Methods of Measuring Sterilization Effect of Acidic Electrolyzed Water for Cleaning the Oral Cavity

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Purpose of the study

Unlike general disinfectants, acidic electrolyzed water is applied by flushing rather than soaking the object to be disinfected. It is supposed to have bactericidal effect. Since it can be safely used as bactericidal solution in great amount for the purpose of cleaning and disinfecting root canals and periodontal pockets for the treatment of disinfected root canals as well as the operative area, impressions and dentures, it is considered to be usable for cleaning and disinfecting the oral cavity with safety.

However, because acidic electrolyzed water is more susceptible to the surrounding environment, its effect can vary depending on the capacity, shape and tissue conditions of the oral cavity as well as individual differences of salivary compositions, hygienic conditions, and changes of the oral environment in the daily life. Therefore, bactericidal effect of acidic electrolyzed water applied to the oral cavity should be evaluated by in vivo testing rather than in vitro. On the other hand, it is difficult to identify all the species and number of microorganisms living in the oral cavity of each individual and examine each of their quantitative changes before and after the disinfection.

Therefore, we came up with a measuring method of evaluating changes in the amount of oral microorganisms by extracting ATP (Adenosine-5'-triphosphate) that derives from oral bacteria contained in the mouth-wash drainage, and compared bactericidal effect of acidic electrolyzed water with different degrees of electrolysis as reported in the following sections.

Test materials and testing method

For the mouth-wash, acidic electrolyzed water produced by an electrolyzer CXM-1500 (by Corona Industries). This device has a mode switch that can select 'high', 'medium', 'low' and 'no electrolysis'. Concentration of hydrogen ions, ORP and effective chlorine concentration applied in this testing are shown in Table 1. As to the electrolytic mode, only 'high', 'medium' and 'low' were tested.

Table 1: pH, ORP and effective chlorine concentration of tested acidic electrolyzed water

Electrolytic mode	pH	ORP (mV)	Cl (ppm)
High	2.3	1,230	45.0
Medium	2.7	1,214	29.0
Low	3.2	1,158	9.7
No electrolysis	3.3	1,027	3.3

Five paper cups per subject for one-time mouth-wash were prepared, and the first, second, fourth and fifth cups were used to test 10 ml each of physiological saline, and the third cup was used for 10 ml of acidic electrolyzed water. With each of these samples in this order, the mouth was rinsed for 10 seconds to conduct a bacteria count. Samples of 100 μl each of waste water were prepared using a cuvette and mixed with NRS (somatic ATP extracting agent) or Somase (ATP lytic enzyme) to incubate at room temperature for 45 minutes. For the ATP assay, the temperature of

the photometry chamber of Lumac bio-counter (by 3M) was set at 25℃, and NRB (microbial ATP extracting agent) was added followed by Lumit-PM 30 seconds later. The amount of light generated by a Luciferin-Lciferase reaction was assayed by the integration method for 10 seconds to obtain a RLU (Relative Light Unit) value to represent the amount of microorganisms. For the testing agent, a generic IMC kit (Lumac by Gunze) was used. The tested subjects were 41 adults, and all the measurements were repeated twice, and the second data were adopted only when the first data showed abnormal values.

Test results and analysis

In order to test bactericidal effect using mouth-wash, quantitative changes of oral bacteria should be examined first. It is known that the amount of oral bacteria is rapidly reduced after the meal, and the amount of bacteria in the waste water of mouth wash by physiological saline fluctuates during the day. Therefore, all the tests were conducted two hours after the meal.

Among the tested samples in 5 paper cups, the first waste water of mouth-wash by physiological saline showed maximum RLU of 1,766, with minimum 0 and average 203. The second sample showed maximum 462, minimum 0 and average 102. Since the first sample contained some bits of food washed away from the mouth, the second sample was considered to be appropriate to set the basic values for measuring bactericidal effects.

In case of the third sample using acidic electrolyzed water, the RLU rapidly dropped. The larger the extent of electrolysis, the smaller the RLU values. However, since bactericidal effect is likely to be maintained during the minutes before the testing, we considered it inappropriate to compare bactericidal effects just for oral bacteria.

Because the fourth sample showed similar values to those of the fifth, the measurement of waste water of the fourth sample using physiological saline was considered to represent bactericidal effect of acidic electrolyzed water. Also, it was found that the effect of residual water in the oral cavity is negligible in testing acidic electrolyzed water.

In summary, by assaying waste water of the second and fourth samples using this method, quantitative analysis of acidic electrolyzed water's bactericidal effect was made possible as shown in Fig.1. In addition, bactericidal effects were compared among different degrees of electrolysis.

Fig. 1: Comparison of RLUs among different acidic electrolyzed water before and after the mouth-wash