Use of ATP Bioluminescence to Survey the Spread of Aerosol and Splatter during Dental Treatment

Running title: **Survey of Dissemination of Aerosol and Splatter**

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Summary

Aerosol and splatter produced during dental treatment (ultrasonic scaling and professional mechanical tooth cleaning) are potential sources of infection. Contamination patterns on the operators' masks, goggles, chests and gowned right arms, and on the patients' goggles, before and after dental treatment were investigated by using ATP bioluminescence analysis. Contamination on every surface tested increased significantly after dental treatment. Maximum contamination was found on patients' goggles. Aerosol and splatter produced during dental treatment thus have the potential to spread infection to operators and patients. ATP bioluminescence is a useful tool for monitoring surface contamination.

Keywords:

Aerosol, ATP bioluminescence, Bacterial contamination, Dental Treatment, Infection control, Splatter

Introduction

Dental treatments often involve surgical procedures that use air or water syringes, high-speed turbines, micromotors and ultrasonic scalers. These devices produce aerosols containing potentially infectious agents, such as those found in the patient's blood, saliva and oral cavity.[1–4] Patient blood and saliva spread as aerosols and droplets during dental treatments are considered potential sources of infectious cross-contamination and have the potential to cause microbial infections in not only patients but also dental staff. The CDC recommends that dentists and dental hygienists use personal protective equipment (PPE) such as masks, gloves, goggles and gowns to protect themselves from exposure to infectious aerosol and splatter containing patient saliva and blood.[5] The distance of spread of aerosol and splatter and the levels of contamination that occur during dental treatment are issues of great concern.

Adenosine triphosphate (ATP), which is present in the cells of all living microorganisms (bacteria, fungi and protozoa), is a good marker of microorganism viability and contamination. The ATP bioluminescence assay, which is based on luciferin–luciferase reactions, has been used in the field for rapid detection in sanitary surveys of hospitals and in the food and beverage industries. [6, 7]

We previously compared the monitoring of bacterial contamination of dental unit waterlines by using the ATP bioluminescence assay and conventional culture-based tests; the results indicated that ATP bioluminescence was potentially useful for rapid and simple monitoring of bacterial contamination.[8]

Here, to evaluate the usefulness of ATP bioluminescence for contamination

monitoring during dental treatment, we used this assay to investigate the contamination patterns produced by aerosol and splatter during ultrasonic scaling followed by professional mechanical tooth cleaning (PMTC).

Methods

Sample collection

The study was performed during the clinical practice sessions of students in a dental hygiene program. An ultrasonic scaler (Solfy F; Morita Mfg. Corp., Tokyo, Japan) was used for ultrasonic scaling and a high-speed turbine/micromotor with dental unit (Model: SIGNO Type G40J; Morita Mfg. Corp.) was used for PMTC. Ultrasonic scaling and PMTC as dental treatments were performed on 10 students, with 10 min for each procedure. Operators and patients wore dental goggles (AS ONE Co., Ltd, Osaka, Japan). Additionally, each operator wore a mask fitted with a surgical face shield (3M Japan Co., Ltd, Tokyo, Japan), as well as a long-sleeved surgical gown made of polyethylene (Whitemax Co., Ltd, Shiga, Japan). The surface of the mask was covered by plastic film (3M Japan Co., Ltd) that could be wiped down easily for measurements. Square areas measuring 5 cm × 5 cm on the surface of the mask, the dental goggles, chest, and right arm of the long-sleeved surgical gown of the operator were wiped with cotton containing 70% alcohol before the dental treatments. As the dental goggles used in this study were not disposable, these surfaces were wiped with cotton containing 70% alcohol after the dental treatments, and were used the next dental treatments.The contamination pattern on each square was evaluated before and after the dental treatments.

ATP bioluminescence assessment

Samples were collected from the abovementioned squares by using a cotton swab from a LuciPac Pen kit (Kikkoman Biochemifa Co., Tokyo, Japan). The samples were immediately analysed by using a Lumitester (Kikkoman Biochemifa Co.) in accordance with the manufacturer's instructions. Contaminating ATP levels, expressed in relative light units (RLUs), were then recorded.

Method of culture of scattered oral bacteria

Areas close to the 5 cm × 5 cm squares sampled for the ATP bioluminescence assessment were rubbed with a sterile cotton swab pre-moistened with sterile 0.01M phosphate-buffered saline (PBS). Each cotton swab was placed in a test tube containing 1 mL of sterilised PBS. One hundred microlitres of the sample solution was then plated on Mitis Salivarius agar (BD, Franklin Lakes, NJ, USA), which is selective for oral streptococci, and incubated at 37 °C for 5 days under anaerobic conditions. The bacterial colonies were Gram stained to distinguish and classify the bacterial species with a microscope (Model: BX51N; OLYMPUS. Corp.).

Statistical analyses

Data were expressed as median values (interquartile range 75%, 25%), and the Wilcoxon signed-rank test was used to assess the differences in contamination patterns between before and after dental treatments. The JMP

software program (version 12, SAS Institute, Cary, NC, USA) was used to conduct the statistical analyses; $P < 0.05$ was considered statistically significant.

Results

ATP values before and after ultrasonic scaling and PMTC

We determined the ATP values (in RLU) on the surfaces of the mask, dental goggles, chest and gowned right arm of the operators, and on the dental goggles of the patients, before and after ultrasonic scaling and PMTC (Table 1). All ATP values before treatment were less than 40 RLU. However, the ATP values [median (25%, 75%)] on the operators' masks [672.0 (448.0 to 938.6)], dental goggles [1106.8 (657.4 to 1580.3)], chests [672.0 (448.0 to 938.6)], and gowned right arms [761.0 (670.8 to 914.3)], and on the patients' dental goggles [1519.5 (913.5 to 1866.7)] increased significantly after the dental treatments ($P < 0.001$). In addition, all of the ATP value of dental goggles were decreased to less than 40 RLU after wiping with cotton containing 70% alcohol.

Distinguishing and classification of oral bacteria

Gram-positive oral streptococci were distinguished and classified from all samples; we surmised that they were derived from the patients' dental plaque and saliva (data not shown).

Discussion

To the best of our knowledge, this is the first study to monitor the bacterial contamination during dental treatment by using ATP bioluminescence analysis.

Previously, we reported that levels of ATP bioluminescence were positively correlated with bacterial counts in dental unit waterlines, and concluded that ATP is an effective indicator of bacterial contamination.[8] In this study, the significantly elevated post-procedure ATP measurements in samples were obtained from the surfaces of the operators' masks, chests, right arms and goggles after ultrasonic scaling and PMTC. The high ATP measurements indicated their constant and extensive exposure to high levels of aerosol and splatter. These results indicated that aerosol and splatter produced during the dental treatments had contaminated these surfaces at high rates. Therefore, it is important to thoroughly protect operators through the use of proper PPE during dental treatments; cleaning and disinfection of the hands and forearms are also necessary before and after treatments.

During dental treatments, both operator and patient are continuously exposed to aerosol and splatter including bacterial contaminants and the patient's body fluids, such as saliva and blood. Contaminating aerosol and splatter can also spread to the surfaces of tools and equipment and the general environment of the dental clinic. This spread is likely to play an important role in the transmission and diffusion of several microorganisms of concern in regard to healthcare and potential infection. Aerosol and splatter generated in dental treatments contain patients' oral bacteria, which contaminate their eyes.[9] In this study, we detected oral streptococci on all surfaces tested, including the goggles. The high levels of ATP were also found on the goggles of both operators and patients, implying that abundant aerosol and splatter were spread to the face. These results indicate that both operator and patient are at risk of exposure with

the patient's plaque and saliva, which including oral microbes via contaminated aerosol and splatter. Eyes contaminated with blood or saliva are at high risk of infection and corneal epithelial exfoliation, conjunctivitis and keratitis.[10] Patients, as well as operators, should need to wear goggles during dental treatments to protect their eyes from trauma or corneal injury, as well as from infection of the ocular mucous membranes and cornea by bacteria and viruses. Dental treatments often employ the use of sharp items, such as scissors and orthodontic wires, as well as harsh agents such as acids. Pieces of tooth or metal from drilled or scraped teeth are occasionally scattered; these items are also contaminated and harmful, making it even more important to protect the eyes with goggles.

According to our results, ATP values of the googles were greatly decreased after the wipes of these surfaces with cotton containing 70% alcohol. It was confirmed that the bacterial contaminations were cleaned from the goggles surface, which was generated in dental treatments. Therefore, the ATP bioluminescence is very effective for prevention of infection of dental treatment, because it is possible to find out the spread and diffusion of aerosol and splatter including pathogenic bacteria within less than a few minutes.

In conclusion, ATP bioluminescence method would be useful for rapid determining and monitoring the cleanliness of the environment without the need for special instruments or techniques. Environmental monitoring of contamination or cleanliness in dental clinics by using simple and quick methods will reduce the health risks to both patients and operators.

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Conflict of interest statement

The authors state that they have no conflict of interest.

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Table 1. Changes in ATP values during dental treatment

Variable	Before		After		*p* value*
	Median	(25%, 75%)	Median	(25%, 75%)	
Chest (RLU)	19.0	(19.0, 19.8)	532.0	(295.8, 612.5)	<0.001
Right arm (RLU)	23.0	(22.0, 23.8)	761.0	(670.8, 914.3)	<0.001
Mask (RLU)	27.0	(27.0, 28.0)	672.0	(448.0, 938.6)	<0.001
Goggles of the operator (RLU)	37.5	(37.0, 38.8)	1106.8	(657.4, 1580.3)	<0.001
Goggles of the patient (RLU)	37.0	(37.0, 38.8)	1519.5	(913.5, 1866.7)	<0.001

*: Wilcoxon signed-rank test

RLU: relative light units