

## Research Report

# The effectiveness of 0.5–0.7% tetracycline gel to reduced subgingival plaque bacteria

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## ABSTRACT

**Background:** The tetracycline was an antimicrobial agent, that a broad spectrum. In addition to the antimicrobial effects, their efficacy was also anticollagenase and removal of the smear layer on the root surface. **Purpose:** The aim of the study was to evaluate effectiveness tetracycline gel 0.5–0.7% to reduction subgingival plaque bacteria. **Method:** A laboratory experimental study was conducted to investigate the effectiveness tetracycline gel 0.5–0.7%. Samples were divided into 5 groups with different concentration. The antimicrobial effect was performed using spectrophotometer. The statistical test was used One-Way ANOVA with significant difference 5% and subsequently Tukey-HSD test. **Result:** The study showed that tetracycline gel 0.5% has the highest antimicrobial effect. **Conclusion:** Tetracycline gel with 0.5% concentration is effective in inhibiting the growth of subgingival plaque bacteria.

**Key words:** subgingival plaque bacteria, tetracycline gel 0.5–0.7%

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## INTRODUCTION

Periodontal disease is accepted as an infection of the periodontium since the primary etiologic factor is bacteria, which triggers the host immune response and tissue destruction.<sup>1</sup> The most important in periodontitis therapy is plaque and calculus removal with scaling and root planning. However recurrence frequently occurs due to the invasion of periodontal pathogen in gingival epithelium, cementum and dentin tubulus which is unreachable by mechanical removal.<sup>2</sup> It recently has been considered that mechanical treatment only is insufficient in the periodontal treatment strategy. Antimicrobial therapy, especially is of importance as an adjunct to mechanical periodontal treatment.<sup>3</sup> The most widely used antibiotics in the treatment of periodontal disease have been tetracycline since they show the highest concentrations in gingival crevicular fluid (GCF) and are highly effective on *Actinobacillus actinomycetemcomitans* (Aa).<sup>4</sup> In addition to the antimicrobial effects, their efficacy was also anticollagenase and removal of the smear layer on the root surface.<sup>5</sup> Regarding the side effects of systemic antibiotic treatments, local delivery system have

been developed in the last quarter of the 20<sup>th</sup> century. Many studies on clinical effects of both systemic and local tetracycline have been performed. In some of these studies, systemic and local tetracycline provided significant decreases even in probing depth, clinical attachment levels and improved clinical parameters.<sup>6</sup> A number of local drug delivery devices have been proposed, including fibers, strips, films, gels, sponges and micro particles. Goodson *et al.*, 2004 developed ethyl vinyl-acetate hollow fibers loaded with tetracycline HCl, but the fibers being nonresorbable, have to be removed after 10 days. These delivery systems have a number of shortcomings, including limited duration of drug release, difficulty in application and poor retention in the periodontal pocket.<sup>7</sup> Tetracycline gel could be effective in the pocket for 48 hours to 14 days.<sup>8</sup> Hydrophilic gel is one of semisolid prepares with some advantages such easily and quickly applied, unsticky and comfort.<sup>9</sup>

Currently, metronidazole gel 25% is sold in Indonesia is metrodanizole gel 25%. Metrodanizole can survive in gingival sulcular fluid only for 24 hours. Metrodanizole is effective for obligate anaerobic bacteria and spirochaeta. As a choice for facultative anaerob bacteria are ampicillin,

chloramphenicol, tetracycline, and clindamycin.<sup>10</sup> The use of local antimicrobial to this time is at 10–25%. In high concentration, tetracycline is toxic towards gingival epithelial cells and fibroblasts, thus the choice for local antimicrobial material needs biocompatible concentration and able to function optimally in inhibition of mouth microorganism, especially subgingival bacteria. Tetracycline is biocompatible to fibroblast cell at below 0.7% but the question whether biocompatible of tetracycline is effective to reduce the subgingival plaque bacteria. While the aim of this research was to obtain certain concentration of tetracycline gel that effective in inhibiting subgingival plaque bacteria.

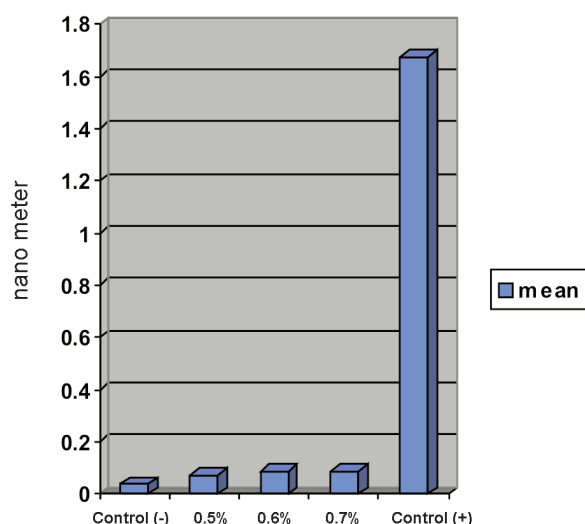
## MATERIALS AND METHODS

The type of this research is experimental laboratory study with research design post test only control group. The sample size was 9 patients. This research is conducted in Periodontics clinic and microbiology laboratory of Faculty of Dentistry, Airlangga University. Material used are mouth mirror, pinset, periodontal probe, spiritus brander, excavator, glass beaker, reaction tube, measuring glass, mortar and pestle, measuring pipette, electric scale, incubator, spectrophotometer. The material used is hydroxypopile methylcellulose, propylene glycol, aquadest, pure tetracycline hydrochloride, Brain Heart Infusion (BHI) media, gas pack. Subgingival plaque bacteria taken from patient's pocket who visit Periodontics clinic Faculty of Dentistry, Airlangga University; with the periodontitis diagnosis criteria as follows: pocket depth 5–7 mm, had not consume any antibiotic for 6 months, had no systemic problems. Nine patients that had already checked according to criteria, had their subgingival plaque extracted using sterile excavator and quickly inserted to Brain Heart Infusion media in reaction tubes. After that it is incubated in the incubator for 48 hours to grow anaerobic bacteria. The research are divided to 5 different treatment groups, that are, groups added tetracycline gel with concentrations of 0.5%, 0.6%, 0.7%, groups with control positive and control negative are each replicated 7 times. Samples are incubated for 24 hours, which then had the inhibition of subgingival plaque bacteria scaled using spectrophotometer uv-vis to see the growth of bacteria marked with the degree of optical density with the wavelength of 570 nm.

To compare the effectivity of 0.5–0.7% tetracycline hydrochloride gel concentration towards the prevention of subgingival plaque bacteria, ANOVA statistic test is used.

## RESULT

The inhibitory capacity of tetracycline towards subgingival plaque bacteria are examined according to the degree of optical density analyzed using spectrophotometer



**Figure 1.** Graphic of mean of optical density 0.5–0.7% tetracycline gel toward subgingival plaque bacteria.

with the wavelength of 570 nm. According to the observed calculation, the result could be seen in table 1 and 2.

**Table 1.** The mean of optical density 0.5–0.7% tetracycline gel toward subgingival plaque bacteria

| Treatment | Control – | 0.5%  | 0.6%  | 0.7%  | Control + |
|-----------|-----------|-------|-------|-------|-----------|
| mean      | 0.04      | 0.073 | 0.088 | 0.088 | 1.672     |

Table 1, it can be seen that the average degree of optical density after the application of tetracycline gel at 0.5% is the lowest. While in positive control, the graphic rises sharply. To identify the difference between each treatments ANOVA test is done, the result is  $p < 0,00$ , since  $p < 0,05$  thus means that there is a significant difference of optical density degree between the application of tetracycline gels in varied concentrations. The next analysis used Tukey-HSD examination with result as shown in table 2.

**Table 2.** The result of Tukey-HSD of optical density 0.5–0.7% tetracycline gel toward subgingival plaque

|           | Control – | 0.5%  | 0.6%  | 0.7%  | Control + |
|-----------|-----------|-------|-------|-------|-----------|
| Control – | -----     | 0,00* | 0,00* | 0,00* | 0,00*     |
| 0.5%      |           | ----- | 0,03* | 0,08* | 0,00*     |
| 0.6%      |           |       | ----- | 1,0   | 0,00*     |
| 0.7%      |           |       |       | ----- | 0,00*     |
| Control + |           |       |       |       | -----     |

\* = significant

## DISCUSSION

Antibiotics are organic or synthetic substance which functions as a way to inhibit or kill bacteria. According to The American Academy of Periodontology, the administering of systemic antibiotic has significant meaning if given to certain types of periodontitis. Those are acute periodontitis, refractory periodontitis, periimplantitis, acute periodontal infection with systemic manifestation or for prophylaxis.<sup>1</sup> Antibiotics are only administered, in case conventional treatments are not effective. While local antimicrobial material can be used as adjunction of scaling and root planning, especially in deep moderate periodontal pocket. Due to unreachable in deep pockets therefore the result of plaque control cannot be predicted.<sup>11</sup>

Tetracycline is one of the antibiotics choice for periodontitis cases. In aggressive periodontitis case, combination of antibiotics of amoxicillin-metronidazole or combinations of tetracycline-metronidazole is often used.<sup>4</sup> Tetracycline can be consumed systemically or locally. Systemic tetracycline 250 mg 4 times a day, it is seen that the concentration reached in the gingival sulcular fluid is between 0 to 8 µg/ml.<sup>12</sup> *Actinobacillus actinomycetemcomitans* bacteria have Minimal Inhibitory Concentration (MIC) and Minimal Bactericidal Concentration (MBC) more than 64 microgram/ml, while *porphyromonas gingivalis* have MIC 0.5 microgram/ml and MBC 16 microgram/ml.<sup>10</sup> Biofilm in plaque bacteria, its extra cellular components will inhibit the diffusion of antimicrobials components. Therefore, subgingival plaque in the form of biofilm need antibiotics with 50 times the amount of concentration. With the local application of antimicrobial preparation, the concentration in sulcus gingival fluid can reach 100 times higher.<sup>12</sup> The effectivity of antimicrobial preparation applied can be reached if the concentration in the pocket is enough and can inhibit the growth of subgingival plaque bacteria.

Previously research, it was concluded that tetracycline gel was biocompatible with fibroblast cells at concentration below 0.7%,<sup>5</sup> but still not known its inhibition capacity towards subgingival plaque bacteria. In this research, inhibition capacity of tetracycline gel is examined with concentration between 0.5–0.7% towards subgingival plaque bacteria. From this research result, it is proven that tetracycline gel with 0.5% concentration is adequate for inhibition of subgingival plaque bacteria compared to the positive control. This proves that tetracycline gel could be applied locally in 0.5% concentration. It shows that the concentration inside the pocket can reach up to 5000 µg/ml. That concentration is far above the MIC or MBC of periodontopathogenic bacteria, and adequately effective to inhibit subgingival plaque bacteria.<sup>10</sup>

The use of tetracycline gel with high concentration that is 10–35% will cause bitterness at application to the patient, and also toxic towards epithelial cell and gingival fibroblast. Nevertheless, the use of tetracycline at 25% in the form of film strips is very difficult and takes a lot of time because it has to be placed below the interproximal contact point and

tied securely with a thread.<sup>13</sup> By applying tetracycline as gel, it can be absorbed into the gingival connective tissue and penetrate the root surface, therefore increasing the antibacterial capacity towards periodontopathogenic bacteria.<sup>8</sup> In addition, Tetracycline also inhibits the activity of matrix metalloproteinase produced by PMN (MMP-8 and MMP-9) by binding  $\text{Ca}^{2+}$  and  $\text{Zn}^{2+}$  which is located at its active side. So it inhibits the resorption of alveolar bones and collagen degeneration.<sup>1</sup> Tetracycline also display a number of independent, pleiotropic activities on inflammatory and immune processes. They have been shown to specifically decrease level of inducible NO synthesis, inhibit phospholipase A2 and cyclooxygenase-2 mediated prostaglandin synthesis.<sup>14</sup> This wide array of biological activities has resulted in the widespread application of tetracycline – based therapy in inflammatory disorders such as periodontitis and rheumatoid arthritis. Following the discovery of a striking variety of non antibiotic properties of tetracycline, the therapeutic indications for these drugs have been successfully extended in the past 15 years to periodontal disease and potential new applications are being explored for conditions as stroke, aortic aneurysm, Parkinson's disease and metastatic cancer. This is certainly in large part due to the tetracyclines' ability to inhibit the release of proinflammatory mediators, including NO, TNF alpha and IL-1 as well as the production and activity of enzymes directly involved in tissue destruction, such as matrix metalloproteinase and stromelysin.

The application of antimicrobial preparation can only be done in certain periodontitis cases. Systemic antibiotic therapy combined with mechanical therapy generally has been noted to be a beneficial therapeutic approach in refractory periodontitis previously unsuccessfully treated with solely conventional therapy. Local antimicrobial preparations is suggested to be applied for chronic periodontitis cases after scaling and root planning 2 times a week for two weeks. It seems clear that mechanical treatment is essential for periodontal therapy. However, it has been noted that if the microbiological or clinical analysis indicate persistent pathogenic infection, so local antibiotic therapy should be considered. Thus, the tetracycline gel formulation along with with scaling root and planning was effective in reducing gingival inflammation, bleeding on probing, pocket depth and increasing clinical attachment.<sup>8</sup> In summary, our result suggest that tetracycline gel with 0.5% concentration is effective in inhibiting the growth of subgingival plaque bacteria.

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