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### The effectiveness of metronidazole gel based chitosan inhibits the growth of bacteria *Aggregatibacter actinomycetemcomitans*, *Porphyromonas gingivalis*, *Fusobacterium nucleatum* (In vitro)

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#### Abstract

Periodontal disease is usually associated with microbial infections caused by biofilms, plaque and calculus. Bacteria *A. actinomycetemcomitans*, *P. gingivalis* and *F. nucleatum* is the dominant bacteria in periodontal disease. The purpose of this study was to determine and analyze the effectiveness of chitosan hydrogel-based metronidazole gel in inhibiting the growth of bacteria *A. actinomycetemcomitans*, *P. gingivalis* and *F. nucleatum* in vitro. Compounding the metronidazole gel 0.125%, 0.25%, 0.5%, 1% and 2% chitosan-based hydrogel and chitosan gel without metronidazole and testing antibacterial activity against bacteria *A. actinomycetemcomitans*, *P. gingivalis* and *F. nucleatum* carried out in the Laboratory of Microbiology oral, Airlangga University, Surabaya. Samples of bacteria in pure culture of *A. actinomycetemcomitans* (ATCC29522), *P. gingivalis* (33 277) and *F. nucleatum* (ATCC25586) were bred in the *Mueller Hinton Agar* which were followed by putting paper disc on top of the media and each paper disc etched with the test material. Then each of the media was incubated on days 1, 2 and 3 in the anaerobic incubator and inhibition zone is measured by using a caliper. The mean diameter of inhibitory zone of all metronidazole gel against bacteria *A. actinomycetemcomitans*, *P. gingivalis* and *F. nucleatum* are more than 22 mm which showed the presence of anti-bacterial activity is strong. Comparison of inhibitory effects of each concentration of metronidazole gel and chitosan gel without metronidazole against bacteria *A. actinomycetemcomitans*, *P. gingivalis* and *F. nucleatum* showed a significant difference ( $p = 0.000$ ). Metronidazole 2% chitosan-based hydrogels have the most powerful antibacterial activity because it shows the largest diameter of inhibition zone. Metronidazole-based chitosan hydrogel with the lowest concentration was 0.125% is also quite effective in inhibiting the growth of bacteria *A. actinomycetemcomitans*, *P. gingivalis* and *F. nucleatum*.

**Keywords:** metronidazole, hydrogel chitosan, periodontal pathogen

#### 1. Introduction

The periodontal disease is a chronic, degenerative disease which is localised on the gingiva, periodontal ligament, cementum and alveolar bone. These diseases are usually associated with microbial infection due to accumulation of a plaque biofilm and calculus. The current concept concerning the etiology of periodontal disease considers 3 groups of factors which determine whether active periodontal disease will occur: a susceptible host, the presence of pathogenic species, and the absence of so-called "beneficial bacteria". Gram negative and motile organisms increase significantly in accordance with increasing severity of disease.<sup>1,2</sup> Several suspected pathogens have been identified to be involved in the destructive periodontal disease. The most important species of which are *Aggregatibacter actinomycetemcomitans* (Aa), *Porphyromonas gingivalis* (Pg), *Tannerella forsythia* (Tf), *Treponema denticola* (Td), *Fusobacterium nucleatum* (Fn), *Prevotella intermedia* (Pi), *Campylobacter rectus* (Cr) dan *Eikenella corrodens* (Ec) [3, 4]

*Aggregatibacter actinomycetemcomitans* is a gram-negative bacteria found in the oral cavity and one of the etiology of aggressive periodontitis. *Aggregatibacter actinomycetemcomitans* was first identified as a possible periodontal pathogen in 1975 in studies of localized juvenile periodontitis, now known as localized aggressive periodontitis (LAP). These bacteria have the ability to produce leukotoksin which can lead damage to periodontal tissues [3]. *Porphyromonas gingivalis* previously known as *Bacteroides gingivalis*, is a strictly anaerobic, gram negative

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rod and black pigmented. P.g is a one of the major periodontopathogen with the ability to adhere, and to invade oral epithelia *in vitro*. Its importance as a periodontal pathogen is also highlighted by the research efforts aimed at developing a vaccine aimed at immunization against this bacterial species and thus preventing a chronic periodontal disease [1, 3] *Fusobacterium nucleatum* is a gram-negative anaerobic bacterium associated with gingivitis and chronic periodontitis. F.n. is an important periodontal pathogen, particularly in the beginning of the rapidly progressive periodontal disease. It creates very strong lipopolysaccharide as well as butyric acid as a metabolic end product [1]. The ability of F.n. to coaggregate with many plaque bacteria suggests that it acts as a *microbial bridge* between early and late colonisers. F.n. has also been described as an important initiator organism by promoting physico-chemical changes in the gingival sulcus, allowing pathogenic successors to establish and proliferate [3].

Elimination or adequate suppression of periodontal pathogens in the subgingival microflora is essential for periodontal healing to take place. Antimicrobial therapy in periodontology includes mechanical debridement of the root surfaces, oral hygiene measures and local and systemic antimicrobial chemotherapy. Numerous studies have shown that mechanical debridement cannot effectively eliminate some types of periodontopathogens, making it difficult to obtain or re-establish periodontal health. Targeted antimicrobial therapy could perhaps suppress or eliminate residual periodontal pathogens, and thus serve as an adjunct to conventional mechanical therapy [4, 5]

Antibiotics can be administered locally (immediate or controlled release) or systemically. Systemically administered antibiotics penetrate the periodontal tissues and the pocket via the serum. Systemic antibiotic therapy also has the potential to suppress any periodontal pathogenic bacteria colonizing the deep crevices of the tongue as well as clinically non diseased sites that could potentially cause chronic re-infection. Systemic antibiotic therapy is therefore advantageous for the eradication and prevention of infections by periodontal pathogenic bacteria that invade the subepithelial periodontal tissues or that colonize extradental areas [6, 7] However, administration of systemic antibiotics have side effects including hypersensitivity reactions, gastrointestinal intolerance and the development of bacterial resistance. Some studies also reported poor results due to the fact that the active product could not achieve an adequate concentration at the site of action and/or due to the inability of the active product to be retained locally for a sufficient period of time. The weakness of systemic antibiotics will be reduced if the antimicrobial agent applied locally [8]. studies focusing on the development of localized drug delivery systems for the release of antibiotics in the periodontal pockets are becoming more frequent. This approach leads to higher concentrations of the drug at the target sites, minimizing the potential systemic side effects [9].

Various local drug delivery system for treating periodontitis is Fibers, Film, Injectable systems, Gels, Strips and compacts, Vesicular systems, Microparticle system, Nanoparticle system [10]. Semisolid or gel formulations can indeed have some advantages. They possess a higher biocompatibility and bioadhesivity, allowing adhesion to the mucosa in the dental pocket and they can be rapidly eliminated through normal catabolic pathways, decreasing the risk of irritative or allergic host reactions at the application site [11]. Systemic antibiotic therapy for periodontal treatment usually involves

monotherapy based on metronidazole, tetracyclines (tetracycline, doxycycline, minocycline), clindamycin, ciprofloxacin and the  $\beta$ -lactams (amoxicillin with or without clavulanic acid) [7].

Metronidazole is a broad-spectrum antimicrobial activity against protozoan infections and anaerobic bacteria. In the late 1950s Metronidazole was first introduced for the treatment of trichomoniasis. At present, the antimicrobial metronidazole is one of the most widely used as antibacterial compounds in the treatment of periodontal disease [9]. Beside the periodontal pocket debridement (subgingival scaling and curettage), topical application of metronidazole seems to be very efficacious. Metronidazole efficiently inhibited anaerobic microorganisms in the periodontal pockets [13].

Sato et al. testing of local stocks on periodontal gel metronidazole *in vivo* performed in Mongrel Dogs have shown that metronidazole may be released in liquid krevikular gingival several times higher than the minimum inhibitory concentration of periodontal pathogens that grow in biofilm subgingival [9]. The association of metronidazole dental gel to scaling

and root planning gave the best results and it certifies that it has an important role in improvement of periodontal condition, preparing best conditions for periodontal surgery in more severe cases [15].

Hydrogels are cross-linked, three dimensional hydrophilic polymeric networks that swell but not dissolve when brought into contact with water. As a result, they are commonly used in clinical practice and experimental medicine for a wide range of applications, including biosensors, tissue engineering and regenerative medicine, separation of biomolecules or cells and barrier materials to regulate biological adhesions. Hydrogels can protect drugs from hostile environments, e.g. the presence of enzymes and low pH within the body fluids. Their porosity permits loading of drugs into the gel matrix and subsequent drug release at a pre-designed rate. [16] The injectable hydrogels can be originated from either natural biomacromolecules such as collagen, chitosan, alginate and hyaluronan, or synthetic materials such as poly(ethylene glycol) (PEG), poly(propyl fumarate) (PPF) and Pluronic F127 [17].

Chitosan is a mucoadhesive natural polysaccharide formed through N-deacylation of chitin obtained from shrimp or crab shells. Chitosan-based hydrogels have been largely described in literature as drug vehicle and its characteristics could be considered as a potentially beneficial approach in the formulation of periodontal systems for local, intra-pocket delivery of antibiotics for periodontitis [18]. Chitosan has advantage over other polysaccharides due to its non-toxicity, biocompatibility and biodegradability [16].

## 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. Metronidazole gel 0,125%, 0,25%, 0,5%, 1% dan 2% chitosan-based made with the preparation method reported by Yellanki S in 2010. Each material for the manufacture of Metronidazole gel based chitosan weighed. Chitosan was added as much as 3 g, Lactic acid 2% and added pure

Metronidazole powder respectively 0.125 g, 0.25 g, 0.5 g, 1 g, 2 g 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.

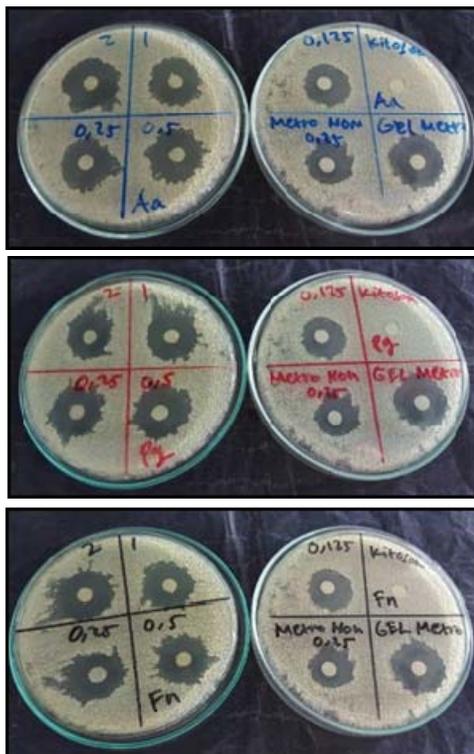
The media for bacteria growth made as much as 12 grams of powdered *Mueller Hinton* To be dissolved in 240 ml of distilled water to 40 petri (20 ml / Petri), then reheated on the stove to boil magnetic. Then the media that have been cooked, sterilized in an autoclave for 15 minutes by air pressure 2 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  $1 \times 10^6$  CFU / ml. BHI (*Brain Heart Infusion*) Broth 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 BHI (*Brain Heart Infusion*) Broth and when opened must be near the bunsen so bacteria *A. actinomycetemcomitans*, *P. gingivalis* and *F. nucleatum* not contaminated, then an incubator at 37 °C for 24 hours.

Bacteria that have been diluted by mixing 1 ose suspension of bacteria in a test tube containing a solution of NaCl which has been standardized and suitable concentration of 0.5 Mc Farland, the bacteria are smeared into *Mueller Hinton Agar* (MHA), planting is done with methods swabbing (equalization bacteria with swab). Petri disk divided into several sections according to the tested concentration is 0.125%, 0.25%, 0.5%, 1%, 2% and controls, then sterile paper disc with a diameter of +6 mm is placed each test material as much as 10 mL using micropipette, 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 paper disc by using a ruler and calipers.

Data from in vitro test of the effectiveness of chitosan-based antimicrobial Metronidazole gel was analyzed using a statistical test of Kruskal-Wallis and Mann Whitney to see the effectiveness of Metronidazole gel 0.125%, 0.25%, 0.5%, 1% and 2% 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 Metronidazole 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*

From figure 1, 2 and 3 show that the Metronidazole gel 0.125%, 0.25%, 0.5%, 1%, 2% based on chitosan, Metronidazole gel 0.25% in MHA and commercial Metronidazole gel to form a clear zone area paper discs on *Mueller Hinton Agar* to be so visible that these materials are effective in inhibiting the growth of bacteria *A. actinomycetemcomitans*, *P. gingivalis* and *F. nucleatum* (Tables 1, 2, 3). Chitosan gel without Metronidazole not form a clear zone area paper disc on *Mueller Hinton Agar* so that the material is not effective in inhibiting the growth of bacteria *A. actinomycetemcomitans*, *P. gingivalis* and *F. nucleatum*, therefore not performed statistical tests on the data inhibition gel chitosan without Metronidazole.

**Table 1:** The mean diameter of the inhibition zone respectively Metronidazole gel and chitosan gel without Metronidazole against bacteria *A. actinomycetemcomitans* on days 1, 2 and 3.

Variable	Mean Inhibitory zone diameter (mm)		
	Day 1	Day 2	Day 3
Metronidazole gel 0,125% chitosan based	19,52	19,52	19,52
Metronidazole gel 0,25% chitosan based	21,22	21,22	21,22
Metronidazole gel 0,5% chitosan based	21,97	21,97	21,97
Metronidazole gel 1% chitosan based	23,47	23,47	23,47
Metronidazole gel 2% chitosan based	24,57	24,57	24,57
Metronidazole gel 0,25% in MHA	24,8	24,8	24,8
Commercial Metronidazole gel	23,8	23,8	23,8
Chitosan gel without Metronidazole	0	0	0

**Table 2:** The mean diameter of the inhibition zone respectively Metronidazole gel and chitosan gel without Metronidazole against bacteria *P. gingivalis* on days 1, 2 and 3.

Variabel	Mean Inhibitory zone diameter (mm)		
	Day 1	Day 2	Day 3
Metronidazole gel 0,125% chitosan based	18,52	18,52	18,52
Metronidazole gel 0,25% chitosan based	20,95	20,95	20,95
Metronidazole gel 0,5% chitosan based	22,17	22,17	22,17
Metronidazole gel 1% chitosan based	23,45	23,45	23,45
Metronidazole gel 2% chitosan based	24,55	24,55	24,55
Metronidazole gel 0,25% in MHA	24,8	24,8	24,8
Commercial Metronidazole gel	23,87	23,87	23,87
Chitosan gel without Metronidazole	0	0	0

**Table 3:** The mean diameter of the inhibition zone respectively Metronidazole gel and chitosan gel without Metronidazole against bacteria *F. nucleatum* on days 1, 2 and 3.

Variabel	Mean Inhibitory zone diameter (mm)		
	Day 1	Day 2	Day 3
Metronidazole gel 0,125% chitosan based	18,02	18,02	18,02
Metronidazole gel 0,25% chitosan based	20,07	20,07	20,07
Metronidazole gel 0,5% chitosan based	21,72	21,72	21,72
Metronidazole gel 1% chitosan based	22,9	22,9	22,9
Metronidazole gel 2% chitosan based	23,95	23,95	23,95
Metronidazole gel 0,25% in MHA	24,82	24,82	24,82
Commercial Metronidazole gel	23,15	23,15	23,15
Chitosan gel without Metronidazole	0	0	0

Table 1, 2 and 3 shows that there is no difference in the diameter of the inhibition zone respectively Metronidazole gel and chitosan gel without Metronidazole on bacterial *A. actinomycetemcomitans*, *P. gingivalis* and *F. nucleatum* on days 1, 2 and 3.

*Kruskal-Wallis* test to compare the respective inhibitory concentration of Metronidazole gel and chitosan gel without Metronidazole against bacteria *A. actinomycetemcomitans*, *P. gingivalis* and *F. nucleatum* (Tables 4, 5, 6).

**Table 4:** The diameter of inhibition zone of each Metronidazole gel and chitosan gel without Metronidazole against bacteria *A. actinomycetemcomitans*.

Variable	Repetition				Median ± Interquartile range	p Value
	1	2	3	4		
Metronidazole gel 0,125% chitosan based	19,6	19,8	19,5	19,2	19,55 ± 0,48	0,000*
Metronidazole gel 0,25% chitosan based	21,2	21,5	21,2	21,0	21,20 ± 0,38	
Metronidazole gel 0,5% chitosan based	22,3	22,0	21,9	21,7	21,95 ± 0,48	
Metronidazole gel 1% chitosan based	23,8	23,6	23,0	23,5	23,55 ± 0,62	
Metronidazole gel 2% chitosan based	24,8	24,2	24,3	25,0	24,5 ± 0,72	
Metronidazole gel 0,25% in MHA	25,0	25,8	25,4	24,8	25,6 ± 0,80	
Commercial Metronidazole gel	23,7	23,8	24,0	23,8	23,80 ± 0,22	
Chitosan gel without Metronidazole	0	0	0	0	-	

*Kruskal-Wallis* test  
(\*) signifikan  $p < 0,05$

The diameter of inhibition zone against bacteria *A. actinomycetemcomitans* at 0.25% Metronidazole gel in MHA, while the smallest is on 0.125% Metronidazole gel-based chitosan. Inhibition zone formed by all gel concentrations Metronidazole has a significant difference ( $p=0.000$ ).

**Table 5:** The diameter of inhibition zone of each Metronidazole gel and chitosan gel without Metronidazole against bacteria *P. gingivalis*.

Variable	Repetition				Median ± Interquartile range	p Value
	1	2	3	4		
Metronidazole gel 0,125% chitosan based	18,2	18,9	18,8	19,1	18,52 ± 0,98	0,000*
Metronidazole gel 0,25% chitosan based	21,1	21,0	20,8	20,9	20,95 ± 0,25	
Metronidazole gel 0,5% chitosan based	22,5	22,1	21,3	21,8	22,17 ± 0,57	
Metronidazole gel 1% chitosan based	23,2	23,9	23,7	23,0	23,45 ± 0,80	
Metronidazole gel 2% chitosan based	24,0	25,1	24,8	25,3	24,55 ± 0,95	
Metronidazole gel 0,25% in MHA	24,8	25,1	24,6	24,7	24,85 ± 0,52	
Commercial Metronidazole gel	23,6	24,0	24,2	23,7	23,85 ± 0,52	
Chitosan gel without Metronidazole	0	0	0	0	-	

*Kruskal-Wallis* test  
(\*) signifikan  $p < 0,05$

The diameter of inhibition zone against bacteria *P. gingivalis* at 0.25% Metronidazole gel in MHA, while the smallest is on 0.125% Metronidazole gel-based chitosan. Inhibition zone formed by all gel concentrations Metronidazole has a significant difference ( $p = 0.000$ ).

**Table 6:** The diameter of inhibition zone of each Metronidazole gel and chitosan gel without Metronidazole against bacteria *F. nucleatum*.

Variable	Repetition				Median ± Interquartile range	p Value
	1	2	3	4		
Metronidazole gel 0,125% chitosan based	17,6	18,6	18,0	17,9	17,95 ± 0,48	0,000*
Metronidazole gel 0,25% chitosan based	19,8	20,6	19,9	20,9	19,95 ± 0,63	
Metronidazole gel 0,5% chitosan based	21,8	21,6	21,8	21,7	21,75 ± 0,18	
Metronidazole gel 1% chitosan based	22,2	22,8	23,2	23,4	23,0 ± 1,0	
Metronidazole gel 2% chitosan based	23,6	23,9	24,1	24,2	24,0 ± 0,5	
Metronidazole gel 0,25% in MHA	24,8	24,6	24,9	25,0	24,75 ± 0,55	
Commercial Metronidazole gel	22,6	22,8	23,7	23,5	23,15 ± 1,0	
Chitosan gel without Metronidazole	0	0	0	0	-	

*Kruskal-Wallis* test  
(\*) signifikan  $p < 0,05$

The diameter of inhibition zone against bacteria *F. nucleatum* at 0.25% Metronidazole gel in MHA, while the smallest is on 0.125% Metronidazole gel-based chitosan. Inhibition zone formed by all gel concentrations Metronidazole has a significant difference ( $p = 0.000$ ).

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 that a *Mann-Whitney*

test to compare the inhibitory zone between the concentration of each Metronidazole gel and chitosan gel without

Metronidazole against bacteria *A. actinomycetemcomitans*, *P. gingivalis* and *F. nucleatum* (Table 7, 8, 9).

**Table 7:** Results of *Mann-Whitney* test analysis comparison of inhibition zone of each concentration of Metronidazole gel and chitosan gel without Metronidazole against bacteria *A.actinomycetemcomitans*.

Variable	p Value							
	Metronidazole gel 0,125%	Metronidazole gel 0,25%	Metronidazole gel 0,5%	Metronidazole gel 1%	Metronidazole gel 2%	Metronidazole gel 0,25% in MHA	Metronidazole gel commercial	Chitosan gel without Metronidazole
Metronidazole gel 0,125%		0,020	0,021	0,021	0,021	0,021	0,020	0,014
Metronidazole gel 0,25%			0,020	0,020	0,020	0,020	0,019	0,013
Metronidazole gel 0,5%				0,021	0,021	0,21	0,020	0,014
Metronidazole gel 1%					0,021	0,021	0,076*	0,014
Metronidazole gel 2%						0,021	0,021	0,014
Metronidazole gel 0,25% in MHA							0,020	0,014
Metronidazole gel commercial								0,013
Chitosan gel without Metronidazole								

*Uji Mann-Whitney*  
 (\*) Not significant  $p > 0,05$

Their significant inhibition zone differences between each concentration of Metronidazole gel and chitosan gel without Metronidazole against bacteria *A. actinomycetemcomitans*, *P.*

*gingivalis* and *F. nucleatum*, but there is no significant difference between the inhibition zone Metronidazole 1% with commercial Metronidazole gel.

**Table 8:** Results of *Mann-Whitney* test analysis comparison of inhibition zone of each concentration of Metronidazole gel and chitosan gel without Metronidazole against bacteria *P. gingivalis*.

Variable	p Value							
	Metronidazole gel 0,125%	Metronidazole gel 0,25%	Metronidazole gel 0,5%	Metronidazole gel 1%	Metronidazole gel 2%	Metronidazole gel 0,25% in MHA	Metronidazole gel commercial	Chitosan gel without Metronidazole
Metronidazole gel 0,125%		0,021	0,021	0,021	0,021	0,021	0,021	0,014
Metronidazole gel 0,25%			0,021	0,021	0,021	0,021	0,021	0,014
Metronidazole gel 0,5%				0,021	0,021	0,021	0,021	0,014
Metronidazole gel 1%					0,021	0,021	0,191*	0,014
Metronidazole gel 2%						0,021	0,059*	0,014
Metronidazole gel 0,25% in MHA							0,021	0,014
Metronidazole gel commercial								0,014
Chitosan gel without Metronidazole								

*Uji Mann-Whitney*  
 (\*) Not significant  $p > 0,05$

Their significant inhibition zone differences between each concentration of Metronidazole gel and chitosan gel without Metronidazole against bacteria *A. actinomycetemcomitans*, *P. gingivalis* and *F. nucleatum*. But there is no significant

difference between the inhibition zone Metronidazole gel 1% with commercial Metronidazole gel and between 2% Metronidazole gel with commercial Metronidazole gel.

**Table 9:** Results of *Mann-Whitney* test analysis comparison of inhibition zone of each concentration of Metronidazole gel and chitosan gel without Metronidazole against bacteria *F. nucleatum*.

Variable	p Value							
	Metronidazole gel 0,125%	Metronidazole gel 0,25%	Metronidazole gel 0,5%	Metronidazole gel 1%	Metronidazole gel 2%	Metronidazole gel 0,25% in MHA	Metronidazole gel commercial	Chitosan gel without Metronidazole
Metronidazole gel 0,125%		0,021	0,020	0,021	0,021	0,021	0,021	0,014
Metronidazole gel 0,25%			0,020	0,021	0,021	0,021	0,021	0,014
Metronidazole gel 0,5%				0,020	0,021	0,020	0,020	0,013
Metronidazole gel 1%					0,021	0,021	0,468*	0,014
Metronidazole gel 2%						0,021	0,043	0,014
Metronidazole gel 0,25% in MHA							0,021	0,014
Metronidazole gel commercial								0,014
Chitosan gel without Metronidazole								

Uji *Mann-Whitney*/(\*) Not significant  $p > 0,05$ 

Their significant inhibition zone differences between each concentration of Metronidazole gel and chitosan gel without Metronidazole against bacteria *A. actinomycetemcomitans*, *P. gingivalis* and *F. nucleatum*. But there is no significant difference between the inhibition zone Metronidazole 1% with commercial Metronidazole gel.

### Discussion

In this study tested the effectiveness of Metronidazole-based chitosan and chitosan gel without Metronidazole on bacterial *A. actinomycetemcomitans*, *P. gingivalis* and *F. nucleatum*, because in the last 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. *P. gingivalis* is a one of the major periodontopathogen with the ability to adhere, and to invade oral epithelia in vitro. Its importance as a periodontal pathogen is also highlighted by the research efforts aimed at developing a vaccine aimed at immunization against this bacterial species and thus preventing a chronic periodontal disease [1, 3]. *F. nucleatum* was able to coaggregate with many plaque bacteria suggests that it acts as a *microbial bridge* between early and late colonisers. In addition to its ability to coaggregate with many oral bacteria, *F. nucleatum* has also been described as an important initiator organism by promoting physico-chemical changes in the gingival sulcus, allowing pathogenic successors to establish and proliferate [3]. This study uses a base of chitosan concentration of 3% and 2% lactic acid, because Popa L, et al 2013 states that the higher the concentration of chitosan will reduce the ability of drug release [18] Yellanki et al research in 2010 using metronidazole 0.25% mixed 2% lactic acid and 3% chitosan results showed a higher concentration of the drug, bioadhesive better and more controlled drug release. Metronidazole gels can be successfully prepared using natural polymers which can be targeted in treatment of the periodontal disease and also reduce dosing frequency, increase bioavailability of Metronidazole that will result in better patient compliance with minimum side effects [19]. Materials used in this study was metronidazole gel 0,125%,

0.25%, 0.5%, 1% and 2% based on chitosan, metronidazole 0.25% in the MHA, commercial metronidazole gel and chitosan gel without metronidazole. Chitosan is a hydrophilic polysaccharide derived from crustacean exoskeletons, crab and belangkas. 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 [20]. 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. 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 effective [21]. 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 toxic. Metronidazole is a nitroimidazole, used to treat protozoal infections. It is bactericidal to anaerobic organisms and is believed to disrupt bacterial DNA synthesis. Metronidazole is not the drug of choice for treating *Actinobacillus actinomycetemcomitans* infections. However, it is effective against *Actinobacillus actinomycetemcomitans* when used in combination with other antibiotics. Metronidazole is also effective against anaerobes such as *Porphyromonas gingivalis* and *Prevotella intermedia* [22]. But in this study, metronidazole gel without combination with other antibiotics effective in inhibiting the growth of bacteria *A. actinomycetemcomitans*. This is possibly because the drugs are directly applied to the test bacteria so that higher drug concentrations can be achieved.

Many studies suggest that the use of metronidazole with the recommended dose for use in dentistry both for local and systemic treatment is very safe. Metronidazole as local drug delivery has been proven to be safer (lower serum concentration) when compared with systemic administration

[20] It can be used as an adjunct to scaling and root planing and for periodontal maintenance therapy. It has been observed that the local route of drug delivery can attain 100-fold higher concentrations of an antimicrobial agent in subgingival sites compared with a systemic drug regimen thereby reducing the total patient dose by over 400 fold avoiding development of drug-resistant at non oral body sites [23]

The test results inhibitory zone Metronidazole gel 0.125%, 0.25%, 0.5%, 1% and 2% based on chitosan, metronidazole 0.25% in the MHA, commercial metronidazole gel against bacteria *A. actinomycetemcomitans*, *P. gingivalis* and *F. nucleatum* showed a clear zone on the media Muller Hinton Agar circular around the paper disc. The clear zone indicates the antibacterial effectiveness of each medicinal concentration. The mean diameter of inhibition zone of each concentration above 22 mm. The results showed each drug substance has a strong inhibitory zone against bacteria *A. actinomycetemcomitans*, *P. gingivalis* and *F. nucleatum*. The conclusion was based on research Greenwood 1995, if the diameter of the inhibition zone formed <10 mm, considered to be weak between 10 mm and 15 mm, moderate between 15 mm and 20 mm, strong if > 20 mm [24] Based on these statements, Metronidazole gel 0.125%, 0.25%, 0.5%, 1% and 2% based on chitosan have a strong antibacterial effect. While chitosan gel without Metronidazole is ineffective in inhibiting the growth of bacteria *A. actinomycetemcomitans*, *P. gingivalis* and *F. nucleatum*. 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 [25].

The result of the inhibition of Metronidazole 2% gel-chitosan based have inhibitory zone the largest among all the concentration of Metronidazole is tested against the bacteria *A. actinomycetemcomitans*, *P. gingivalis* and *F. nucleatum*. Metronidazole 1% gel-chitosan based showed inhibition similar to or not significantly different from the commercial Metronidazole gel to each bacterium tested by inhibition zone > 23 mm. Although there are significant differences between the inhibition zone commercial Metronidazole gel compared to Metronidazole 0.25% gel-chitosan based, but Metronidazole 0.25% gel-chitosan based produce inhibition zone > 20 mm and categorizing them stronger.

This research has shown the effectiveness of chitosan-based antimicrobial Metronidazole 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 Metronidazole gel-chitosan based can be applied clinically as supporting periodontal treatment.

### Conclusion

In this study, we concluded that the chitosan-based metronidazole gel has the effectiveness in inhibiting the growth of some bacteria *A. actinomycetemcomitans*, *P. gingivalis* and *F. nucleatum* in vitro. The mean diameter of inhibitory zone all chitosan-based metronidazole gel more than 22 mm which showed the presence of antibacterial activity Metronidazole 0.125%, 0.25%, 0.5%, 1% and 2% based on chitosan was strong while chitosan gel without Metronidazole has no antibacterial activity. Metronidazole gel 2% based chitosan has antibacterial activity most good because it shows that the inhibition zone diameter greater than 0.125% metronidazole gel, 0.25%, 0.5% and 1% based on chitosan and chitosan gel without metronidazole. Metronidazole gel 0.25% chitosan-based produce inhibition

zone > 20 mm so that the inhibitory zones are also considered strong. Chitosan can be used as a medium for the conductor of the drug topically as chitosan materials can release Metronidazole is in chitosan gel.

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