EVALUATION OF THE EFFICACY OF 0.2 % CHLORHEXIDINE GLUCONATE ON CALCULOBACTERIA AN IN VITRO STUDY

Teresa Mao, Julie Toby Thomas, NP Muralidharan

ABSTRACT
Background: Chlorhexidine is used as pre procedural rinse to avoid the aerosol contamination produced by the ultrasonic scaling which is a potential threat to the operator, dental auxiliary and the patients. Aims and Objectives: To assess the efficacy of Chlorhexidine on the viable bacteria that exists within the calculus. Materials and methods: This in vitro study was conducted on 10 supragingival calculus samples collected from patients with periodontal disease. The samples were washed and soaked in Chlorhexidine for two minutes followed by rinsing. A subculture was taken and examined for the presence of bacteria. The samples were then crushed and a subculture was again taken for assessing the presence of bacteria. The data collected were statistically analyzed for significance. Result: It was found that the surface bacteria on the calculus sample were eliminated following the Chlorhexidine rinse. The crushed calculus samples demonstrated presence of both aerobic and anaerobic bacteria. Conclusion: the present study concluded that the bacteria present in the depth of the calculus were still viable, thereby aerosol contamination is produced during the scaling procedure.

Keywords: Chlorhexidine; Instrumentation; Micro-organism

Introduction
Dental professionals are exposed to a number of occupational hazards such as infections from the environment, incidents of percutaneous exposure, musculoskeletal disorders and eye insults. To prevent such infections, patient positioning, barrier protective utilities, high power suction and good ventilation is required, which will thereby reduce contaminated aerosol and vapor hazards.

Aerosols are suspensions of liquid and/or solid particles in the air generated by coughing, sneezing or any other act that expels oral fluids into the air. They may be in the form of splatter or droplet nuclei. A number of detailed studies have documented the aerosol production from air turbines, ultrasonic and sonic scalers. The ultrastructure of dental calculus and the associated bacteria existing within the calculus has been investigated by Tan B et al in 2004. The bacteria present within the calculus are collectively called the calculobacteria. Aerosol is hazardous and can be the causative factor for many microbial infections. With respect to this article, we have taken bacteria as an indicator. This in vitro study was conducted to demonstrate the efficacy of 0.2% Chlorhexidine gluconate on the bacterial coat on the calculus as well as on the calculobacteria.

Materials and Methods
This in vitro study was conducted after obtaining written consent from the study participants. A total of 10 samples of supragingival calculus were collected from patients diagnosed with periodontal disease with clinical attachment loss of more than 3 mm. The calculus samples collected were about 5-8mm in surface area. The patients who were excluded from the study were (i) those having history of any systemic disease, (ii) those who underwent antimicrobial therapy in the last one month, (iii) those who underwent oral prophylaxis for at least six months prior to harvesting the sample, (iv) those who were pregnant or lactating mothers and (v) those with salivary gland disease or xerostomia. The materials used for this study were listed on table 1.

The calculus samples were taken from the lingual aspect of lower central incisors using a sterile hand scaler. The samples were transported to the microbiology lab packed in a sterile aluminum foil. They were then placed in sterile test tubes containing 1ml of sterile saline for the purpose of rinsing. The calculus was rinsed twice in the test tube and then put in a centrifuge for two minutes at 3000 rpm, following which the saline was discarded. This is to ensure removal of unattached plaque present on the surface of the calculus. A separate sterile test tube containing 0.2% chlorhexidine mouthwash was taken. The calculus sample was then transferred to this container using a sterile tweezer. The sample was then mixed in the container mechanically for two minutes and was then centrifuged for another two minutes to ensure thorough mixing. The calculus sample was then further transferred to a sterile test tube containing 1 ml of saline and shaken vigorously and centrifuged for two minutes to remove chlorhexidine, which was on the surface of the sample. Then from the sediment 10 µL of saline was taken using a micropipette on a culture plate containing brain heart infusion media. It was streaked well on the surface of the plate containing brain heart infusion media. It was streaked well on the surface of the plate using a sterile inoculation loop. This was incubated for 24 hours and examined to detect the presence of surface bacteria following chlorhexidine rinse. The calculus sample was then crushed into powder using a sterile glass rod.

Table 1. Materials used

| 1. Calculus collected from the patients |
| 2. Saline |
| 3. 0.2% chlorhexidine gluconate mouthwash |
| 4. Gas pack ( Himedia LE 002A Anaerogas Pack 3.5L) |
| 5. Brain heart infusion agar (BHI) (Himedia M211) |
| 6. Anaerobic jar ( Himedia) |
| 7. Micropipette |
Evaluation of the Efficacy of 0.2 % Chlorhexidine Gluconate on Calculobacteria An In vitro Study

<table>
<thead>
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<th>Sample</th>
<th>BC*</th>
<th>AC a** (Per Ml)</th>
<th>ACaa *** (Per Ml)</th>
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<td>1200</td>
<td>3500</td>
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Mean 0 2400 1530 3930

Table 2. Bacterial Count
*Before Crushing, **After Crushing Aerobic, ***After Crushing Anaerobic, ****Total Count

Discussion

One of the commonest occupational hazards faced in dentistry is over exposure to infected aerosol. Aerosols are created by dental procedures during usage of ultrasonic and sonic vibrating instruments.

The oral cavity of a patient having periodontal disease harbors both aerobic and anaerobic microorganisms such as A. actinomycetem comitans, P. gingivalis, P. intermedia, B. forsythus, C. rectus, E. nodatum, S. intermedium and Treponema species. These microbes can be transported in the aerosol produced during dental procedures leading to respiratory infections, skin infections and other systemic diseases, and also contaminates the environment and other instruments.

Dental plaque is defined clinically as a structured, resilient yellow-grayish structure that adheres tenaciously to the intraoral hard surfaces including removable and fixed restorations.

Patients infected with periodontal disease harbor pathogenic bacteria, which are present in several niches of the oral cavity. Dental plaque is comprised of most of such bacteria, which gets calcified to form calculus. Plaque control is the removal of microbial plaque and the prevention of its accumulation on the teeth and adjacent gingival tissues. Preprocedural rinse is an antimicrobial or antiseptic rinse used before a treatment procedure to reduce the number of bacteria introduced into the patient’s bloodstream and to control contaminated aerosols from being released into the surrounding environment.

Calculus consists of mineralized bacterial plaque that forms on the surfaces of natural teeth and dental prosthesis. The bacteria present within the calculus are collectively called the calculobacteria. This calculus, once disrupted from the tooth surface during the dental procedures, gets mixed with the water spray emitted from the dental instrument creating a contaminated aerosol.

Furthermore, long term exposure of this contaminated aerosol can produce certain skin and eye infections. Various steps have been undertaken to prevent this aerosol contamination such as preprocedural antiseptic mouth rinse, high volume suction apparatus, flushing of water from ultrasonic scaler device and turbine handpieces for 5-10 minutes in the beginning of the day and for 2 minutes before treatment.

Several mouthwashes have been used as preprocedural rinse such as Chlorhexidine (tempered and non tempered), and herbal mouthwashes containing Salvadora persica, Terminalia bellierca etc.

Even though studies on Chlorhexidine as a preprocedural rinse found that there was reduction in bacterial load in aerosol, it eliminates bacteria only from the saliva. There was still presence of bacteria in the aerosol emitted during the dental procedure in spite of all the measures taken to avoid contamination. This can be due to the existence of viable calculobacteria that gets exposed only by disruption of calculus. The calculus gets broken into smaller pieces during the pro-

Figure 1, 2. Growth of Bacteria in Culture

and made as a suspension with the remaining saline. 10 µL of this suspension was transferred to surface of 2 BHI agar plates and was spread uniformly with an inoculation loop. One plate was incubated aerobically at 37°C for 24 hours to detect aerobic bacteria and the other plate was placed in the anaerobic chamber and was spread uniformly with an inoculation loop. One plate was incubated aerobically at 37°C for 24 hours to detect aerobic bacteria and the other plate was placed in the anaerobic chamber and was spread uniformly with an inoculation loop.

Results

A total of 10 supragingival calculus samples were collected and the efficacy of 0.2% chlorhexidine on the surface bacteria as well as the bacteria present within the calculus was estimated. The results of the study showed that there was a 100% reduction in the surface bacterial count, when rinsed with 0.2% Chlorhexidine. All the samples were thoroughly crushed and the effectiveness of Chlorhexidine on the calculobacteria was estimated. The culture plates containing 10µL of the samples were incubated for determining growth of both aerobic and anaerobic bacteria present within the calculus. (Figure 1, 2)

It was found that there was significant growth of aerobic bacteria after crushing of the calculus samples. Anaerobic growth of bacteria was also detected using BHI agar (Table 2).

Shows the presence of bacteria in suspension having calculus after treating with Chlorhexidine before and after crushing.
cess of ultrasonic scaling thereby exposing the inner bacterial content. This emphasizes the need for developing new means of preventing microbial aerosols in dentistry.

Though numerous studies have been done to prove the efficacy of 0.2% Chlorhexidine on the surface bacteria of dental calculus, there has been no significant study done to show its effectiveness on the bacteria within the hard structure of calculus, which may be a possible aerosol contaminant during the procedure. Usage of 0.2% Chlorhexidine as a preprocedural rinse in our study has been proved to have a definite benefit in the reduction of bacterial load in saliva similar to the previous studies.16 But there was no significant decrease in the bacteria present in the aerosol, which may possibly be due to viable bacteria within the calculus.

Conclusion
In conclusion, even though a definite benefit in the reduction of bacterial load in saliva was observed following the preprocedural rinse of 2% Chlorhexidine, the bacteria was still present inside the calculus. Further study is warranted to determine methods to effectively destroy the calculobacteria, thereby reducing the risk of aerosol contamination.

Acknowledgement
The authors would like to thank the Department of Microbiology, Saveetha Dental College for all the help rendered.

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Conflict of Interest: None Declared