【Original】

Effects of nano-bubble water on periodontal disease

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The objective of the study is to investigate the clinical effects of ozone nano-bubble water used as a mouthwash on periodontal diseases.

The subjects were four patients with mild periodontal disease. The patients were instructed to rinse their mouth with ozone nano-bubble water twice a day for two weeks. Probing depth (PD) and bleeding on probing (BOP) were measured at six different sites in each patient at the beginning of the study, and one and two weeks after.

A significant difference was noted between the PD values at the beginning of the study and those at one week ($p<0.001$), as well as at the beginning of study and at two weeks ($p<0.001$). A significant difference was also noted between the BOP ratio at the beginning of the study and at one week ($p<0.001$), as well as at the beginning of the study and at two weeks ($p<0.001$).

The clinical parameters of periodontal disease showed improvement after two weeks of mouth rinsing with ozone nano-bubble water. This method is promising because it is easily applied, causes less mechanical damage than brushing and will not trigger the emergence of resistant bacteria.

keywords nano-bubble water, ozone, antimicrobial, antiseptic, oral rinse

Introduction

Lifelong self care is essential for preventing periodontal diseases. Although they have traditionally been considered to be diseases localized within the oral cavity, recent studies indicate that they are closely related to risk factors of type 2 diabetes[1)2)] and ischemic heart disease[3~5)].

Elimination of plaque formed by periodontopathic bacteria (biofilm) is essential for the prevention and treatment of periodontal diseases[6)]. Daily self care is classified into mechanical methods such as brushing and chemical methods such as disinfectant applications. As mechanical elimination of plaque is the most effective against biofilm, brushing has been the method of choice. Chemical methods are less effective and are generally used as an adjuvant[7)]. Many adults fail to brush their teeth as frequently as required because of their lifestyle. And in general, appropriate brushing is a difficult practice. Due to these conditions, brushing is less effective than one would expect[8)].

The authors have focused on the strong antimicrobial activity of ozone nano-bubble water[9)10)]. The concept of nano-bubble water emerged through the research on bubble formation of decompression sickness[11)12)]. Ozone nano-bubble water contains ozone bubble nuclei in a nanometer order. The superiority of nano to micro-

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Table 1 Changes in Probing depth

	Pre	1 week	2 weeks
Whole	2.32±0.30	2.06±0.26	2.02±0.25
Maxilla	2.30±0.38	2.08±0.32	2.00±0.29
Mandible	2.34±0.47	2.05±0.41	2.03±0.41
Left jaws	2.36±0.46	2.07±0.40	2.04±0.39
Right jaws	2.29±0.06	2.06±0.35	1.99±0.33

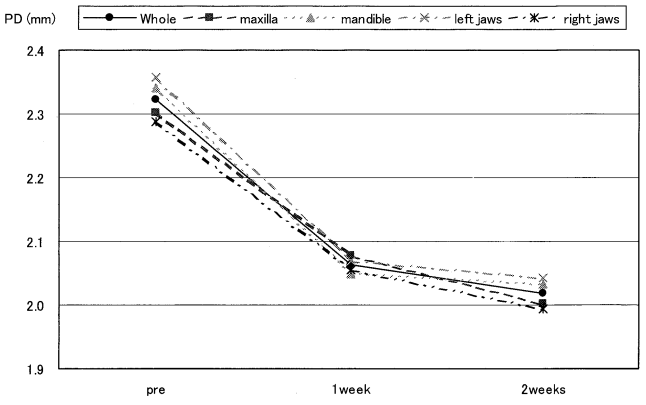

Fig. 1 Changes in Probing depth

bubble water is that it can attack and dislodge many microorganisms, including viruses[9]. Studies have reported a strong antimicrobial and antiseptic activity of ozone[13)14)]. Also, it is stabilized and stored over a long storage period[15]. Although ozone nano-bubble water with these properties is considered to be suitable for the treatment and prevention of periodontal diseases, its application in clinical practice has not been reported.

In the present study, the clinical effects of ozone nano-bubble water against periodontal diseases were examined.

Materials and Methods

The present study was approved by the Institutional Review Board (IRB) of Tokyo Medical and Dental University, School of Dentistry.

Subjects

The subjects were four patients with mild periodontal disease (2 males and 2 females, age: 31.8 ± 2.8 (mean ± SD) y. o.). Mild periodontal disease is the condition of a slight change of color tone of the gingiva and form of the gingival surface[16].

Test product (Ozone nano-bubble water)[17]

Micro-bubbles at a diameter of less than 50 μm were generated in hard water (underground water) at a saline concentration of 1.0 mass%. The micro-bubbles were rapidly crushed[15] into bubbles at a diameter of less than 200 nm. And it was used as "ozone nano-bubble water" in the study.

Experimental procedures (Rinsing and brushing)

The subjects were instructed to rinse their mouths for 20 seconds with 20 mL of ozone nano-bubble water twice a day (morning and evening) for two weeks. They did not receive any instructions regarding appropriate brushing methods.

Clinical examination

All teeth from the subjects were monitored. Clinical examinations were conducted at six different sites at the beginning of the study, and at one and two weeks after.

The following parameters were measured accord-ing to the method presented below.

·Probing depth (PD)

PD was measured in 0.5 of millimeters increments at six sites on each tooth.

·Bleeding on probing (BOP)

Bleeding from the gingival tissue on probing was examined.

Statistical tests

An intrasubject comparison of the changes in PD

Table 2 Improvement by more than 1mm in depth of the sites with Probing depth of 3mm and more

	Improvement rate	improved sites/total sites
Whole	61.8%	139/225
Maxilla	66.7%	74/111
Mandible	58.8%	67/114
Left jaws	64.1%	75/117
Right jaws	59.3%	64/108

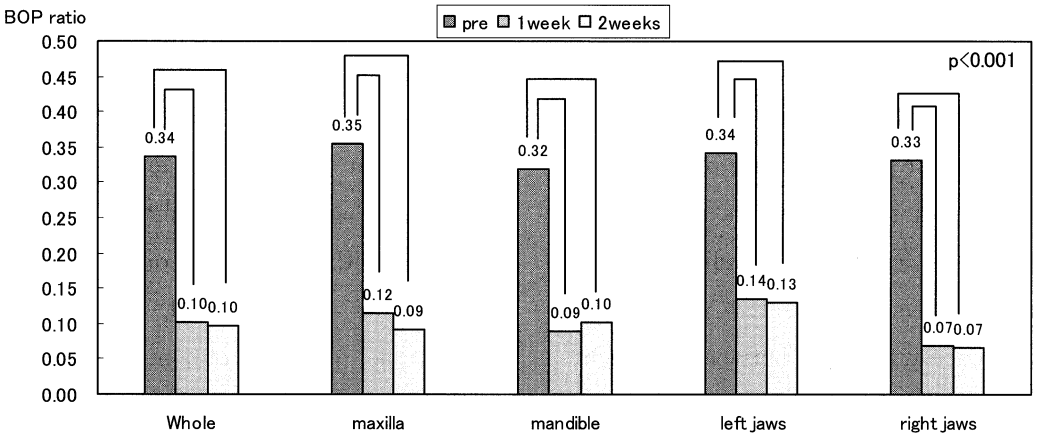

Fig. 2 Changes in Bleeding on probing ratio

at different measurement times was conducted using the paired t-test, and BOP ratio (number of bleeding points/number of probed sites) at different measurement times was done using the Wilcoxon test. A comparison of changes in PD and BOP ratio over time between the maxilla and mandible and between the right and left jaws was done using repeated-ANOVA.

Analysis was performed using statistical software SPSS for Windows version 13.

Results

1) Changes in PD

PD values at the beginning of the study, and at one and two weeks after rinsing with ozone nano-bubble water are shown in **Table 1** and **Fig. 1**.

In the intrasubject comparison, a significant difference was noted between the PD values at the beginning of the study and those at one week ($p<0.001$), as well as at the beginning of the study and at two weeks ($p<0.001$). A significant difference was also noted between the PD values at the beginning of the study and at one week ($p<0.001$), as well as at the beginning of the study and at two weeks ($p<0.001$), in separate comparisons of the maxilla and mandible, and of the right and left jaws. In repeated-ANOVA, no significant difference was found between the right and left jaws and between the maxilla and mandible.

The number of points with a PD of 3 mm or more, which is a sign of significant periodontal disease,

are shown in **Table 2** An improvement of more than 1 mm at two weeks was noted in 61.8% of the sampling places.

2) Changes in BOP ratio

BOP ratios at the beginning of the study, and at one and two weeks are shown in **Fig. 2.**

In the intrasubject comparison, a significant difference was noted between the BOP ratio at the beginning of the study and that at one week ($p<0.001$), as well as at the beginning of the study and at two weeks ($p<0.001$). A significant difference was also noted between the BOP ratio at the beginning of the study and at one week ($p<0.001$), as well as at the beginning of the study and at two weeks ($p<0.001$), in separate comparisons of the maxilla and mandible, and of the right and left jaws. In repeated-ANOVA, no significant difference was found between the right and left jaws or between the maxilla and mandible.

Discussion

The Ministry of Health, Labor and Welfare of Japan is currently promoting a campaign called "Health Japan 21" and proposes the prevention of periodontal disease[18]. Periodontal disease is chronic inflammation closely related to systemic lifestyle diseases such as type 2 diabetes and cerebral and heart diseases[1~5]. In adult health care, dental health should be included in prevention activities aimed at fighting systemic diseases.

Conventional self care for periodontal disease has traditionally been brushing. However, correct knowledge and technique are required for appropriate brushing[19]. Inappropriate brushing and device use may cause damage to the teeth and oral tissues, as well as causing a reduction in the effectiveness of brushing[20][21]. In addition, place, time and opportunity are necessary for proper brushing. Japanese workers are said to be too busy to perform brushing as frequently as desired[22]. Chemical methods are generally used as a supplementary method. However, their effect is limited because biofilm is not destroyed by usual chemical plaque control [23][24].

In the present study, the subjects did not receive any instructions regarding appropriate brushing methods in order to accurately evaluate the effect of the ozone nano-bubble water. Ozone water has an approximate ten-fold antiseptic activity to that of chloride[9]. However, the ozone in conventional ozone water disappears in an hour. Ozone nano-bubble water can be stored for several months[15].

The results of this study show that PD markedly improved with the use of ozone nano-bubble water during the study period. These results are comparable to those obtained from a study on tooth brushing among Japanese[25][26]. The results also show that BOP ratio improved markedly. The guidelines for periodontal disease define objective indicators for complete resolution as being periodontal pockets of less than 3 mm in depth without gingivitis or BOP[16]. When inflammation is not resolved in the bottom of pockets due to remaining periodontopathic bacteria, BOP will not improve[27]. The early improvement of BOP ratio in this study may be the effect of the strong antiseptic activities of ozone nano-bubble water and the enhancement of regeneration and restoration of the gingival tissue[28].

No difference in PD and BOP ratio curves for two weeks was found between the maxilla and mandible and right and left jaws. Brushing on the dominant arm side (right side) is generally difficult, however, clinical symptoms in the right jaw also was significantly improved. These results suggests that oral rinse with ozone nano-bubble water could

immediately reach the bottom of periodontal pockets and provide complete care regardless individual technique, which is entirely different from brushing. To be specific, ozone nano-bubble water provides easy and certain cleaning of the whole oral cavity without special knowledge or technique.

Ozone nano-bubble water has the possibility of having an effect on biofilm that cannot be eliminated by other chemical methods. Even when biofilm is eliminated mechanically by brushing, bacterial flora can be reconstructed in the periodontal pockets again, resulting in a recurrence of periodontal disease[16]. In contrast, ozone nano-bubble water has high permeability due to its bubble size, each of which is surrounded by a kind of inorganic ion hull, and is able to infiltrate into biofilm constructed of many microorganisms and break them up[17)29)]. Future studies should be conducted to elucidate, in a much more detailed manner, the mechanisms involved.

Regarding the safety of clinical use, oral rinsing with ozone nano-bubble water rarely causes damage to the teeth and periodontal tissues, like brushing. Differently from other chemical methods, when ozone bubble nuclei disappear in water, free radicals (·OH: hydroxyl radical) are generated which sterilize bacteria, including periodontal bacteria[30]. As the ozone dissolves, ozone water simply becomes water, therefore, no secondary products are left in ozone nano-bubble water[9)10)]. Furthermore, it is noteworthy to mention that ozone nano-bubble water breaks down bacteria and virus colonies at the gene level and no resistant bacteria are produced[9)10)]. It is generally considered that ozone is harmful to our health, however, the safety of oral rinsing with ozone water has been reported.[31] The ozone nano-bubble water used in the present study might be considered to induce no health problems. This poses great advantages for safety of self care. Therefore, safe ozone water is a sterilizer that is very easy and safe to use.

In conclusion, ozone nano-bubble water improves mild periodontal disease. The effects are comparable to conventional brushing methods. Further studies should be conducted to elucidate the microbial activities of ozone nano-bubble water and its clinical effects utilizing larger populations and more precise evaluations.

Conclusions

The clinical parameters of periodontal disease were improved by rinsing the oral cavity with ozone nano-bubble water. The results suggest that ozone nano-bubble water has the potential to be used in the prevention and treatment of periodontal diseases.

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