

Efficacy of Mouth Rinse Formulation Based On Cetyl Pyridinium Chloride in the Control of Plaque as an Early Onset of Dental Calculus Built Up

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Abstract

This study aimed at comparing the antiplaque and antigingivitis potential of a mouth rinse containing Essential Oil, zinc and fluoride with a CPC-containing mouth rinse over a short term period. This study is a double blind randomized cross over clinical trial with a 3 days run in phase (wash out period). A non-brushing 3 days intervention was conducted with 18 females with mean age of 21 years old and a single examiner throughout the study. Prophylaxis was done after each intervention. Four mouth rinse were tested on 4 groups; 1: negative control, 2: mouthwash with alcohol, 3: mouthwash with CPC 0.1%, and 4: mouthwash with CPC 0.07%. Oral hygiene scores which consist of Plaque Index (PI), Gingival Index (GI), and bacterial load measurement using CariScreen were taken and analyzed.

The result shows that the most effective to the less effective mouth rinse in inhibiting bacterial built up (antiplaque) are mouthwash 2, 3, 4 and 1 respectively. However, but with no statistically significant effect in antigingivitis.

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Introduction

Protective factors have been of increasing interest over the last several decades as dentistry has shifted from treating the existing disease to preventing future disease. Oral biofilms have been related to the development and severity of both gingivitis and periodontitis. Therefore, oral biofilm control plays a key role in prevention, treatment, and decrease of recurrence of periodontal diseases.¹ Although this relationship has long been recognized, appropriate self-performed biofilm control still remains an area with opportunity for improvement. Non-invasive techniques such as rinsing with adequate mouthwash for preventing plaque build up which indicates early onset of

calculus build up are low cost, feasible and sustainable.² Therefore research on mouthwash efficacy is important to be able to give adequate information to consumers. Hence diagnosis and evaluation of plaque build up are also important to evaluate the effectiveness of the non-invasive treatments.

Cetylpyridinium chloride (CPC)-containing mouth rinses are indicated for regular daily use, aiming at preventing and controlling biofilm and gingivitis.³ Since the choices of the CPC, concentration may lead to different patterns of clinical use, comparative studies can better support the choice between different concentrations of CPC.⁴ CPC is a quaternary ammonium compound that has demonstrated plaque and gingivitis reducing benefits when compared with placebos.⁵

A systematic review of CPC-containing mouth rinses supported additional benefit in reducing plaque accumulation and gingival inflammation provided by this agent when used in combination with either supervised or unsupervised oral hygiene. As mentioned before,

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in an attempt to meet professional and consumer needs, new products have been formulated providing multiple benefits and consumer value.⁶

The purpose of this study is to test if this CPC containing mouthwash show the same pattern of progressive cumulative clinical benefits. To test this new formula, a group of Indonesians were selected to be the study population, considering that literature has shown higher prevalence and severity of gingivitis in developing countries than in populations in developed countries.

Materials and methods

This study is a before and after cross over randomized double blind clinical study. Female subjects aged 18 years and over were recruited. Subjects were given a prophylaxis after each run in phase and then use a silica fluoride toothpaste to use for 3 days as the run in phase. Oral hygiene scores which consist of Plaque Index (PI), Gingival Index (GI), and bacterial load measurement were taken and analyzed.⁷ Each respondent were randomly allocated to the test product. Subjects used the study products as part of their normal oral hygiene regimen. Oral soft tissues will be assessed at each examination for detecting any adverse event such as allergic affect. Adverse events were monitored throughout the study. Any concomitant medication, including food supplements and prophylactic treatments were recorded. A Single Examiner did the examinations to minimize potential biases. Intra rater examiner were analyzed with 10% of samples to optimize the reliability of examinations.

At baseline and after 3 days of mouth rinse, subjects received the oral examination. Following randomization, subjects received a prophylaxis and will begin brushing twice daily with the provided fluoride toothpaste and rinsing twice daily with 15 mL of the assigned mouth rinse for 30 seconds. Convenient sampling will be employed, concordant with the stated inclusion and exclusion criteria. Subjects will be monitored for compliance by keeping good contact with them by regular texting. Moreover, mouthwash are bottled for a weekly proportion, so then subjects need to refill with new mouthwash bottles, meeting the research administrator. Motivational interviewing on meetings will conducted and potential adverse

events will be well monitored. Further, remuneration were periodic.

The inclusion criteria were female subjects aged 18-30 years, willing to participate and have signed the informed consent, able to comply with the study procedures, no medical conditions that prevent a person from brushing their teeth, has minimum of 20 natural healthy teeth with no indication of extraction, has Turesky modification of the Quigley and Hein Index more than 1.5, has Loe and Silness Index more than 1, has Class 3 Calculus in lower lingual teeth 321|123, non-smoker.⁸ Exclusion Criteria were occurrence of adverse event, withdrawal, sickness that could bias the results, not complying with the study procedures that could bias the research results, such as using xilytol gum, CCP ACP, gargling solution and other medication.

Subjects were monitored for the primary outcomes of Gingival Index (GI) and Plaque Index (PI) at baseline and after 3 days.⁹ Subjects were randomly assigned for mouth rinse groups to do unsupervised rinsing twice a day use with CPC or positive or negative control rinse in conjunction with normal brushing. Subjects may discontinue from the clinical study at any time. Subjects are participating this study with their freewill, with full consciousness and without any coercion. In addition, the Principal Investigator has the right to withdraw a subject for any reason that is stated in the exclusion criteria and also for any reason that is in the best interests of the subject, such as force major. The study protocol was considered and approved by the Ethics Committee of the Faculty of Dentistry, Universitas Indonesia (reference number 35/VII/15, No. Protocol: 09760715).

Clinical assessment of mouth rinse efficacy will be assessed before and after 3 days after tested mouthwash usage by using CariScreen Meter and intra oral health indexes. Swab sample will be taken of the plaque from the subjects' teeth, which when combined with special bioluminescence reagents within the swab, will create a reaction which is then measured with the meter. The CariScreen will give a score between 0 and 9,999. A score under 1,500 is considering relatively healthy, while above that shows considerable high bacterial load. A study using oral clinical specimens from pediatric patients demonstrate that ATP-driven bioluminescence can be used in the direct

determination of bacterial numbers, and can serve as a general assessment indicator for oral hygiene.¹⁰ ATP-driven bioluminescence may potentially serve as a component of tartar built up risk assessment.

Results

No side effect nor adverse events occurred, nonetheless respondents complained of the strong flavor of the mouth rinse number 2. The data were assessed for a normal distribution using the Shapiro-Wilk test for normality using the computer software SPSS Statistics. Parametric or nonparametric tests were used as appropriate. All of the statistical tests were two-tailed, and the significance level was set at 0.05. This means that any p-value lower than 0.05 showed statistically significant difference. Research results represented in Table 1, showed the mean of Cariscreen measurements results before and after mouth rinse usage. The CariScreen will give a score between 0 and 9,999. The higher the score the higher the bacterial load. The coding were 1 = T-10; 2 = T-20; 3 = T-30; 4 = T-40.

Mouth rinse	Before Mean (SD)	After Mean (SD)	Delta (After-Before) Mean (SD)	P-value Before VS After (Wilcoxon)
1	4305 (3070)	7619 (1836)	3314 (2173)	0.001
2	5155 (2413)	3092 (2448)	-2063 (2277)	0.003
3	5604 (2469)	6654 (3048)	1050 (2470)	0.112

Table 1. Mean (Standard deviation), of the pre- and post-treatment Cariscreen Measurement Result and reduction by treatment group.

Mouth rinse 2 showed the highest reduction in bacterial load, followed by mouth rinse 3, 4 and 1. This means that mouth rinse 2 has the best capability in inhibiting bacterial growth in vivo, followed by mouth rinse 3, 4 and 1. Further results were regarding the Plaque Index. Table 2 showed the description of the Plaque Index. Research results represented in Table 2, showed the mean of Plaque Index measurements results before and after mouth rinse usage.

Score	Description
0	No plaque
1	Dot Plaque on gingival margin
2	Line Plaque reaching 1 mm from gingival margin
3	Plaque more than 1 mm to 1/3 tooth surface
4	Plaque from 1/3-2/3 tooth surface
5	Plaque more than 2/3 tooth surface

Table 2. Description of Plaque Index.

Mouthwash	Before Mean (SD)	After Mean (SD)	Delta (After-Before) Mean (SD)	P-value Before VS After (Wilcoxon)
1	1.46 (0.38)	2.47 (0.48)	1.01 (0.35)	0.001
2	1.77 (0.54)	2.25 (0.53)	0.49 (0.25)	0.001
3	1.78 (0.39)	2.39 (0.40)	0.61 (0.27)	0.001
4	1.69 (0.54)	2.42 (0.70)	0.74 (0.52)	0.001
P-value	0.079 (Kruskal Wallis)	0.652 (One-Way ANOVA)	0.001 (Kruskal Wallis)	

Table 3. Mean (Standard deviation), of the pre- and post-treatment Plaque Index Measurement Result and reduction by treatment group.

Mouth rinse 2 showed the lowest growth of plaque, followed by mouth rinse 3, 4 and 1. This means that mouth rinse 2 has the best capability in controlling for plaque in vivo, followed by mouth rinse 3, 4 and 1. Further results were regarding the Gingival Index. Table 4 showed the description of the Gingival Index. Research results represented in Table 5, showed the mean of Gingival Index measurements results before and after mouth rinse usage.

Score	Description
0	Normal
1	Light Inflammation, mild edema, mild color change, no Bleeding on Probing
2	Moderate Inflammation, redness, edema, shiny, Bleeding on Probing

Table 4. Description of Gingival Index.

Mouthwash	Before Mean (SD)	After Mean (SD)	Delta (After-Before) Mean (SD)	P-value Before VS After (Wilcoxon)
1	0.009 (0.022)	0.018 (0.036)	0.009 (0.042)	0.380
2	0.009 (0.017)	0.004 (0.013)	-0.004 (0.013)	0.157
3	0.007 (0.015)	0.004 (0.013)	-0.002 (0.022)	0.655
4	0.002 (0.009)	0.009 (0.022)	0.007 (0.025)	0.257
P-value	0.575 (Kruskal-Wallis)	0.438 (Kruskal-Wallis)	0.293 (Kruskal-Wallis)	

Table 5. Mean (Standard deviation), of the pre- and post-treatment Gingival Index Measurement Result and reduction by treatment group.

Gingival Index showed no difference in any groups. This might be caused by the short period of the use of the mouth rinse that might not affect the gingiva nor the periodontal tissue. The bacterial growth might be not enough to evoke for inflammation.

Discussion

Previous studies measured biofilm cell survival using adenosine triphosphate (ATP) bioluminescence. Rapid adenosine triphosphate (ATP) driven bioluminescence assays have long been used in the quantitative determination of bacterial numbers and most recently in dental plaque assessment studies. Using the luciferin substrate and luciferase enzyme, bacterial ATP can be quantified by measuring the release of visible light. This study deployed CariScreen to measure intra oral bacterial load. CariScreen Testing Swabs were used in conjunction with the CariScreen Testing Meter for a simple 1-minute chair-side bacterial test for assessing biofilm cell survival on patients, particularly in measuring the adenosine triphosphate (ATP) bioluminescence. CariScreen uses ATP bioluminescence to identify oral bacterial load. The test is quick and painless. CariScreen Testing meter has been proven to be one of the most effective and accurate way to detect the level of bacterial load in vivo.¹⁰

Calculus formation is the result of petrification of dental plaque biofilm, with mineral ions provided by bathing saliva or crevicular fluids.¹¹ Supra gingival calculus formation can be controlled by chemical mineralization inhibitors, applied in toothpastes or mouth rinses.¹² These agents act to delay plaque calcification, keeping deposits in an amorphous non-hardened state to facilitate removal with regular hygiene.¹³ Clinical efficacy for these agents is typically assessed as

the reduction in tartar area coverage on the teeth between dental cleaning.¹⁴ Research shows that topically applied mineralization inhibitors can also influence adhesion and hardness of calculus deposits on the tooth surface, facilitating removal.^{15,16}

Due to the fact that evidence based dentistry which analyzes the efficacy of over the counter mouth rinse in Indonesia are not yet widely published, this study poses an interesting method for Public Health Service to help reduce the incidence of gingivitis and periodontitis amongst the population in general. Moreover this study demonstrate the adenosine triphosphate (ATP) bioluminescence method may be considered a useful tool, and is an appropriate tool to assess the efficacy of antiseptic mouthrinses in terms of quantitative reductions of total viable microbial counts in mixed biofilm populations in vivo.¹⁷ This study showed that the tested mouth rinse containing CPC has high efficacy in preventing dental calculus than the control mouthwash.

Conclusions

This study aimed at comparing the antiplaque and antigingivitis potential of a mouth rinse containing EO, zinc and fluoride with a CPC-containing mouth rinse over a short term period. In conclusion, the clinical trial showed that the most effective to the less effective mouth rinse in inhibiting bacterial built up (antiplaque), which were analyzed with Cariscreen and Plaque Index are mouthwash 2 (mouth rinse with alcohol), 3 (mouth rinse with CPC 0.1%), 4 (mouth rinse with CPC 0.07%) and 1 (mouthwash as negative control) respectively, but with no statistically significant effect in antigingivitis which was measured by Gingival Index.

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Declaration of Interest

The authors report no conflict of interest.

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