

Super Acidic Electrolyzed Water and Dental Care Materials

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In recent years, not only SAEW but also alkaline ionized water has been studied in various ways and its usage is becoming widespread. I am going to talk about the impact of applying this alkaline ionized water to dental treatment in comparison with SAEW.

Testing materials and equipment

Acidic and alkaline water was produced by National electrolyzer PJ-D20 repeatedly for several times as necessary. Table 1 shows properties of super acidic and super alkaline electrolyzed water. In these two types of water, dental alloys as well as brass pieces used for piping of dental equipment and stainless steel alloys used in dental devices and tools as listed in Table 2 were soaked. About these samples, changes in color, weight, pH and ORP after soaking are reported comparing acidic and alkaline water.

Table 1: Functional water tested

	Acidic	Alkaline
PH	2.48	11.67
ORP	1137	-905

Table 2: Metals and alloys

Gold alloy	20K, 18K, 14K, Sofird, Casting Gold, Altop, Light Gold, shape memory alloy	
Silver alloy	Gold-Palladium	18 Gold Palla, Kimpalla Ace, Pallatop 12, Cast Well, Ishifuku Kimpalla
	Silver alloy	Milo Silver, Super Silver, PI Silver, Mlo Bright, Milo Three, Dent Solder
Silver-copper alloy	Cu, 10Ag-Cu, 20Ag-Cu, 30Ag-Cu, 40Ag-Cu, 50Ag-Cu, 60Ag-Cu, 70Ag-Cu, 80Ag-Cu, 90Ag-Cu, 100Ag-Cu	
Silver-zinc alloy	5Zn-Ag, 10Zn-Ag, 15Zn-A& 20Zn-Ag, 25Zn-Ag, 30Zn-Ag	
Silver-indium, silver-tin alloys were added in the same way as silver-zinc alloy.		
Copper alloy	K-metal, GP-metal, Olden, PP-metal	
Chrome-added alloy	Ni-Cu alloy, Co-Cu-Ti alloy, 18-8 stainless steel	
Titanium alloy	Pure titanium, Ti-6Al-4V	

Impact of Super Electrolyzed Water on Intra-unit Piping System

•by Shuhei Mizogami, Mizogami Dental Clinic

1. Purpose of the study

In the fifth Conference of the Study Group for Dental Application of Super Electrolyzed Water, I expressed my view to the effect that disinfection of the intra-unit piping system using SAEW is effective in preventing nosocomial infections via air-turbine handpieces. As I showed clinical examples in the presentation, some surgical cases indicated distinctively positive effects right after disinfection of internal piping, although bacteria culturing tests conducted one month after disinfection on some handpieces without anti-suckback circuits turned out to be positive despite PFD (pulse flushing drive) by 7:3 mix solution had been conducted after treating every patient. Judging from the report that there are about 2% of HCV carriers, disinfection of internal circuit after treating every patient is ideal but unrealistic because it is so time-consuming and requires too much effort.

Then, an alternative would be to supply disinfectant to the unit all the time, but the biggest stumbling block is corrosive effect of SAEW against metals. However, if SAEW is mixed with super alkaline ionized water, its bactericidal effect can be maintained with its pH at around neutral until the mix ratio gets a little more than 1:1, and its corrosive effect against metals can be diminished to a negligible level. This solution does not cause decalcification of the enamel, either. As to residual chlorine concentration, it is reduced to about 2/3 of SAEW at the initial stage, but this level is long maintained thereafter which gives a sense of security. Since this solution does not have strong taste and odor unlike SAEW, patients can use it for gargling without much noticing it except sensitive few. There is a report that mixing at 1:1 ratio sacrifices the instantaneous bactericidal effect, one of the major characteristics of SAEW, but practically speaking, this does not create a problem since disinfection of internal piping is just enough. In the seventh conference of the Study Group, I reported on the status of the mixed water supply via piping one year after it was installed, and I would like to talk about any further problems now that it is almost 3 years since then.

2. Testing method

This is the same method as I presented in the seventh conference. Stable water pressure at around 1.5 kgf/cm^2 is maintained by sending air from a compressor which was a little modified from a manual pressure-accumulation type sprayer. Connection to the unit was via the service coupler, the same system as I presented in the fifth conference.

The tested super electrolyzed water was produced by Oasis Bio-Half manufactured by Asahi Engineering. The source water is tap water of the City of Osaka.

3. Analysis

1) Samples were randomly taken from the handpiece and coupling to conduct bacteria culturing tests that resulted in negative by which the purpose of disinfecting internal piping was achieved.

2) In minor operations such as extraction of mandibular wisdom teeth buried horizontally, the said mixed water was supplied from the air-turbine and micro-motor during the teeth incision and bone grinding without giving any

unpleasant feeling to the patient. Swelling after the operation differed depending on the patients, but in general it was minor. In three cases, swelling obviously caused by infections was identified including that of mandibular lymph, which seemed unlikely to have been caused by water jet from the handpiece. Since then, we have been more careful about handling the surgical devices, and there has been no problem so far.

3) hi our clinic, we have been using a supersonic sealer with an end tip, and I believe the mixed water supplied to it can disinfect the root canal more effectively.

4) Supply of mixed water for scaling and route planing is done by Suprason p-max via another route, and there has been no rust found on the tip and the wounds after FOp is especially very clean.

5) Regarding rust of the turbine handpiece, the one by Nakanish had slight change of color only at the piping, but no such change was identified on the one by KaVo. I did not have any impression that the life of the rotor was shortened. No abnormality was observed at the head of either handpiece.

6) The silicon tube of the internal piping was often ruptured when water pressure was adjusted to 2.0 kgf/cm² or more. Currently, it is stably operated at 1.5 kgf/cm².

7) A patient complained about crystals of calcium carbonate deposited in alkaline ionized water in the cup for gargling. These crystals sometimes clog the valve within the unit to reduce flow of water from the 3-way syringe or block the pullback valve to have leak in the turbine head which requires repairs. The piping of the Nakanish's turbine easily gets clogged by crystals, and needs to be taken apart for cleaning once a month. The biggest problem now is accumulation of calcium carbonate in the piping, and to completely solve it, it seems like a water-softening device should be installed just before the electrolyzer.

To be honest with you, I would not like to use tap water ever again for the treatment despite many of these minor problems.

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Symposium II: Metal Corrosion by Functional Water and Dental Decalcification

(Translator's note: second appearance)

Dental Solubility and Surface Layers Affected by Super Electrolyzed Water

-by Motoo Niwa, Nippon Dental College, Dept of Dentistry, Div. of Hygieneology

It is well known that the main component of our teeth is apatite which is in turn composed of hydroxyapatite (HAp) for more than 95%. Especially 97% of the enamel that covers the surface of the crown is composed of HAp, most of which is an aggregate of apatite crystals aligned in the same direction.

Many fluorine ions are found in the top layer of the enamel up to the depth of 0.7 to 4µm, and part of HAp is replaced by fluoro-apatite (FAP).¹⁾ Therefore, solubility of teeth can be studied by examining reactivity between SAEW and HAp or FAp.

In order to examine solubility of HAp, baked samples of 98% HAp (baked at 1200°C) and 70% HAp (baked at 1000°C) in the size of 3 x 3 x 3 mm were created to have them react with SAEW. The solubility was calculated by measuring the weights of HAp in consecutive days after contacting the samples with each acid solution and comparing them with the original weight before testing.

As a result, lactic acid with pH 2.45 showed the highest solubility both in cases of 98% HAp and 70% HAp, followed by SAEW and lactic acid with pH 1.05. Solubility of HAp has increased by continuously stirring the SAEW solution in all the tested groups.²⁾ Additional tests were conducted with extracted teeth to find out the highest solubility of HAp was achieved by lactic acid with pH 1.05 followed by lactic acid with pH 2.45 and SAEW. These differences seem to derive from the crystal structure of HAp.

In the next step, X-ray analysis was conducted with the tested HAp to find out it is very similar to secondary calcium phosphate anhydride (monetite) which indicates concentration of phosphoric ions and calcium ions has increased locally to result in re-crystallization.³⁾ In light of these results, comparing SAEW with the same pH and lactic acid solution, SAEW showed higher solubility than lactic acid, which means less decalcification when applied to dental treatment. The same trend is identified with FAp and extracted teeth (Table 1).

Table 1: Comparison of solubility of different acidic solution

70% HAp	pH 2.5 lactic acid > pH 1.05 lactic acid > SAEW
98% HAp	pH 2.5 lactic acid > SAEW > pH 1.05 lactic acid
Tooth enamel	pH 1.05 lactic acid > pH 2.5 lactic acid > SAEW
FAp	pH 2.5 lactic acid > pH 1.05 lactic acid > SAEW

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Prevention of Dental Decalcification by Super Acidic Electrolyzed Water - Endodontic Irrigation Solution Both In-vitro and In-vivo

-by Takakazu Yoshida, Asahi Univ. School of Dentistry, Dept. of Operative Dentistry

Dental decalcification is caused by deteriorating quality of tooth and harmful chemical reactions that cause caries, but enamel etching with acids or chelating agents in the process of composite resin filling as well as softening treatment of root canal dentine at the time of root canal expansion are acknowledged as effective treatments.

Meanwhile, it is known that ground powder of dentine generated in the process of root canal expansion is mixed with the cleaning agent to form pasty substance adhered to the root canal walls as residues. To remove this smear layer, a chemical agent with non-organic solubility is used to decalcify and dissolve its main component, ground powder of dentine. The presence of the smear layer not only prevents the cleaning agent from permeating into the lesion and affects the tightness of the root canal filler, but also contaminate the root canal with bacteria, so that removal of the smear layer is considered to be an important process of operation.

The most effective clinical method to remove the smear layer is to apply supersonic vibration in conjunction with 15% EDTA solution. However, it is reported that the solution overreacts to decalcify and soften healthy teeth after removal of the smear layer depending on the way of application. It is not clear yet as to how much the impact of softening of the root canal walls is, but it surely is an unstable factor. It was made clear by the same kind of test using EDTA solution less than 15% that it has less antibacterial resistance although it does not affect healthy teeth as much as the 15% solution, which remains to be a clinical problem.[^] Therefore, we have focused on super acidic water that can be used under stable conditions.

First of all, in order to know more about non-organic solubility of super acidic water, we conducted a basic test by dissolving dentine powder taken from extracted teeth with super acidic water and examined the changes in decalcification effect, pH, ORP and residual chlorine concentration of the solution. As a result, we found this solution has non-organic solubility which can be further enhanced by renewing it. All the values of pH, ORP and residual chlorine concentration drop down by time, although by having the solution react with the dentine continuously, these values did not go down.

Mandibular central incisors extracted from the human oral cavity were used to test lower root canal expansion by alternately cleaning them with sodium hypochlorite solution and hydrogen peroxide solution. After the canal expansion, super acidic water was continually jetted with a device called ENAC 6 to clean root canals to visually examine the effect of removing smear layers by means of SEM observation.

The duration of cleaning was set at 1,5 and 10 minutes. Two kinds of tips were connected to the supersonic system, prototype cleaning tips soldered with a plugger and with a root canal probe (Clean Washing Needle by Nipro). As a control group, 7.5% EDTA solution and purified water was used. The result showed that purified water did not remove the smear layers during 10 minute cleaning, whereas super acidic water did remove them, and especially using the prototype tip, the smear layers were removed in 1 minute exhibiting almost the same effect as using 7.5% EDTA solution.

Next, a clinical evaluation was made as to the effect of super acidic water. In the conventional way of cleaning by alternately using sodium hypochlorite and hydrogen peroxide solution, the root canal that proved to be negative in the bacteria culturing test just after the canal expansion is very likely to result in positive transference at the next visit of the patient. This is because, as is explained in the document 1) and 2) listed below, smear layers still remain in the root canal, and their removal is essential to obtain real negativity. On these assumptions, conducting bacteria culturing tests in the root canal, we examined the effect of smear layers on the result of bacteria culturing. From the root canal of a single-root tooth diagnosed as chronic apical periodontitis, the contents were collected three times, before and after the canal expansion as well as at the next visit, to culture bacteria and compare the results. Canal cleaning was done after completing the canal expansion by continuously irrigating the solution under supersonic vibration. As a control group, 7.5% solution of EDTA was used. As a result, we found high possibility of negativity right after the canal expansion and at the next visit in both cases of super acidic water and EDTA solution, which indicates removal of smear layers is profoundly related to the rate of positive transference.

From all of these results, it is now clear that application of super acidic water is clinically useful in removing smear layers on the root canal walls after the canal expansion and contributes to disinfecting the root canal. We are planning to continue our effort to study the relationship with the clinical results.

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Experimental Studies on Therapeutic Effect of Super Acidic Electrolyzed Gel on Wounds

-by Akihiro Qhyama, Akihiko Shiba, Emiko Kimura, Tomoki Saitou, Shintaro Miyatani, Hiroyuki Yanagisawa,
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Translator's note: SAEW = Soper Acidic Electrolyzed Water

Purpose of the study

Wounds are defined as conditions where severance or deficit of organs or tissues exists caused by external forces, but in a broader sense, they are supposed to mean defective tissues and necrosis. If an organism get wounds, they are treated according to its regenerative functions, but in the process of healing, inflammation is caused even if the lesion is not infected.

SAEW produced by electrolyzing tap water with a pinch of salt added has intensive and instantaneous bactericidal effect¹⁾ and has a wide range of antibacterial spectrum. However, based on a series of in-vitro experiments, our lab has reported that SAEW does little harm to living organisms.²⁾ We have also reported that, clinically speaking, SAEW is effective in cleaning the gingiva, gingival pocket and root canal.³⁾

As I presented in the eighth Conference of the Study Group for Dental Application of Super Electrolyzed Water, we found that the most effective of SAEW gel, SAEW solution and isodine applied to rats' experimental wounds for 7 days turned out to be SAEW gel. We gained some insights from our visual and histological examination of the healing process using SAEW gel as compared to the control gel, which I am going to talk about.

Test materials and testing method

For the testing, twelve 4-week old Wistar rats (male) were used to test SAEW gel and the control gel.

The back skin of the rats was sterilized by Hibiten before shaving the fur, and wounds were made with a Dispo punch with a diameter of 6 mm and the depth of about 1 mm at four locations per rat. The wounds were cleaned and disinfected with approximately 10 ml of SAEW gel and the control gel twice a day (9:00AM and 9:00PM). After measuring the size of the wounds 3, 5, 7 and 14 days after making the wounds in a group of 3 rats, they were all killed and immediately preserved in formalin. According to the conventional method, the wounds were paraffin-wrapped, stained with hematoxyline-eosin (H-E) to create sample pieces. H-E stained samples were comparatively examined from the standpoint of 1) regeneration of epithelial cells, and 2) multiplication and differentiation of granulation.

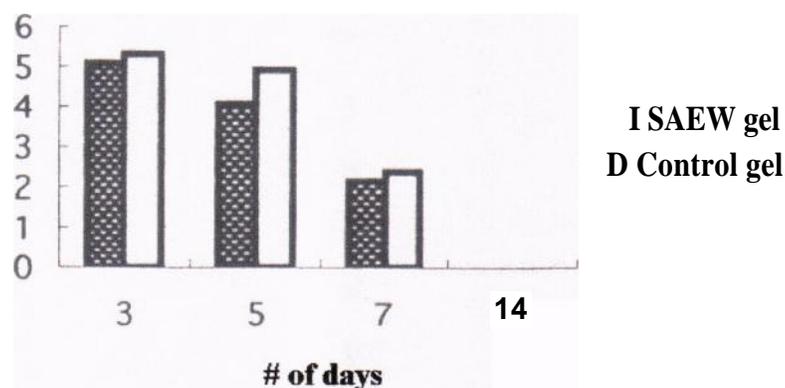
Test results and analysis

According to visual observation, no difference of curing was identified among 3-day samples, although 5-day and 7-day samples showed better curing using SAEW gel than the control gel. In case of 14-day samples, the entire lesion was covered by the epithelium to make the measurement impossible.

According to histological examination, no distinct difference was found among 3-day samples. However, 5-day samples showed earlier regeneration of epithelial cells using SAEW gel than the control gel, and multiplication and differentiation of granulation appeared good in case of the former. The same was true for 7-day samples, plus fibrinogenesis was found in some area. In case of 14-day samples, the capillary in the granulation was reduced using SAEW gel as compared to the control gel, and fibrinogenesis has further progressed.

All of these results indicate that SAEW gel works as a facilitator to cure the wounds, and is effective in cleaning and disinfecting them.

Test results



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Impact of Functional Water on Treatment of Periodontitis Tissue Wounds in Early Stage

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Purpose of the study

Functional water is supposed to have intensive bactericidal effect with little harm to the organism, and for its known effect and safety, it has been clinically applied as a supplemental agent to burns and atopic dermatitis in the field of dermatology. Many reports have been issued on its effect of using it for oral wounds, although there are only few histological reports on its effect on curing wounds after surgical periodontal treatments.

Therefore, this study focused on the regeneration of periodontal tissues in the early stage after applying SAEW to surgical wounds of periodontia near the rats' molars from histo-pathological and immunological point of view.

Test materials and testing method

1. Subject animals

Twenty-seven 5-week old Wistar rats weighing 150g each.

2. Testing method

From the first maxillary molar to the third on the left side of rats' oral cavity, palate-side marginal gingivae were ablated to artificially make deficits with a round bar. For the group of 9 rats, Aqua acidic water (pH 2.6 by Oxilyzer OXM-01, Miura Electronic) was applied to the samples using algin acid as the carrier. The new periodontal tissue regeneration agent Emdogain® was applied to the samples of the control group composed of other 9 rats, and the rest of 9 were restored their gingival flaps without applying anything.

To examine the division activities of each cell, 3 rats per group were given BrdU 1, 3 and 7 days after the operation before being killed. After extracting the maxillae for histo-pathological examination, they were fixed, decalcified and wrapped with paraffin in the conventional way to make sample pieces. These pieces were observed under an optical microscope after hematoxiline-eosin stain.

Next, for immunological examination, murine monoclonal antibody against BrdU as the primary antibody, and murine peroxidase antibody against IgG2a as the secondary antibody were used for marking. The samples were stained with 3,3'-diaminobenzidine (DAB) and further stained with methyl-green to examine the ongoing division of the cells. The examined parts were gingival epithelia of the oral cavity and the upper edge of the periodontal ligament. Statistic analysis was conducted for temporal changes by calculating the marker-cell rate in these tissues.

Test results and analysis

- 1) Multiplication of the deep area of the epithelia was repressed for the treated groups, and in comparison with one of the control groups without any treatment, the wounds of periodontal connective tissues tend to be cured faster. However, on the seventh day of testing, adhesive connective tissues were found on the treated periodontal base of the control group that was given Emdogain®, which were not found in the treated group.
- 2) The marker-cell rate of the regenerated gingival epithelia using BrdU was lower in the treated group than in the control group, and almost at the same level as the Emdogain-applied control group.
- 3) The marker-cell rate of the gingival epithelia in the oral cavity showed almost the same value as that of the untreated control group, and higher than that of the Emdogain-applied control group.
- 4) The marker-cell rate of the upper periodontal ligament turned out to be almost the same as that of the untreated control group and lower than that of the Emdogain-applied control group.

From these results, functional water does not do any harm to periodontal tissues and repressed multiplication of deep epithelial tissue earlier than untreated control group, although the cure of wounds was delayed compared to the Emdogain-applied control group. Further studies are expected on this issue of curing mechanism of oral wounds.

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Application of Super Acidic Water to Dental Maxillary Sinusitis

-by Hideaki Takeuchi, Takeuchi Denial Clinic, Nagasaki

Test results and analysis

SAEW with pH 2.3 to 2.7, ORP 1,100 mV or Mg²⁺, and hypochlorous acid concentration of 10 ppm was produced by electrolyzing tap water containing 17.1 ppm (annual average) of chlorine ions mixed with Aquatid NDX 250KMW (by Omco) using electrolytic additive (sodium chloride + diluted hydrochloric acid) and was used to clean the dental maxillary sinus on the right side of the oral cavity by pressurized tidal jet. It was applied in the amount of 1000 cc per one round of cleaning at the temperature between 30°C to the body temperature, and was repeated 35 times.

It seemed to be highly effective against *Candida albicans*, *Candida glabrata* (*torulopsis glabrata*) and *Actinomyces israelii* generated in the sinus.

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Cleaning and Sterilization of Interim Implant Prosthesis with SAEW Immediately After Abutment Surgery

-by Masahiko Ozeki, Aldhiko Shiba, Hiroshi Shimizu, Chika Kase, Azusa Kanaishi, Hiroaki Tsukasaki,
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I. Purposes of the test

In prosthetic treatments using the two-step osseo-integrated implant, not only restoration of occlusal and masticatory functions but also aesthetics improvements are required. To get adaptation of maxillary bones by progressive loading and good emergence profile after connecting the abutment, it is desirable to wear interim prosthesis from the early stage which requires as bacteristatic and aseptic treatment as possible.

In order to enhance the patient's QOL by conducting a temporary implant prosthetics immediately after the abutment surgery in a hygienic way, we create a temporary prosthesis with an ideal crown-shape at the time of the abutment surgery (2nd operation), and clean and disinfect it with electrolyzed water (super alkaline and super acidic) to apply it as an implant prosthesis. I am going to present its overview and clinical evaluations.

II. Test materials and testing method

Test materials

The tested subjects were 17 patients treated with prosthetics using Branemark implant (12 upper jaw and 5 lower jaw cases), 30 abutments, and 17 implant superstructures. The temporary prostheses were made of temporary cylinders (DCA 157, DCA 468 by Nobel Biocare), temporary crowns made of polycarbonate resin (by Nisshin), and immediate-type polymerized resin (UniFirst by GC). As to electrolyzed water, super alkaline ionized water (pH 10.5, ORP -827 mV, effective chlorine concentration 0.05 ppm) and SAEW (pH 2.6 or less, ORP 1, 137 mV, effective chlorine concentration 30 ppm) was produced by an electrolyzer called Oxilyzer (by Miura Electronics).

Testing method

(D After a curing period of 6 months (for upper jaw) or 4 months (for lower jaw) since the fixture had been buried, abutment connection operation was conducted. The periosteal flap of gingival mucosa was flipped to the labial and lingual sides by alveolar crest incision, and the abutment was installed onto the fixture.

(D After placing a temporary cylinder to the abutment with dental pins, a temporary crown was connected to the temporary cylinder using immediate-type polymerized resin.

(D When the resin is dried, the connected temporary cylinder and temporary crown were taken out of the oral cavity to clean the attached blood paste with super alkaline ionized water, and the crown-shape (temporary prosthesis) was created by brushing over the immediate-type polymerized resin. Before inserting the temporary prosthesis into the oral cavity, it was disinfected with SAEW for a minute.

© After connecting the temporary prosthesis to the abutment with gold screws, the periosteal flap of gingival mucosa was stitched.

(D) After the operation, curing process of gingival mucosa was visually observed. Temporal changes of marginal bones around the implant was examined by oral X-ray.

III. Test results

In any of these cases, abnormal bleeding or infection was not found after the temporary prosthesis. The operation left minimum pain. On the gingival mucosa around the temporary prosthesis, minor reddish swell was identified one week after the operation (after removing stitches). The swell has receded in 2-3 weeks and the gingival mucosa re-

sumed normal in 4 weeks after the operation to form a junction of marginal gingiva compatible with the temporary prosthesis. Four or more weeks after satisfactory curing of gingival mucosa, a precise impression was taken as needed. Mucosa around the implant after the final prosthesis looked healthy and aesthetically great. No image of abnormal absorption was found by oral X-ray in the marginal bones around the implant.

IV. Analysis

1. This hygienic method of interim implant prosthesis immediately after abutment surgery can achieve highly aesthetic emergence profile and allows progressive loading from the early stage, which is very useful for enhancing the patient's QOL.

2. To alleviate the patient's mental and physical stresses accompanying the extended hours of the operation with bleeding, it seems necessary to reduce the time spent on making the temporary prosthesis.

Inhibitory effect of super acidic electrolyzed water against halitosis

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Purpose of the study

In recent years, it is reported that there are increasing number of patients who visit dental clinics for halitosis, which is becoming growing social concerns. It is known that the main cause of halitosis is volatile sulfuric compounds (VSC) such as hydrogen sulfide (H₂S), methyl mercaptan (MMP) and dimethyl sulfide (DMS) generated by oral bacteria. Also the culprit of halitosis is considered to be bacteria on the coated tongue or in the periodontal pockets, and all of them are Gram negative anaerobic. We have tested inhibitory effect of rinsing the oral cavity with SAEW against halitosis by examining its effect on concentration of volatile sulfide compounds and bactericidal effect of cleaning periodontal pockets.

Test materials and testing method

SAEW was produced by Asitron (by MAC Japan), and used for testing right after production. I.

Measuring VSC concentration by gas chromatography Testing equipment and conditions

GC-14B gas chromatograph by Shimadzu Corporation equipped with a flame photometric detector (FPD) was used for measuring VSC concentration. For the VSC separation column, CDPN 25% Uniport HP 60/80 Glass 3 mm 1.D. x 3 m (by G.L. Sciences) was used. The testing conditions were column temperature at 70*0, inlet temperature at 100°C, detector temperature at ISO'C, and helium was used at the flow rate of 40 ml/min. as carrier gas. Flow rates of hydrogen and air were both 50 ml/min. Creating calibration curve and calculating VSC concentration

Three calibration curves were created for H₂S, MMP and DMS by a chromatography data station Vstation (by GL. Science) based on the given peak area measured against the standard gas which has different concentration adjusted by each VSC's permeation tube using the Standard Gas Generator (Permeator by Gastech).

2. Effect of SAEW on VSC concentration

Three kinds of VSC's permeation tubes were placed in the Permeator where diluted gas (nitrogen) was sent at the flow rate of 5 L/min. Generated H₂S (570.66 ppb), MMP (492.51 ppb) and DMS (82.28 ppb) was collected in a Ted-dler bag to store as VSC sample gas. 150 ml of this VSC sample gas was placed in a sampling bag and mixed with SAEW or tap water (0.5 to 50 ml) and was shaken for 30 seconds. 5 ml of this gas was collected with a gas-tight syringe to measure each SSC concentration by gas chromatograph and the reduction rate was calculated.

3. Bactericidal effect of cleaning periodontal pockets

Fifteen patients diagnosed as adult periodontitis at the Periodontics Division of Kagoshima Univ. School of Dentistry Hospital were tested as subjects. Two single-root teeth with periodontal pockets in the size of 4 mm or more were examined. Subgingival plaque was collected by a paper point for 30 seconds, and periodontal pockets were

cleaned with 5 ml of SAEW twice for 30 seconds each. One hour after, subgingival plaque was collected again to calculate the value of colony forming unit (CFU). Seven days after then, CFU was calculated again in the same way. Physiological saline was used as a control group.

4. VSC concentration changes of oral gas after cleaning the oral cavity with SAEW

Seven volunteers who were identified to have halitosis were tested for inhibitory effect of SAEW against halitosis. They cleaned their oral cavities and gargled with 100ml each of SAEW and tap water for a minute. Changes in VSC concentration of oral gas were examined. Collection of the oral gas was done by the direct expiration method. That is, a hematocrit capillary with its one end closed by paraffin film was inserted in the oral cavity and let the patient aspirate with the mouth closed for a minute. Then 5 ml of oral gas was collected to send to the gas chromatograph. Tap water was used as a control group.

Test results

Effect of SAEW on concentration of VSC

H₂S showed volume-dependent reduction of concentration with both SAEW and tap water, but the reduction rate was greater in case of SAEW. The reduction rate of MMP concentration was much larger than that of tap water, with 100% reduction by adding 2.5 ml of SAEW whereas 50ml addition was required to get the same reduction rate for tap water. The reduction rate of DMS was also much larger in case of SAEW than tap water, with 100% reduction by adding 5 ml of SAEW whereas the same rate was achieved by 50 ml addition of tap water.

Bactericidal effect of cleaning the oral cavity

One hour after cleaning periodontal pocket, its CFU tended to drop down with physiological saline, but SAEW showed more rapid reduction of CFU. CFU of SAEW was still lower than that of physiological saline one week later.

3. VSC concentration changes of oral gas after cleaning the oral cavity with SAEW

By cleaning the oral cavity with SAEW, VSC concentration in the oral gas has drastically dropped, and concentration of each component was lower than using tap water one hour after the test. All three kinds of gas have lowered its concentration, although no idiosyncratic inhibition was identified.

Analysis

Three kinds of VSC, H₂S, MMP and DMS are considered important substances to cause bad breath. Above all, it is confirmed that MMP has the closest correlation with the intensity of bad breath in terms of its exponential concentration and the foul odor. In our experiments, SAEW showed bactericidal and deodorant effects, which indicates it has a great potential as a mouth-wash agent.

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Property changes of powder-type acidulous functional water under different storing conditions

•Especially pH, ORP, available free chlorine concentration, and bactericidal effect

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Purpose of the study

Due to the effect against oral microorganisms of acidulous functional water produced by electrolyzing mixed solution of NaCl and HCl, its usefulness as a plaque control agent has been studied in recent years. However, since electrolyzers to produce acidulous functional water are still expensive, their clinical use in general treatments as well as domestic use have some difficulties.

Now, we have noticed that powder-type acidulous functional water developed for disinfecting food is easy to handle and costs less. If used as a chemical agent to control plaque, its property changes can vary depending on the storing conditions, so that their temporal changes of pH, ORP, effective free chlorine concentration, and bactericidal effect under 3 different storing conditions were examined.

Test materials and testing conditions

Preparation of functional water

Two packages of acidulous functional water powder (Aqua Sun 70 by Japan Aqua) was dissolved in distilled water according to the manufacturer's guidelines.

Bacteria specimen

Streptococcus mutans PS 14, Streptococcus sanguis ATCC 10556, Porphyromonas gingivalis 381, and Actinobacillus actinomycetemcomitans ATCC 33384 were used for testing.

Media and culturing

Facultative anaerobes were cultured in brain heart infusion (GHI) broth under aerobic conditions, and obligate anaerobes in BHI broth mixed with yeast extract under anaerobic conditions both at 37°C until the late period of logarithmic propagation phase.

Examination of bactericidal effect

1 ml of bacteria broth adjusted at 10^9 CFU/ml (suspended by 50 ml of sodium phosphate buffer with pH 7.0) was mixed with 1 ml of acidulous functional water, and the control sample was added 1 ml of distilled water and let stand to react for 30, 60 and 600 seconds. 50ul of 0.5% $\text{Na}_2\text{S}_2\text{O}_3$ was added as an deactivator to make 10-time dilution steps and 10 ul of this solution was applied on the agar plate using a Conradi stick. Facultative anaerobes were aerobically cultured at 37°C for 48 hours, and obligate anaerobes were anaerobically cultured at 37°C for 5 days before conducting the CFU count. In every test, n=5 was applied.

Changes in pH, ORP, free effective chlorine concentration and bactericidal effect by storing period and conditions

1 ml of bacteria broth adjusted to 10^9 CFU/ml in the same way as described above was mixed with 1ml of acidulous functional water stored for different period of time under different conditions and was let react for 30 seconds. Then, the same deactivation, application, culturing and cfu count was conducted. The storing period was 0,7,14,21, 28, 42 and 56 days, and the storing conditions were 1) in a shaded air-tight container at 4° (S4), 2) in a shaded air-tight container at room temperature (SRT), and 3) in an air-tight container at room temperature (RT). The values of pH, ORP were measured by a pH meter, and free effective chlorine concentration was measured by the o-tolidine method. In every test, n=5 was applied.

Test results and analysis

Bactericidal effect of acidulous functional water just after production

The CFU count was less than 2.0×10^2 CFU/ml for all the reaction time and bacteria specimen, which indicates intensive bactericidal effect.

Changes in pH, ORP, free effective chlorine concentration and bactericidal effect by storing period and conditions

The values of pH and ORP did not change much, but free effective chlorine concentration showed a tendency of declining day by day. S4 group showed more significant decline than RT group. As to bactericidal effect, the CFU count was less than 2.0×10^2 CFU/ml up to 56th day of storing, which indicates enough bactericidal effect. All the control groups allowed microbial growth.

From all of these results, it was proven that powder-type acidulous functional water is easy to produce at minimum cost and retain bactericidal effect for relatively long time. However, in clinically applying it, protein contained in saliva can give some impact. Since this study found its bactericidal effect against oral bacteria, it is expected to be applied as a chemical plaque control agent.

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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 electrolysed 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 ul 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

tile photometry chamber of Lumac bio-counter (by 3M) was set at 25*0, 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.

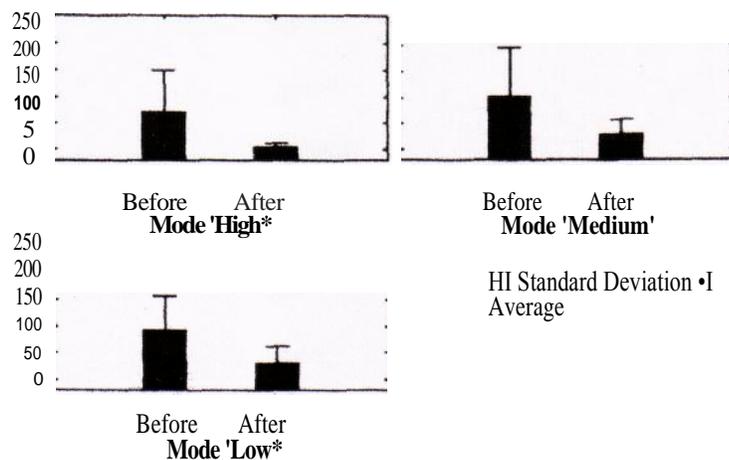
Among the tested samples hi 5 paper cups, the first waste water of mouth-wash by physiological saline showed maximum RLU of 1,766, with minimum 0 and average 203. The second sample showed maximum 462, minimum 0 and average 102. Since the first sample contained some bits of food washed away from the mouth, 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



Disinfection of Water by Granular Polyaniline

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Purpose of the study

Oxygen abounds in the air and water, but its reductive molecular types such as super oxide (O_2^-) and hydroxyl radicals (OH) are highly reactive and are called 'active oxygen'. Active oxygen, which is said to cause cancer and senility, generates in the body of animals to protect them from bacteria. Due to its lack of cumulative effect, it is considered to be the ideal disinfectant with no environmental hazard.

We found that polyaniline that has been actively studied as a dielectric polymer all over the world produces active oxygen by releasing electrons when it is contacted by oxygen dissolved in water.⁰ This report is about bactericidal effect of granular polyaniline that is rather easy to handle. Some methods have already been developed to produce active oxygen in aqueous solution such as the photo-catalytic method by applying light to titanium oxide or ozone photolysis, but our tests require no light.

Test materials and testing method

'Anilide' by Nitto Electric was selected as polyaniline, and granulation was done with a non-organic binder to make granulation of 0.7 to 2.0 mm diameter containing 73% of polyaniline.

Granular polyaniline was put in pure water and stirred to generate active oxygen. Polyaniline was dried indoor or dried overnight in a desiccator using anhydride phosphoric acid.

Bactericidal tests were conducted in the following manner. 10^4 CFU/ml (control) of E.coli was added to sterilized physiological saline together with granular polyaniline and shaken lightly for 20 minutes. Part of this solution and its diluent was applied to agar media to culture at 37°C for 24 hours. Generated colonies were counted to compare the number of surviving colonies compared to the control group.

Decomposition of formaldehyde and sulfonic compounds was conducted in gas phase. About 20g of granulated polyaniline was placed in a column and the above substances contained in approximately 4 liter of air was let through it to measure change in concentration.

Test results and analysis

Integrated amount of super oxide generated by stirring the mixture of granular polyaniline and pure water for about 20 minutes is shown in Fig. 1. Assuming that 2 molecules of super oxide are generated at the same time as a molecule of hydrogen peroxide, integrated amount of super oxide was calculated from the amount of generated hydrogen peroxide. The drier the polyaniline is, the greater the amount of generated super oxide. It was also found that polyaniline is repeatedly usable by drying.

Relationship between the amount of granulated polyaniline added and its bactericidal effect against E.coli is shown in Fig. 2. When the granule was added to the bacteria broth, less than 1 weight % was enough to kill the bacteria if it is completely dried. 2.5 wt % polyaniline solution was stirred for 10 minutes to generate active oxygen, and