid 1% and added Tetracycline respectively 0.25 g, 0.35 g, 0.5 g which had been crushed and then stirred in a glass beaker until a homogeneous gel and then inserted into the syringe sterile. Material made at the time will try to test the sensitivity of bacteria in Surabaya to maintain the stability of the material.

The media for bacteria growth made as much as 12 grams of powdered Mueller Hinton To be dissolved in 240 ml of distilled water to 40 petri (20 ml / Petri), then reheated on the stove to boil magnetic. Then the media that have been cooked, sterilized in an autoclave for 15 minutes by air pressure 2 atm a temperature of 121 °C. Once sterilized, the media is stored in a refrigerator. If it will be reused, media reheated to a boil and then poured into each petri and allowed to cool. Activity breeding specimens was performed in anaerobic atmosphere in CO2 incubator. *A actinomycetemcomitans, P gingivalis* and *F nucleatum* used is each stem-cell specimens bacteria that have been bred purely on media Muller Hinton Agar (MHA), which had been prepared in the previous procedure in anaerobic atmosphere. A total of 1-2 ose of pure cultures of test bacteria that has been cultured and thrive suspended using 0.9% NaCl solution to obtain turbidity of Mc Farland 0.6 as standard or proportional to the number of bacteria 1 x 106 CFU / ml. Muller Hinton Agar (MHA) sterile taken in the refrigerator and removed and left in the room. Turn bunsen and heat ose ose that can be sterilized, heat until simmering in order ose ose sterile. Afterwards *A actinomycetemcomitans, P gingivalis* and *F nucleatum* taken from Muller Hinton Agar (MHA) and when opened must be near the bunsen so bacteria *A actinomycetemcomitans, P gingivalis* and *F nucleatum* not contaminated. MHA media that have been inoculated bacteria that make holes using a sterile metal ring with a diameter adjusted later try input material used consisted of concentration of 0.5%, 0.7%, 1% and controls using a micropipette into each hole in the media MHA, each of these actions were repeated four times and then incubated in the incubator at 37 ° C for 48 hours to observe and measure the diameter of the light zone (clear zone) that is shaped around the hole by using a ruler and calipers.

Data from *in vitro* test of the effectiveness of chitosan-based antimicrobial tetracyclines gel was analyzed using a statistical test of Kruskal-Wallis and Mann Whitney to see the effectiveness of tetracyclines gel 0.5%, 0.7%, 1% chitosan based on the growth of bacteria *A. actinomycetemcomitans, P. gingivalis and F. nucleatum.*

Results

Inhibitory zone diameters were measured to determine the effectiveness of tetracyclines gel against bacteria tested. Clear inhibition zone is a circular area which showed no bacterial growth in the surrounding area of drugs. The wider the circle diameter clear zone is the zone of inhibition greater. This can be seen in Figures 1, 2 and 3.

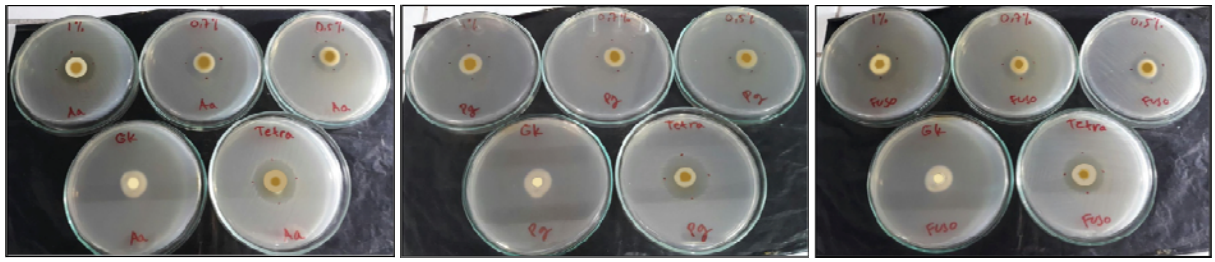

Fig 1, 2, 3: Zone inhibitory activity test each Tetracycline-based chitosan gel against bacteria *A. actinomycetemcomitans, P. gingivalis, F. nucleatum*

The diameter of inhibition zone against bacteria *A. actinomycetemcomitans* is in gel tetracyclines in MHA, while the smallest is in a concentration of 0.5%. Inhibition zone formed by all gel concentrations Tetracycline has a significant difference (P = 0.001) (Table 1).

Table 1: The diameter of inhibition zone of each gel Tetracycline and chitosan gel without tetracycline against bacteria *A. actinomycetemcomitans.*

Variable	Repetition				Mean	Median Interquartile range	P value
	1	2	3	4			
1% concentration	28,2	28,7	27,6	27,6	28,22	28,30 ± 0,88	0,001*
0,7% concentration	26,0	26,5	26,4	26,1	26,25	26,25± 0,45	
0,5% concentration	24,8	24,1	24,3	24,5	24,42	24,40± 0,57	
Chitosan Gel without Tetracycline	0	0	0	0	0	0	
Tetracycline 0,7% in MHA	29,8	30,2	30,1	29,6	29,92	29,95± 0,50	

Kruskal-Wallis test
(*) significant p<0,05

The diameter of inhibition zone against bacteria *P. gingivalis* is in gel tetracyclines in MHA, while the smallest is in a concentration of 0.5%. Inhibition zone formed by all gel concentrations Tetracycline has a significant difference (P= 0.001) (Table 2).

Table 2: The diameter of inhibition zone of each gel Tetracycline and chitosan gel without tetracycline against bacteria *P. gingivalis*.

Variable	Repetition				Mean	Median Interquartile range	P value
	1	2	3	4			
1% concentration	27,8	27,4	28,0	27,5	28,35	28,33 ± 0,78	0,001*
0,7% concentration	25,8	25,7	25,7	25,5	27,25	27,15± 0,47	
0,5% concentration	23,0	22,8	23,1	22,6	25,65	25,50± 0,66	
Chitosan Gel without Tetracycline	0	0	0	0	0	0	
Tetracycline 0,7% in MHA	29,6	29,4	29,7	29,8	29,97	29,95± 0,57	

Kruskal-Wallis test
(*) significant p<0,05

The diameter of inhibition zone against bacteria *F. nucleatum* is in gel tetracyclines in MHA, while the smallest is in a concentration of 0.5%. Inhibition zone formed by all gel concentrations Tetracycline has a significant difference (P = 0.001) (Table 3).

Table 3: The diameter of inhibition zone of each gel Tetracycline and chitosan gel without tetracycline against bacteria *F. nucleatum*.

Variable	Repetition				Mean	Median Interquartile range	P value
	1	2	3	4			
1% concentration	27,2	27,1	27,5	27,6	27,72	27,35 ± 0,88	0,001*
0,7% concentration	25,3	24,8	25,0	24,9	25,34	24,95± 0,40	
0,5% concentration	23,1	22,6	22,9	22,4	22,75	22,75± 0,60	
Chitosan Gel without Tetracycline	0	0	0	0	0	0	
Tetracycline 0,7% in MHA	28,9	28,6	28,7	28,6	28,70	28,65± 0,25	

Kruskal-Wallis test
(*) significant p<0,05

The data have been obtained by the normality test using the Shapiro-Wilk normality test. The results show the data distribution is not normal (P <0.05), so the results do not use a mean value but using the median value. Comparison of inhibitory zone Tetracycline gel 0.5%, 0.7%, 1%, chitosan gel without Tetracycline and Tetracycline in MHA against bacteria *A. actinomycetemcomitans*, *P. gingivalis*, *F. nucleatum* performed with the Mann-Whitney test after found significant differences the concentration of the Kruskal-Wallis test.

The diameter of inhibition zone against bacteria *A. actinomycetemcomitans* is in gel Tetracycline in MHA and our significant inhibition zone to a 1% concentration, 0.7% concentration, 0.5% concentration. Whereas in the group of tetracyclines based chitosan gel, the largest inhibition zone diameter is 1% and a significant difference seen with a concentration of 0.7% and 0.5% concentrations (Table 4).

Table 4: Comparison of inhibitory zone diameter of each test Tetracycline gel and chitosan gel without tetracyclines against bacteria *A. actinomycetemcomitans*.

Variable	Mean	Median Interquartile range	P value				
			1%	0,7%	0,5%	Chitosan	Tetrasiklin in MHA
1% concentration	28,22	28,30 ± 0,88	-	0,021*	0,021*	0,021*	0,021*
0,7% concentration	26,25	26,25± 0,45	0,021*	-	0,021*	0,021*	0,021*
0,5% concentration	24,42	24,40± 0,57	0,021*	0,021*	-	0,021*	0,021*
Chitosan Gel without Tetracycline	0	0	-	-	-	-	-
Tetracycline 0,7% in MHA	29,92	29,95± 0,50	0,021*	0,021*	0,021*	0,021*	-

Mann-Whitney test
(*) significant p<0,05

The diameter of inhibition zone against bacteria *P. gingivalis* is in gel Tetracycline in MHA and our significant inhibition zone to a 1% concentration, 0.7% concentration, 0.5% concentration. Whereas in the group of tetracyclines based chitosan gel, the largest inhibition zone diameter is 1% and a significant difference seen with a concentration of 0.7% and 0.5% concentrations (Table 5).

Table 5: Comparison of inhibitory zone diameter of each test Tetracycline gel and chitosan gel without tetracyclines against bacteria *P. gingivalis.*

Variable	Mean	Median Interquartile range	P value				
			1%	0,7%	0,5%	Chitosan	Tetrasiklin in MHA
1% concentration	28,35	28,33 ± 0,78	-	0,021*	0,021*	0,021*	0,021*
0,7% concentration	27,25	27,15± 0,47	0,021*	-	0,021*	0,021*	0,021*
0,5% concentration	25,65	25,50± 0,66	0,021*	0,021*	-	0,021*	0,021*
Chitosan Gel without Tetracycline	0	0	-	-	-	-	-
Tetracycline 0,7% in MHA	29,97	29,95± 0,57	0,021*	0,021*	0,021*	0,021*	-

Mann-Whitney test
(*) significant p<0,05

The diameter of inhibition zone against bacteria *F. nucleatums* is in gel Tetracycline in MHA and our significant inhibition zone to a 1% concentration, 0.7% concentration, 0.5% concentration. Whereas in the group of tetracyclines based chitosan gel, the largest inhibition zone diameter is 1% and a significant difference seen with a concentration of 0.7% and 0.5% concentrations (Table 6).

Table 6: Comparison of inhibitory zone diameter of each test Tetracycline gel and chitosan gel without tetracyclines against bacteria *F. nucleatums.*

Variable	Mean	Median Interquartile range	P value				
			1%	0,7%	0,5%	Chitosan	Tetrasiklin in MHA
1% concentration	27,72	27,35 ± 0,88	-	0,021*	0,021*	0,021*	0,021*
0,7% concentration	25,34	24,95± 0,40	0,021*	-	0,021*	0,021*	0,021*
0,5% concentration	22,75	22,75± 0,60	0,021*	0,021*	-	0,021*	0,021*
Chitosan Gel without Tetracycline	0	0	-	-	-	-	-
Tetracycline 0,7% in MHA	28,70	28,65± 0,25	0,020*	0,020*	0,020*	0,020*	-

Mann-Whitney test
(*) significant p<0,05

Discussion

The test results inhibitory zone Tetracycline gel 1%, 0.7%, 0.5% based on chitosan and chitosan gel without tetracyclines against bacteria *A. actinomycetemcomitans*, *P. gingivalis* and *F. nucleatum* showed a clear zone on the media Muller Hinton Agar circular around the wells. The clear zone indicates the antibacterial effectiveness of each medicinal concentration. The mean diameter of inhibition zone of each concentration above 27 mm. The results showed each drug substance has a strong inhibitory zone against bacteria *A. actinomycetemcomitans*, *P. gingivalis* and *F.nucleatum*. Tetracycline 1% gel-based chitosan showed the greatest inhibition zone diameter while Tetracycline 0.5% gel showed the smallest diameter of inhibition zone. Davis and Stout stated that the inhibition zone measuring less than 5 mm were formed on the agar diffusion test showed inhibition zone categorized as weak whereas inhibition zone measuring 5-10 mm is average, 10-19 mm and 20 mm are categorized strong or very strong categorized.[22] based on these statements, Tetracycline gel 1%, 0.7%, 0.5% based on chitosan have very strong antibacterial effect. These results are consistent with Ernie 2008 studies which stated that tetracyclines gel 0.7% cellulose-based biocompatible properties of the gingival fibroblasts cells and is effective against pathogenic bacteria periodontal [20].

Popa L, *et al* 2013 states that the higher the concentration of chitosan will reduce the ability of drug release. 3% chitosan base can act as a conductor of drugs modulating the most optimal [20]. This study uses chitosan base 4%, due to the necessary concentration of the drug is more viscous to be applied in periodontal pocket so as to increase the base of chitosan to 4%, the consistency can be achieved. Nevertheless Tetracycline-based chitosan gel has a very strong antibacterial effect. Tetracycline inhibition zone of 0.7% in the medium MHA greater than Tetracycline gel 1%, 0.7%, 0.5% chitosan-based and differ significantly. This is probably caused by the material of chitosan as a base drug carrier will release the drug slowly over time. Harry Research 2008 said chitosan as a base conductor sustained release drug has properties that will last for 5 hari [23]. Rodigues *et al* 2012 study showed that chitosan is able to become a local basis to deliver stable drug delivery and drug slowly over 7 hari [24].

In the past 25 years there have been more than 100 studies that compared the bacteria in plaque associated with periodontal health. The results of these studies indicate that the anaerobic bacteria, such as *A. actinomycetemcomitans* dominate in plaque and are closely related to the occurrence periodontitis.[25] In this study the inhibition zone test Tetracycline gel 1%, 0.7% and 0.5% chitosan-based against bacteria *A. actinomycetemcomitans*, *P. gingivalis* and *F. nucleatum.*

Muller Hinton agar is a medium that is used to study the effectiveness of test bacteria. The media has several advantages such as the ability to demonstrate the effectiveness of the test process on an ongoing basis with good bacteria, does not act as an inhibitor based drugs Sulfonamite, Trimethoprime and Tetracycline, and able to support the growth of almost all pathogenic bacteria that do not grow fast [19]. This research uses Muller Hinton media as periodontal pathogenic bacteria growth media.

Wells diffusion method is a method that enables easy measurement of inhibitory zone and show more tangible results for bacteria isolated not only move in the top surface of the media but also to bottom media [22]. This study uses the method because the test material is semi-solid material sinks to the method the test can be directly placed in bacterial media. Eko P 2009 in the research states that by wells method will demonstrate inhibition zone diameter larger than the disc diffusion method because the test material is placed directly so that the effect of the test material becomes stronger [23].

Chitosan is a hydrophilic polysaccharide derived from crustacean exoskeletons, crab and belangkas [18]. This study used chitosan derived from crab. Chitosan is a substance that is not toxic, biodegradable, biocompatible, inexpensive, and effective in releasing the drug. Chitosan has mucoadhesive properties, and the ability of gelling at low pH state, in addition to the chitosan has an antacid and antiulcer properties that may reduce the irritation of drug [16].

Chitosan is a polysaccharide that is very basic so that chitosan can form salts polyoxy, film, chelate metal ions, and the optical structure. Chitosan can be dissolved using dilute acid such as acetic acid and citric. Chitosan has the characteristic of forming hydrogels, hydrophilic polymer network that is highly capable of absorbing water, therefore the chitosan is very often used in drug delivery systems [17]. Chitosan is a good matrix-forming material in the form of micro and nano particles so that chitosan is able to be a release system controlled drug was very good and efective [18].

George and Abraham Research in 2006 regarding the toxicological properties of chitosan claim that chitosan is a hydrophilic polymer which is biodegradable and does not toxsic [24] Dionisio Rodrigues S and M study in 2012 stating that chitosan is an effective drug conducting material as an ingredient chitosan hydrophilic polymer stabil [23].

In this study, chitosan gel without Tetracycline is not effective at inhibiting the growth of bacteria *A. actinomycetemcomitans*, *P. gingivalis* and *F. nucleatum* which is a gram-negative anaerobic bacteria. These results are consistent with the statement Goy *et al* stated that the chitosan has a higher antibacterial effect against gram-positive bacteria than Gram negative [15].

Tetracycline is an antibiotic that bakteriostatic against gram negatve and positive, Tetracycline is also active against *A. actinomycetemcomitans* and other periodontal pathogens. Antibiotics also have anticollagenase properties and can reduce tissue destruction and bone resorption. Tetracycline also can directly inhibit MMPs (Matrix Metalloproteinase) issued by host immune cells in response to microorganism [16]. O Connor *et al* 1990 study stated that tetracyclines very effective against anaerobic bacteria such as bacteria *A. actinomycetemcomitans*. Golub *et al* 2005 study showed that Tetracycline, Doxycycline and minocycline can suppress the activity of the enzyme collagenase measured by using sulcus gingiva liquid [20]. The use of systemic antibiotics in the long time can disrupt the normal system of the body and cause bacterial resistance. Local antibiotics are a new approach to the management of localized periodontal infection. The main advantage is locally antibiotics can be incorporated directly into the pocket, avoiding side effects, increasing the exposure

to microorganisms targeted by higher concentration so the treatment becomes more terapeutic [14].

Tetracycline is the usual dose given to the patient as much as 250 mg 4 times daily so that makes patient compliance in taking the drug further reduced. The development of drug delivery locally will cover the weaknesses in the administration of tetracyclines in sistemic [17]. Hung and Douglass stated that non-surgical periodontal therapy in combination with antibiotics locally will provide better clinical outcomes compared to only non-surgical periodontal therapy [25]. Sachdva and Agarwal in research on the use of tetracyclines locally combined with scaling and root planing compared to scaling and root planing showed that a decrease in pocket depth and attachment of epithelial unifying improvement in patients who received local Tetracycline therapy combined with scaling and root planing [18]. same thing also stated by Thomas *et al* [19].

Based on the theory of systemic administration of Tetracycline, Tetracycline more effective against gram-positive bacteria, but in this study, gel Tetracycline is effective in inhibiting the growth of bacteria *A. actinomycetemcomitans*, *P. gingivalis* and *F. nucleatum*. This was probably due to the provision of locally can achieve higher drug concentration in the area of applied compared to systemic administration. Some researchers have observed drug delivery locally may reach concentrations 100-fold higher in the region given the drug compared with systemic administration, so as to reduce the total dose of more than 400 times that given to patients and drug resistance can be avoided [24]. Rodrigues *et al* 2004 states that Tetracycline is administered locally to have little likelihood of bacterial resistance as compared with the use of sistemically [25].

This research has shown the effectiveness of chitosan-based antimicrobial tetracyclines gel against bacteria *A. actinomycetemcomitans*, *P. gingivalis* and *F. nucleatum* which is periodontal pathogenic bacteria. This study could be the basis for further research, so that later Tetracycline-based chitosan gel can be applied clinically as supporting periodontal treatment.

Conclusion

This study concluded that the chitosan based Tetracycline gel as effective in inhibiting the growth of some bacteria *A. actinomycetemcomitans*, *P. gingivalis* and *F. nucleatum in vitro*. The mean diameter of inhibitory zone all Tetracycline gel more than 27 mm which showed the presence of antibacterial activity of the tetracyclines gel 1%, 0.7% and 0.5% chitosan-based is very strong while chitosan gel without tetracyclines have anti-bacterial activity. Tetracycline 1% gel-based chitosan has the most antibacterial activity because it shows that the greatest inhibition zone diameter compared with Tetracycline gel 0.7%, 0.5% based on chitosan and chitosan gel without Tetracycline. Chitosan can be used as a medium for the conductor of the drug topically, chitosan materials can release Tetracycline that in chitosan gel.

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