

Clinical Evaluation of Root Canal Irrigation with Strong Acidic Water

Tatsuya Saito, Ryui-chi Yoshida, Tetsu Kawano, Yasuhiro Oka, Tsuguhiko Okuga, and Ichiro Sekine
(Section of Conservative Dentistry, School of Dentistry, Asahi University)

Purpose

Infected root canals have been endodontically treated to kill all bacteria in the root canals, and in this treatment bacterial culture tests have been clinically used to evaluate the bacterial microbicidal efficacy objectively. In previous studies the cases were reported, where the bacterial culture test was negative immediately after mechanical and chemical cleanings of the root canals, but positive in next visit by the patients, suggesting the smear layer may be formed. Meanwhile, it has been known that the alternate irrigation with sodium hypochlorite and hydrogen peroxide used routinely in clinical practice cannot remove the majority of the smear layer, where both necrotic tissues and bacteria are present, resulting in the formation of residual smear layer on the root canal walls. Various methods have been investigated to remove the smear layer. It has been reported that among the removal methods, an EDTA solution is effective in removing the smear layer and when used in irrigation of the root canals, the less positive results in the bacterial culture test were found in next visit of the patients as compared to the conventional methods. Removal of the smear layer is thus presumed to be critical for the sterilization of the root canal because its success is one of critical factors to kill all bacteria in the root canals. We previously reported that in ultrasonic cleaning of the infected root canals, the strong acidic water was as effective as an EDTA solution in removal of the smear layer. In the present study, we used the strong acidic water for the root canal irrigation in clinical practice together with the culture test of bacteria in the root canals and evaluated the potential of clinical use.

Experimental Materials and Method

(1) Subjects and Irrigation Solution

Thirty single-rooted teeth used in this study were selected from the patients, who visited the Section of Conservative Dentistry in Asahi University Hospital, showed willingness to cooperate, and were diagnosed with chronic periapical periodontitis. The root canals were irrigated with the strong acidic water to form a strong acidic water group, whereas irrigation with an EDTA solution formed the control group. A needle-shaped ultrasonic probe was used to assure the complete immersion of the root canals in a fresh solution. Teeth on which the rubber dam could not be mounted were excluded from the experiment.

(2) Sample Collection and Irrigation Method

Samples were collected at three different times. First and second samples were collected from the patients on their first visit. Controlling a moisture level with a rubber dam, the infected teeth were disinfected with iodine tincture, and after the pulp chamber was enlarged according to a routine procedure, the working length was determined to enlarge the root canal to a size of a #25 K-file and the root canal substances adhered to the file were inoculated into a culture medium. Second samples were

collected after completing the enlargement of the root canals. The root canals were enlarged to a size of the #50-#70 K-files while irrigating alternately with a 5% sodium hypochlorite solution and a 3% hydrogen peroxide solution, and the root canals were then ultrasonically cleansed for one minute with each irrigation solution. The irrigation solution remained in the root canals was then washed with purified water, and after drying, the root canal substances were collected with the largest sterilized file used in final enlargement to inoculate into a culture medium. Without intracanal medication a sterilized cotton plug was then inserted into the root canals, which were temporarily sealed with a gutta percha stopping and a zinc oxide-eugenol cement, completing the treatment of the patients on their first visit.

Third samples were collected from the patients on their next visit (approximately one week later). Controlling the moisture level with a rubber dam, infected teeth were disinfected with iodine tincture to remove the temporary seal materials. The largest sterilized file used in final enlargement was inserted into the root canals to collect the root canal substances to inoculate into a culture medium.

(3) Culture Medium and Culture Method

All samples obtained from teeth of the subjects were inoculated into CDC blood agar and incubated anaerobically in an anaerobic box at 37°C for 48 hours.

(4) Results and Discussion

The bacterial culture test of second and third collection samples were negative in both the strong acidic water group and the control group. This result indicates use of the strong acidic water in the clinical irrigation of the root canals can remove the smear layer, suggesting the strong acidic water is useful as a root canal irrigation solution.

Disinfection of Root Canal by Irrigation with Functional Water

Masako Nakano, Yoshiko Ozawa, Koji Yokota, Takayuki Koshino, and Jiro Nakamura (Second Section of Conservative Dentistry, School of Dentistry, Tsurumi University)

Purpose

Mechanical enlargement and irrigation of the root canals are important in the endodontic treatment. In particular treatment of the infected root canals is intended to remove the bacteria as a possible source of infection, pulp tissue fragments, and dentin fragments to kill all bacteria in the root canal. Sodium hypochlorite (NaClO) is an effective root canal irrigant, but is a strong irritant to tissues, thus requiring adequate care to prevent it from spilling over the apical foramen and flowing in and out of the oral cavities. In recent years there is strong interest in various functional waters because they can inactivate bacteria and virus, have low adverse effect to living organisms, and is inexpensive. Our study revealed that irrigation with strong acidic electrolyzed water can remove the smear layer on the root canal walls. In the present study, we investigated the efficacy of various functional waters in the root canal irrigation.

Materials and Method

Root canals of the avulsed teeth (working length; 10 mm and root canal enlargement; #70 K-file) and a same size root canal model made from an epoxy resin were used in present study. A bacterial culture medium ($5 \mu\ell$, 10^{6-8} CFU/ml) was injected into each root canal of the avulsed teeth, which was irrigated with functional waters (3, 6, and 12 ml) and the irrigants remained were then removed with a paper point, followed by addition of $5 \mu\ell$ of sterile distilled water to the root canals and insertion of the paper point into the root canals for sampling. 3% NaClO and sterile distilled water were used as the root canal irrigant for the control.

Experiment 1

As the root canal irrigant the strong acidic electrolyzed water, weak acidic electrolyzed water, neutral electrolyzed water, and strong alkaline electrolyzed water were used to evaluate the antiseptic effect to *Enterococcus faecalis* (E.f.). The paper points used to collect samples were placed in Tryptic soy broth to incubate. The opaque appearance and no change in the culture medium after 72 hours of incubation at 37°C were assigned as negative and positive for its antiseptic effect. This was quantified as the percent negative rate.

Experiment 2

Strong acidic electrolyzed water was used in the root canal irrigation to evaluate the antiseptic effect to *Staphylococcus aureus* (S.a.), *Streptococcus mutans* (S.m.), and *Candida albicans* (C.a.). Tryptic soy broth containing the paper points used for sampling was diluted serially to incubate in an agar medium at 37°C for 72 hours. A colony count (CFU/ml) was then determined to assign as the residual bacteria count after the root canal irrigation.

Results

In Experiments 1 and 2, 3% NaClO was 100% effective in the antiseptic effect on the root canals of avulsed teeth and the root canal model, but distilled water was 0% effective. Results in Experiment 1 were demonstrated in Tables 1 and 2. The functional waters except the strong alkaline electrolyzed water were significantly less effective in the root canal irrigation of avulsed teeth than that of the root canal model.

Table 1 Antiseptic Effect of Various Functional Waters in Root Canal Model (E.f.)

	Strong acidic water	Weak acidic solution	Neutral water	Strong alkaline water	Strong alkaline water + Strong acidic water
3 ml	80%	70%	40%	0%	60%
6 ml	80%	90%	50%	0%	80%
12 ml	90%	100%	70%	0%	90%

8th Workshop for Use of Strong Electrolyzed Water in Dental Field

March 6 (Saturday), 1999

**Auditorium on 4th floor of Building #5, School of Dentistry, Tokyo Medical and Dental
University**

Chairman; Takashi Miyazaki

Organizer; Study Group for Use of Strong Electrolyzed Water in Dental Field

Benefactor; Foundation for Study and Promotion of Functional Water

Program of Workshop

- 9.00 Opening speech; Takashi Miyazaki (Chairman of 8th Workshop)
Speech by President; Akihiko Shiba (President of Study Group)
Speech by Guest of Honor; Jun-ichi Urata (Director of Foundation for Study
and Promotion of Functional Water)

General Session (10 Presentations)

9.15-9.35 Chair; Ryu-ichi Yoshida (Section of Dental Materials and Devices, School of Dentistry, Nippon Dental University)

- (1) Prevention of Corrosion by Reduced Water Produced by Electrolysis

p 16 and 17

Shuhei Mizogami* (Mizogami Dental Clinic)

- (2) Corrosivity of Electrolyzed Water against Dental Metals -Report 3; Study on Corrosion with Weak Alkaline Electrolyzed Water

p 18 and 19

Takeo Maida*, Kazuhiko Endo¹⁾, Osamu Tanaka (Research Institute of Health Science, Health Science University of Hokkaido, ¹⁾ Section of Dental Materials and Devices, School of Dentistry, Health Science University of Hokkaido)

9.35-9.55 Chair; Osamu Tanaka (Research Institute of Health Science, Health Science University of Hokkaido)

- (3) Manufacture and Use of Non-woven Tissue Impregnated with Weak Acidic Electrolyzed Water for Trial

p 20 and 21

Hiroshi Iwamoto* (Iwamoto Dentistry)

- (4) Reaction of Oxidized Water with Reducing Agent

p 22 and 23

Takeo Igarashi*, Naoto Yoshinuma, Katsumi Yokose¹⁾, Ko-ichi Ito, Nariaki Nomoto¹⁾, and Shodai Murai (Section of Periodontology, Department of Conservative Dentistry, School of Dentistry, Nippon University, ¹⁾ Section of Chemistry, Department of General Education, School of Dentistry, Nippon University)

* Speaker

9.55-10.15 Chair; Teruo Obana (Obana Dental Clinic)

- (5) Use of Strong Acidic Electrolyzed Water in Orthodontic Field

p 24 and 25

Hideki Kitaura*, Kazuhide Kobayashi (Section of Orthodontics, School of Dentistry, Nagasaki University)

- (6) Antiseptic and Cleaning Effects of Strong Acidic Water on Alginate Impression

p 26 and 27

Akihiro Nishimura*, Azusa Kanaishi, Akihiko Shiba, Toshihiro Sakai, Hiroaki Shiozaki, Hiroyuki Nakane, Chika Kase, Kazuhito Nimi, Shiho Enomoto, and Yoshiori Hyodo (Third Section of Prosthodontics, School of Dentistry, Showa University)

10.15-10.35 Chair: Takeshi Sueda (Second Section of Conservative Dentistry, School of Dentistry, Kagoshima University)

- (7) Fundamental Study on Denture Cleaning by Home-care Electrolyzed Water

P 28 and 29

Toshiya Kashiwara*, Yoko Terada, Fumiaki Kohno, Tetsuo Ichikawa, Katsuhiko Hirota¹⁾, and Yo-ichiro Miyake¹⁾ (First Section of Prosthodontics, School of Dentistry, Tokushima University, ¹⁾ Section of Oral Bacteriology, School of Dentistry, Tokushima University)

- (8) Usefulness of Strong Acidic Electrolyzed Water in Dental Care for Disabled

P 30 and 31

Atsushi Yamazaki* (Section of Dental Care for Disabled, School of Dentistry, Tokyo Medical and Dental University)

10.35-10.55 Chair: Noriki Takahashi (Takahashi Dental Clinic)

- (9) Disinfection of Hands and Fingers with Electrolyzed Water p 32 and 33

Tatsushi Kohno*, Hiroyuki Manaka, Tomohisa Ogawa, Hiroshi Nakatani, and Kyu-ichi Kamoi (Section of Periodontology, School of Dentistry, Nippon Dental University)

- (10) Installation and Operation of Continuous Discharging System for Electrolyzed Functional Water, “Sebics” from Nobel Dental Clinic p 34 and 35

Katsumi Kimura*, Kazuyoshi Yoshimura¹⁾, Kohei Morita¹⁾, Kazuaki Shimada¹⁾, Chizuru Namiki¹⁾, and Hidehito Kaneko¹⁾ (Nobel Dental Clinic, ¹⁾ Okegawa Sakata Hospital, Seiseki Kai)

Break

Plenary Lecture

11.00-11.45 Chair: Takashi Miyazaki (Section of Dental Materials and Devices, School of Dentistry, Showa University)

Use of Electrolyzed Functional Water in Food Industry and its Issue p 8 and 9

Tetsuya Suzuki* (Section of Food and Biodynamics, Department of Marine Bioresources Chemistry, School of Fisheries Science, Hokkaido University)

Plenary Lecture

11.45-11.55 Chair: Hiroshi Iwamoto (Iwamoto Dentistry)

- C1) Review on Acidic Water Generator, “TFS400A” from TOTO p 54 and 55

Masanao Hamazaki* (Development of Water Faucets and Equipments, TOTO, Ltd.)

Lunch (Board Meeting)

13.00 General Meeting

Lecture on Commercial Products

13.30-14.15 Chair; Akihiko Shiba (Third Section of Prosthodontics, School of Dentistry, Showa University)

Findings in Use of Acidic Electrolyzed Water in Medical Front p 10 and 11

Katsuhiko Muramatsu (Department of Medical Treatment Technologies, Ihda Hospital, Kuriyama Kai)

Plenary Lecture

14.15-14.35 Chair; Kyu-ichi Kamoi (Section of Periodontology, School of Dentistry, Nippon Dental University)

(11) Sensitivity of Two Kinds of Strains of Oral Indigenous Microbe Isolated from Infected Root Canal against Electrolyzed Water p 36 and 37

Yo-ichi Ito*, Teruo Obana, Kengo Mirua, and Noriki Takahashi (Dentist Association of Kanagawa Prefecture)

(12) Effect of Strong Acidic Water on Cleaning of Root Canal p 38 and 39

Tetsu Kohno*, Yasuhiro Oka, Tatsuya Takahashi, Ryu-ichi Yoshida, and Ichiro Sekine (Section of Conservative Dentistry, School of Dentistry, Asahi University)

General Session (4 Presentations)

14.35-14.55 Chair; Hisashi Hisamitsu (Second Section of Conservative Dentistry, School of Dentistry, Showa University)

(13) Effect of Strong Acidic Electrolyzed Water on Avulsed Teeth p 40 and 41

Tsutomu Sato*, Hatsuko Kamoi, Tomoko Tanaka, Moto-o Niwa, and Hidehiro Aoki¹⁾ (Section of Hygiene, School of Dentistry, Nippon Dental University, ¹⁾ Research Institute of Tokyo Bioceramics, Ltd.)

(14) Effect of Fragrance-added High Oxidation Potential Water on Cleaning of Root Canal

p 42 and 43

Masaki Fukada*, Hiroko Suzuki, Satoru Ando, Masashi Komatsu, and Rei-ichi Okuda (Second Section of Conservative Dentistry, School of Dentistry, Tohoku University)

Break

General Session (4 Presentations)

15.05-15.25 Chair; Nariaki Nomoto (Section of Chemistry, Department of General Education, School of Dentistry, Nippon University)

(15) Use of Electrolyzed Water in Disinfection of Commercial Trays for Dental Impression

p 44 and 45

Yukimichi Tamaki*, Sohta Cho¹⁾, Takashi Miyazaki (Section of Dental Materials and Devices, School of Dentistry, Showa University, ¹⁾ Second Section of Conservative Dentistry, School of Dentistry, Showa University)

(16) Dental Alloy and Functional Water-In Case of Au-Ag-Cu Ternary Alloy

p 46 and 47

Akihiro Shimizu*, Ryu-ichi Yoshida (Section of Dental Materials and Devices, School of Dentistry, Nippon Dental University)

15.25-15.45 Chair; Tetsuo Ichikawa (First Section of Prosthodontics, School of Dentistry, Tokushima University)

(17) Effect of Super-oxidized Water on Prevention of Halitosis p 48 and 49

Miho Machito*, Hisashi Setoguchi, Maiko Kita, Hidekazu Kameyama, Ken-ichi Waizumi, and Takeshi Sueda (Second Section of Conservative Dentistry, School of Dentistry, Kagoshima University)

(18) Effect of Strong Acidic Electrolyzed Water on Healing of Wound p 50 and 51

Akihiro Ohyama*, Yuriko Hara, Akihiko Shiba, Hiroko Matsumoto, Emiko Kimura, Fuminori Iwasa, Ken-ichi Taguchi, Katsumi Ihda, and Yukie Shina (Third Section of Prosthodontics, School of Dentistry, Showa University)

Plenary Lecture

15.45-16.30 Chair; Shodai Murai (Section of Periodontology, Department of Conservative Dentistry, School of Dentistry, Nippon University)

Recent Progress in Electrolyzed Water

p 12 and 13

Kunimoto Hotta* (Section of Genetic Biochemistry, Department of Bioactive Molecules, National Institute of Infectious Diseases)

16.30 Closing Remarks Moto-o Li (Chairman of Preparatory Committee for 8th Conference)

Exhibit of Commercial Products

9.30-16.30

First Conference Room on 3rd floor

Sensitivity of Two Kinds of Strains of Oral Indigenous Microbe Isolated from Infected Root Canal against
Electrolyzed Water

Yo-ichi Ito, Teruo Obana, Kengo Mirua, and Noriki Takahashi
(Dentist Association of Kanagawa Prefecture)

Nosocomial infection has become a big issue in recent years because of increase of immunocompromized hosts, transition in responsible microorganisms, and increase of viral infectious diseases. In the dental treatment countermeasures to prevent the nosocomial infection have further become an urgent issue, because a frequent use of the invasive procedure, a more frequent contact with saliva as body fluid, and exposure to aerosols, and a frequent use of sharp instruments may create a more chance of the infection.

Countermeasures to prevent the nosocomial infection have been hitherto mainly targeted to the cross infection, but we consider that the endogenous infection caused by the dental treatment and the opportunistic infection should also be part of the nosocomial infection.

Based on this view we have investigated the sensitivity of microorganisms involving the oral infectious diseases against the electrolyzed water, completed preliminary experiments with streptococci and staphylococci derived from the oral cavity, and will report its finding.

Streptococcus and Staphylococcus strains used in this study were isolated from the infected root canals and assigned to Genus Streptococcus and Genus Staphylococcus as judged from the morphology and arrangement of cells and the gram reaction although their identification was not performed.

Staphylococcus was further assigned to Staphylococcus aureus as judged from the pigment production ability, whereas Streptococcus was assigned to hemolytic streptococcus as judged from the action on erythrocytes.

Both microorganisms were inoculated into an agar plate, collected by a platinum loop while preventing contamination with the medium components, and uniformly suspended in strong acidic electrolyzed water, Hycrosoft oxidized water, and sterile saline as a control. The suspension was inoculated by means of a platinum loop on the agar plate while varying intervals of time and incubated at 37°C for 48 hours to examine if the microorganisms were grown. The blood agar medium for hemolytic streptococcus and the nutrient agar medium for the Staphylococcus aureus were used, respectively, as a culture medium. In treatment with sterile saline, the growth of colony was observed along the inoculation line in both hemolytic streptococcus and Staphylococcus aureus, but in shortest treatment for 20 seconds with the strong acidic electrolyzed water and the Hycrosoft oxidized water, the growth of colony was not observed in both hemolytic streptococcus and Staphylococcus aureus. There was no significant difference in the growth of colony between the strong acidic electrolyzed water and the Hydrosoft oxidized water. It can be therefore concluded that both strong acidic electrolyzed water and Hydrosoft oxidized water have the antiseptic activity against two kinds of the microorganisms possibly causing the purulent diseases and are very effective within a short period of time after treatment. We could not evaluate their effectiveness in a shorter time than 20 seconds, because an operation time to prepare a uniform suspension with a pipet could not be reduced to shorter than 20 seconds.

Effect of Strong Acidic Water on Cleaning of Root Canal

Tetsu Kohno, Yasuhiro Oka, Tatsuya Takahashi, Ryu-ichi Yoshida, and Ichiro Sekine (Section of Conservative Dentistry, School of Dentistry, Asahi University)

Purpose

In recent years it has been known that electrolysis of water containing the minimal quantity of a salt produces strong acidic water. The strong acidic water has pH lower than 2.7 and an oxidation-reduction potential (ORP) higher than 1,100 mV and contains residual chlorine. It has been applied experimentally to the dental field because of little adverse effect to living organisms and the strong microbicidal effect. To use the strong acid water in the root canal treatment, we investigated the effect of this water on decalcification of inorganic substances and its efficacy in the root canal irrigation when used as an ultrasonicated solution.

Experimental Materials and Method

Experiment 1

100 mg of dried dentin powder (after removal of organic contaminants) with a particle size of 75-150 mesh was precisely weighed and added to 30 ml each of the strong acidic water generated by LOBO S-II from Aoi Engineering Co., Ltd. and distilled water. This mixture was stirred for 1, 5, 10, and 30 minutes to designate a group with constant volume. Meanwhile strong acidic water or distilled water was added with stirring to this mixture at 30 ml/min such that one minute of stirring was performed for a 30 ml of volume, 5 minutes of stirring for volume of 150 ml, 10 minutes of stirring for volume of 300 ml, and 30 minutes of stirring for 900 ml, which were designated a group with increased volume. The mixtures were filtered under reduced pressure to dry the residual dentin powder and weigh. Weight loss of the dentin powder was assigned as a decalcification rate. At the same time, a pH value, an oxidation-reduction potential (ORP), and the residual chlorine concentration after treating with the strong acidic water were determined.

Experiment 2

After removal of a crown, a working length of the avulsed human lower incisor teeth was established to be 1mm short of the root apex and the root canals were shaped with K-files with a size up to # 45 K-file according to a routine procedure. Setting an ENAC 6 at a flow rate of 30 ml/min and scale 3, strong acidic water and distilled water were injected to the root canal for 1, 5, and 10 minutes for irrigation. Two kinds of tips were used. One was a root canal plugger tip #40 (hereinafter referred to as a plugger group). Other was a trial tip, to which a needle for root canal irrigation (washing needle from Nippro Inc.) was brazed (hereinafter referred to as a trial tip group) to assure filling of the root canal with a fresh solution.. The root was immediately sectioned vertically to dehydrate with a series of a graded alcohol according to a routine procedure. The samples were dried by a critical-point drying method, onto which a Pd-Pt alloy was vacuum-deposited. The surface of the root canal wall near the apex 1 mm closer to the crown was inspected by a scanning electron microscope to evaluate the cleaning efficacy..

Results

Experiment 1

In the group with constant volume, use of the strong acidic water resulted in the decalcification rate of approximately 17% after treating for one minute and this value little changed after 5 minutes. On the other hand, the decalcification rate in the group with increased volume was increased with extension of a treating time. Use of distilled water little decalcified dentin in both groups. A pH value after treatment was increased with extension of a treating time in the group with constant volume and approached to the value just after its preparation in the group with increased volume. An ORP value was decreased in the group with constant volume, but approached the value just after preparation in the group with increased volume. The concentration of residual chlorine was decreased with time in the group with constant volume, but similar to the value after treating for one minute in the group with increased volume.

Experiment 2

The smear layer could be removed by treatment with strong acidic water for 10 minutes in the plugger group and for one minute in the trial tip group, respectively. An opening of the dentinal tubules was clearly observed. The smear layer could not be removed by treatment with distilled water for 10 minutes in both groups.

Discussion

The strong acidic water may decalcify inorganic substances, but the action was not sustained suggesting that replenishing the fresh strong acidic water can maintain more effective cleaning of the root canal.

7th Workshop for Use of Strong Acidic Water in Dental Field
Abstract

March 7 (Saturday), 1998

Auditorium of Building #3, Kudan Hall, School of Dentistry, Nippon Dental University

Chairman; Kyu-ichi Kamoi

Organizer; Study Group for Use of Strong Electrolyzed Water in Dental Field

Benefactor; Foundation for Study and Promotion of Functional Water

9.00-9.20

Opening speech ; Kyu-ichi Kamoi (Chairman of 7th Workshop for Use of Strong Acidic Water in Dental Field)

Speech by President; Akihiko Shiba (President of Study Group for Use of Strong Electrolyzed Water in Dental Field)

Speech by Guest of Honor; Jun-ichi Urata (Director of Foundation for Study and Promotion of Functional Water)

General Session (Morning)

9.20-9.50 Chair; Hisashi Kume (Second Section of Conservative Dentistry, School of Dentistry, Showa University)

(1) Effect of Acidic Water on Bacteria in Air-water Syringe

Yukie Takemoto*, Tusomu Sato, Moto-o Niwa (Section of Hygiene, School of Dentistry, Nippon Dental University)

(2) Sterilization of Gypsum Model-Immersion in Functional Water

Akihiro Shimizu*, Ryu-ichi Yoshida (Section of Dental Materials and Devices, School of Dentistry, Nippon Dental University)

(3) Oxidation-reduction Potential of Oxidized Water-Electrochemical Reduction of Strong Oxidized Water and Soft Water on Platinum Electrode

Takeo Igarashi*, Ko-ichi Itoh, Nariaki Nomoto¹⁾, and Shodai Murai (Section of Conservative Dentistry and Periodontology, School of Dentistry, Nippon University, ¹⁾ Section of Chemistry, School of Dentistry, Nippon University)

* Speaker

9.50-10.20 Chair; Yukihiro Numabe (Section of Periodontology, School of Dentistry, Nippon Dental University)

(4) Effect of Strong Acidic Electrolyzed Water on Cultured Cells Derived from Human Gingival and Periodontal Ligament

Tsutomu Sato*, Tomoko Tanaka, Hatsuko Kamoi, and Moto-o Niwa (Section of Hygiene, School of Dentistry, Nippon Dental University)

(5) Effect of Oxidized Water on Tongue Wound in Rats

Kazuhiro Kawamoto*, Hideyasu Akutagawa, Noriko Ohta, Taro Kojima, Ko-ichi Itoh, and Shodai Murai (Section of Conservative Dentistry and Periodontology, Department of, School of Dentistry, Nippon University)

(6) Effect of Super-oxidized Water on Healing of Wound

Chiayuki Nyurai-in*, Miho Machto, Koji Matsuyama, Hisashi Setoguchi, Toshikazu Obata, Maiko Kita, Katsuya Kinoshita, Yu-ichi Izumi, and Takeshi Sueda (Second Section of Conservative Dentistry,

School of Dentistry, Kagoshima University)

10.20-10.50 Chair; Tetsuo Ichikawa (First Section of Prosthodontics, School of Dentistry, Tokushima University)

(7) Effect of Strong Electrolyzed Water in Cleaning and Sterilization of Cutting Device for Dentistry

Azusa Kanaishi*, Chika Kase, Akihiko Shiba, Toshihiro Sakai, Hiroaki Tsukazaki, Hiroyuki Nakane, Izu Nimi¹⁾, Akihiro Nishimura, and Noboru Okamura¹⁾ (Third Section of Prosthodontics, School of Dentistry, Showa University, ¹⁾ Section of Health and Hygiene, School of Medicine, Tokyo Medical and Dental University)

(8) Effect of Strong Acidic Electrolyzed Water on Dental Impression Materials

Yukimichi Tamaki, Takashi Miyazaki, Akihiko Shiba¹⁾ (Section of Dental Materials and Devices, School of Dentistry, Showa University, ¹⁾ Third Section of Prosthodontics, Third Section of Prosthodontics, School of Dentistry, Showa University)

(9) Metal Corrosion by Neutral Electrolyzed Water-Comparison with Strong Acidic

Electrolyzed Water-Report 2; Effect on Dental Base Metal Alloy

Yu Yamada*, Kazuhiko Endo,¹⁾ Takeo Maida, and Osamu Tanaka (Medical and Dental Clinic, School of Dentistry, Health Science University of Hokkaido, ¹⁾ Section of Dental Materials and Devices, School of Dentistry, Health Science University of Hokkaido)

10.50-11.50

Plenary Lecture 1 Chair; Akihiko Shiba (Third Section of Prosthodontics, School of Dentistry, Showa University)

Why Did We Air the Water with Terrific Properties in News Special?

Yo Matsumoto (News Bureau, Nippon Television Network)

11.50-12.50 Lunch (Board Meeting)

12.50-13.10 General Meeting

13.10-13.30 Session for Commercial Products

Chair; Masahiko Ozeki (Third Section of Prosthodontics, School of Dentistry, Showa University)

(10) Use of Soft Water in Medial Field

Yasumitsu Koshino* (Aqua Japan Co., Ltd.)

(11) Properties of Oxidized Water Generated in Three-Compartment Electrolysis Cell

Shusei Sumida* (Coherent Technology Ltd.)

13.30-14.30

Plenary Lecture 2 Chair; Shodai Murai (Section of Conservative Dentistry and Periodontology, School of

Dentistry, Nippon University)

Use of Strong Acidic Water in Health Care and its Future

Atsushi Okada* (Manager of Clinical Laboratory, Postal Service Agency Hospital of Kanto)

General Session (Afternoon)

14.30-15.00 Chair; Takeshi Sueda (Second Section of Conservative Dentistry, School of Dentistry, Kagoshima University)

(12) Clinical Use of Soft Oxidized Water in Dentistry-Report 2

Hiroshi Iwamoto*, Yo-ichiro Kitano (Iwamoto Dentistry)

(13) Assessment of One Year Use of Strong Electrolyzed Water Fed to Dental Unit

Shuhei Mizogami* (Mizogami Dental Clinic)

(14) Antiseptic Effect of Functional Water on Dental Gloves

Mami Suzuki*, Keiko Kazuzaki, Kyu-ichi Kamoi (Section of Periodontology, School of Dentistry, Nippon Dental University)

15.00-15.30 Chair; Moto-o Niwa (Section of Hygiene, Nippon Dental University)

(15) Overview of Hand Toothbrush with Cleaning and Draining Functions Using Strong Acidic Electrolyzed Water as Cleaning Fluid

Atsushi Yamazaki* (Section of Dentistry for Disabled, School of Dentistry, Tokyo Medical and Dental University)

(16) Denture Plaque and Use of Strong Electrolyzed Water

Toshiya Kashihara*, Katsuhiko Hirokawa¹⁾, Yoko Terada, Hiroshi Nagao, Tatsuya Ito, Fumiaki Kohno, Tetsuo Ichikawa, and Yo-ichiro Miyake¹⁾ (First Section of Prosthodontics, School of Dentistry, Tokushima University, ¹⁾ Section of Oral Bacteriology, School of Dentistry, Tokushima University)

(17) Effect of Strong Acidic Water and Soft Oxidized Water on Supragingival Plaque

Hiroki Kawamura*, Hiroshi Ito, Nobuyasu Asaki, Yo-ichi Saito, and Kyu-ichi Kamoi (Section of Periodontology, School of Dentistry, Nippon Dental University)

15.30-16.00 Chair; Atsushi Yamazaki (Section of Dentistry for Disabled, School of Dentistry, Tokyo Medical and Dental University)

(18) Antiseptic Effect of Weak Alkaline Electrolyzed Water on Bacteria in Periodontal Pockets

Hirohiko Nakamura*, Tomohisa Ogawa, Hiroyuki Fujita, Hiroshi Nakatani, and Kyu-ichi Kamoi (Section of Periodontology, School of Dentistry, Tokyo Dental University)

(19) Use of Topical Fluoride Solution Using Strong Acidic Electrolyzed Water -Bacterial Count in Oral Cavity after Gargling

Kazunaru Kimoto* Yukio Hirata, Ki-ichi Ihzuka (Section of Dental Hygiene, School of Dentistry, Kanagawa Dental College)

(20) Reaction of Strong Acidic Electrolyzed Water with Sintered Hydroxyapatite

Hatsuko Kamoi*, Tustomu Sato, Tomoko Tanaka, Yugo Nasu¹⁾, and Moto-o Niwa (Section of Hygiene, School of Dentistry, Nippon Dental University, ¹⁾ Cooperative Research Institute, School of Dentistry, Nippon Dental University)

16.00-17.00

Plenary Lecture 3 Chari; Osamu Tanaka (Research Institute of Health Care, School of Dentistry, Health Care University of Hokkaido)

Functional Water and Dental Metals

Ryu-ichi Yoshida* (Section of Dental Materials and Devices, School of Dentistry, Nippon Dental University)

17.00 Closing Remark

Hiroshi Nakatani (Chairman of Preparatory Committee for 7th Workshop for Use of Strong Electrolyzed Water in Dental Field)

Exhibition of Commercial Products 9.00-16.00 Memorial Hall, Building #1, School of Dentistry, Nippon Dental University

Effect of Strong Acidic Electrolyzed Water on Cultured Cells Derived from Human Gingival and Periodontal Ligament

Tsutomu Sato, Tomoko Tanaka, Hatsuko Kamoi, and Moto-o Niwa (Section of Hygiene, School of Dentistry, Nippon Dental University)

1. Purpose

In use of strong acidic electrolyzed water (AEW), its safety has to be reviewed thoroughly. In recent years use of AEW has been widely tried in the dental field. In this study we investigated the effect of AEW on soft tissues in oral cavity using fibroblasts derived from the adult gingival and the periodontal ligament.

2. Materials and Method

Cells used were the fibroblasts and keratinocytes isolated from the healthy gingival of males and females at age of twenties and fibroblasts isolated from the periodontal ligament. Culture media used were Eagle's MEW containing 10% fetal bovine serum for the fibroblasts and serum-free media for the keratinocytes, respectively. All cells were incubated at 37°C under an atmosphere of 5% CO₂ and 100% humidity. Subcultures which had undergone several passages were used in the experiment. The cell cultures were inoculated into a 24-well multiwall plate to incubate for 24 hours, followed by removing the culture media and adding AEW to contact with the cells at ambient temperature for 30 seconds to 5 minutes. AEW applied were undiluted AEW and the solutions diluted with distilled water to 10% AEW, 20% AEW, 30% AEW, 40% AEW, and 50% AEW, respectively. Distilled water was used for a control. Effect of AEW on the cells was evaluated by determining the cellular activity of the DNA synthesis after application of AEW and an activity change of LDH released to the culture.

3. Results and Discussion

Figures 1-3 demonstrate part of the results of ¹H-thymidine uptake in the cells, to which AEW was applied. Treatment with undiluted AEW for 30 seconds completely prevented all three kinds of cells from uptake. Thymidine uptake in all groups was decreased with increasing a contact time of AEW. However, all cells in the groups treated with 10% AEW or 20% AEW for 30 seconds also demonstrated more than 90% uptake of the control group. An increase in the LDH activity was consistent with increased AEW concentrations and increased AEW contact time. The LDH activity of the cells in the groups treated with 10% AEW or 20% AEW for 30 seconds was almost same as that in the control group. In the present study the effect of AEW on the cells derived from human oral tissues was evaluated by determining the activity of DNA synthesis and a change of LDH activity of the cells, confirming low cytotoxicity when treated for 30 seconds with AEW diluted below 20%.

Denture Plaque and Use of Strong Electrolyzed Water

Mineya Kashihara, Katsuhiko Hirokawa¹⁾, Yoko Terada, Hiroshi Nagao, Tatsuya Ito, Fumiaki Kohno, Tetsuo Ichikawa, and Yo-ichiro Miyake¹⁾ (First Section of Prosthodontics, School of Dentistry, Tokushima University, ¹⁾ Section of Oral Bacteriology, School of Dentistry, Tokushima University)

1. Purpose

Application of strong electrolyzed water in the dental field has been advanced in the clinical fronts since Shiba et al reported its use in 1990 at the Conference of the Japan Prosthodontic Society. Its application has been mostly in the field of endodontic and periodontal procedures and presumed to be valuable and important in the prosthodontic field. We investigated whether or not the strong acidic electrolyzed water and neutral electrolyzed water can be used to clean the dentures using denture plaques which were adhered to the mucosal surface of the denture currently used. We found the cleaning effect of the strong electrolyzed water was similar to that of commercial denture cleaners. However, the microbicidal effect of the strong electrolyzed water was not as strong as one found in an in vivo condition. This observation was explained by the fact that microorganisms adhered to the mucosal surface of denture form a biofilm, possibly preventing the electrolyzed water from penetrating deep into it. We prepared a biofilm of *Candida albicans*, a strain of pathogenic bacteria for denture stomatitis in vivo to investigate the effect of the strong electrolyzed water on it. Previous study suggested that to improve the microbicidal and cleaning effects of the strong electrolyzed water on the biofilm of *C. albicans*, the frequency of immersion in the strong electrolyzed water has to be increased to assure its penetration very deep into the biofilm.

In the present study we investigated the sterilization effect of the strong electrolyzed water to *C. albicans* present in the denture plaque adhered to the mucosal surface of the denture currently used by the elderly people. There is also a lot of interest in *Helicobacter pylori* as a pathogen for gastric inflammation, gastric ulcer, and stomach cancer. A positive PCR reaction to this fungus was also found in saliva, dental plaque, and exudates from the periodontal pocket. However, there was few report on whether this fungus could be found in the denture plaque. In the present study the denture plaque collected from the mucosal surface of the denture in the elderly people was investigated by a nested PCR method using a primer specific to *H. pylori* 16S ribosomal RNA.

2. Experimental Materials and Method

1. Effect on Sterilization of *C. albicans*

Two conditions were used in the present study. In condition (1) dentures currently used by 10 elderly subjects were independently immersed in 200 ml of strong acidic electrolyzed water in a beaker, followed by replacing every minute with fresh strong acidic electrolyzed water five times. A total time of washing was 5 minutes. In condition (2) the dentures were immersed in 200 ml of the strong acidic electrolyzed water in a beaker placed in an ultrasonic cleaner, followed by repeating immersion and ultrasonic washing under condition (1).

Denture plaques were sampled by a disc with a diameter of 15 mm from anterior teeth, molar teeth, and palate in the mucosal surface of denture before and after cleaning of the denture with the strong acidic

electrolyzed water. The discs were attached to the medium for selection and isolation of the fungi and then removed. It was reported that use of the medium for selection and isolation of the fungi allowed for identifying the fungi from color and morphology of the colonies. CHROMager Candia (manufactured by CHROMager Inc.) was used. The fungi were incubated at 37°C for 48 hours. The fungi were identified and the sterilization effect was investigated.

2. Detection of *H. pylori* in Denture Plaque

(1) Collection and Treatment of Sample

Denture plaques were collected from 16 subjects, from which DNA was extracted to run nested PCR. *H. pylori* ATCC42504 standard strain was used as a control.

(2) Nested PCR

Three kinds of primers specific to *H. pylori* 16S ribosomal RNA were used for nested PCR. They were

HP1: CTG GAG AGA CTA AGC CCT CC, position 834-853

HP2: ATT ACT GAC GCT GAT TGT GC, position 744-763

HP3: AGG ATG AAG GTT TAA GGA TT, position 407-426

Using HP1 and HP3, the 446-bp region was amplified by the PCR method. Using HP1 and HP2, the 109-bp region of the PCR amplified product was amplified. The PCR amplified products obtained were analyzed by agarose gel electrophoresis.

3. Results and Discussion

Before cleaning *C. albicans* were isolated from the denture plaques on the mucosal surface of denture near the anterior teeth, molar teeth, and palate. Among three sites less *C. albicans* were found in the denture plaques near the palate. The isolated colony count of *C. albicans* was decreased after cleaning under condition (1), suggesting the strong acidic electrolyzed water has the sterilization effect. The isolated colony count of *C. albicans* was further decreased before and after cleaning under condition (2) as compared with the cleaning under condition (1). It was suggested that use of ultrasonic wave together with immersion is more effective for the sterilization by the strong acidic electrolyzed water. The positive PCR reaction was found in 20% cases of the denture plaques. The denture plaques adhered to the mucosal surface of denture are therefore likely to act as a reservoir of *H. pylori*. Effect of the strong acidic water on *H. pylori* appears to be an interesting subject for further study.

The present results suggest that the oral cavity may be utilized as a reservoir of pathogenic fungi for various diseases including the respiratory infection and digestive system diseases. They also suggest control of immunocompromised hosts in the root canal is important particularly in dentistry. We believe a further study is required after increasing the number of subjects.

4. References

- (1) Toshiya Kashihara, Tetsuo Ichiwaka, Naeko Kawamoto, Hideo Kanitani, Masanobu Hori-uchi, Katsuhiko Hirota, Yo-ichiro Miyake, Naoyuki Matsumoto, (1996) "Oral cavity of patients hospitalized in geriatrics hospital and composition of fungi in denture plaques-Report 1- Preliminary results",

Journal of Prosthodontic Research, 40, 446-453.

- (2) Naeko Kawamoto, Tetsuo Ichihawa, Hideo Kanitani, Toshiya Kashihara, Masanobu Hori-uchi, Katsuhiko Hirota, Yo-ichiro Miyake, Naoyuki Matsumoto, (1996), "Cleaning Effect of Electrolyzed Water on Denture-Bacteriological Study", Journal of Prosthodontic Research, 40, 574-579.

Effect of Strong Acidic Water and Soft Oxidized Water on Supragingival Plaque

Hiroki Kawamura, Hiroshi Ito, Nobuyasu Asaki, Yo-ichi Saito, and Kyu-ichi Kamoi (Section of Periodontology, School of Dentistry, Nippon Dental University)

Purpose

In recent years a role of gargles has been reconsidered for improvement of the oral health. However, there are many unknowns about the effect of gargles on mature plaques. In this study the mature plaques were exposed to LISTERINE (Warner Lambert Inc., USA), one of commercial gargles, strong acidic water (HOW) with notable microbicidal activity, and soft oxidized water (SOW) to examine a morphological change of plaques by an electron microscope and some findings will be reported.

Materials and Method

As the subject eighteen healthy individuals were chosen from students and medical staffs in the School of Dentistry, Nippon Dental University. A film was prepared from an epoxy resin (**Kutol**, Nisshin Co., Ltd., Tokyo) and temporarily bonded to the interdental space between first and second premolars in either right or left maxillary using an instantaneous polymerizing resin. Plaques formed on the epoxy resin film were collected in a course of 6 to 120 hours after plaque formation. These films were divided into four groups; the control group was the films immersed in physiological saline (Otsuka Pharmaceutical Co., Ltd.) for 30 seconds; the gargle group was the films immersed in LISTERINE; the strong acidic water group was the films immersed in OXIDIZER OXM-01 (pH 2.6, Miura Electronics Ltd., Japan) for 30 seconds; the soft oxidized water group was the films immersed in Actide NDS-60KMW (pH 5.5, Omuko Ltd., Japan). All films were fixed with 2.5% glutaldehyde and 1% OsO₄, followed by washing with a graded concentration of an alcohol for dehydration. A routine procedure was used to prepare the specimen for SEM. These films were also embedded in **Kutol** resin according to a routine procedure, followed by slicing with a microtome and staining for inspection by TEM.

Results

Formation of clear plaque was not observed in all groups 6 hours after initiation of the plaque formation. Plaque formation was confirmed by SEM 12 hours after initiation of the plaque formation.

In the control group the colony consisting mainly of cocci was observed 12 hours later and the plaques consisting mainly of cocci were found 24-72 hours later. The surface of the plaques was rough. The surface of some plaques was covered with substances other than the fungi.

Filamentous fungi were observed together with filamentous substances other fungi in the colony of cocci and bacilli 120 hours after initiation of the plaque formation..

To the contrary, 12 hours after initiation of plaque formation less colonies were formed in all of the gargle group, HOW group, and SOW group than the control group. Decrease of fungi was significant particularly in the gargle group.

The plaques mainly consisting of cocci were observed in all three groups 24-72 hours after initiation of the plaque formation and the surface of fungi became smoother than that in the control group.

Filamentous fungi were observed in the colony of cocci and bacilli 120 hours after initiation of the plaque formation and the fungi surface became smoother, but few substances other than fungi were found.

The plaque distribution in the control group 72 hours after initiation of the plaque formation was observed by TEM to show a space between fungi was narrow and substances with high electron density was present in the space between fungi. It was observed that fungi were densely distributed 120 hours after initiation of the plaque formation and the filamentous fungi extended to the upper part of cocci and bacilli. It was also observed that substances were densely distributed in the space between fungi.

To the contrary, there was a wide space between fungi in the plaque surface 72 hours after initiation of the plaque formation in the gargle group and HOW group. It was observed that distribution of the substances in the space between fungi was limited.

There was a space between fungi in the gargle group and HOW group 120 hours after initiation of the plaque formation. It was observed that substances in the space between fungi were limited, whereas a space between fungi became slightly narrower in the SOW group and an amount of substances in the space between fungi in the SOW group was second to that in the control group.

Discussion

These results indicate LESTRINE, HOW, and SOW cleanse significantly the plaque in early stage. They also affect the surface morphology of fungi. In mature plaques gargle, HOW, and SOW affect mainly the substances other than fungi on the plaque surface. However, SOW is slightly less effective than gargle and HOW.

These results indicate that LESTRINE, HOW, and SOW are particularly effective to sterilize the plaque in early stage and useful in improving the oral health.

Use of Topical Fluoride Solution Using Strong Acidic Electrolyzed Water -Bacterial Count in Oral Cavity after Gargling

Kazunaru Kimoto Yukio Hirata, Ki-ichi Ihzuka (Section of Dental Hygiene, School of Dentistry, Kanagawa Dental College)

1. Purpose

In the previous report we found that a topical fluoride solution (OF) prepared with the strong acidic electrolyzed water improves the resistance of enamel to acids because of the presence of a fluoride (F) for gargling. We also investigated other application based on the microbicidal activity of the strong acidic electrolyzed water and determined an oxidation-reduction potential (ORP) and pH of the spitting fluid after gargling to find a decrease of ORP by 60-70% in single gargling for 60 seconds, resulting in loss of properties of the strong acidic electrolyzed water in the OF solution by gargling and suggesting weakening of the oxidative power of the OF solution. However, the OPR value was reduced only by approximately 40% when gargled for 30 seconds twice in series. We investigated the effect of the OF solution on oral bacteria by determining the counts of aerobic and anaerobic bacteria in the spitting fluid after gargling with the OF solution twice in series.

2. Materials and Method

As the test solution, OF solutions with 250, 500, and 1,000 ppm F were prepared using the strong oxidized electrolyze water with OPR of 1,120-1,150 mV and pH 2.5 when sampled and sodium fluoride (NaF). 0.9% sodium chloride (NaCl) solution was used as a control solution. Subjects (3 males and 4 female, average age; 32.3) washed mouth and brushed teeth freely before mouth was gargled with the test solutions once a day between 9.30 a.m. and 11.00 a.m. Mouth was gargled with 10 ml of 0.9% NaCl solution for 30 seconds each twice in series (control solution) to determine the counts of aerobic and anaerobic bacteria (CFU/ml) in the second spitting fluid (previous value). Trypticase soy II agar with 5% sheep blood (manufactured by Japan Becton Dickinson Inc.) was used as a culture medium to inoculate the bacteria aerobically at 37°C for 24 hours or anaerobically for 48 hours to determine the colony counts on the plate. To investigate the effect of test solutions on saliva secretion after gargling, saliva was collected 60 seconds after gargling twice in series and rest for 3 minutes to determine the rate of saliva secretion (ml/min). Fifteen minutes later, mouth was gargled with the test solutions or 0.9% NaCl solution for 30 seconds twice in series to determine the counts of aerobic and anaerobic bacteria in the second spitting fluid (subsequent value). The rate of saliva secretion after gargling was similarly determined. The strong oxidized electrolyzed water was prepared using **Sedent Pymizer** SD-1000 (manufactured by Sekimura Co., Ltd.).

3. Results and Discussion

Increase of the F concentration in the OF solution used for gargling resulted in decrease of the ORP value and increase of the pH value (Table 1). The CFU values of the spitting fluid in second gargling with the control solution were in a range of $1.5-33 \times 10^5$ CFU/ml in the aerobic bacteria and $6-112 \times 10^5$ CFU/ml

in the anaerobic bacteria, respectively (previous value). The bacteria count was also widely varied in the same subject depending on the day of experiments. The CFU values of the spitting fluid 15 minutes after gargling twice in series (subsequent value) were decreased more in all OF solutions than those in the control solution (previous values). However, in some cases the subsequent values were increased though not in the case of the OF solutions. We calculated a CFU ratio (subsequent values/ previous values) of each solution (Table 2). The CFU ratios in the OF solutions were decreased in both aerobic and anaerobic bacteria with decreasing the F concentration, but increased gradually in both bacteria with increasing the F concentration. As the F concentration of the OF solution was kept below 250 ppm, the ORP and pH values of the OF solution were higher than 1,000 V and lower than 4, respectively, suggesting the OF solution has the microbicidal power to kill roughly a half of bacteria. Compared from the previous values, the rate of saliva secretion after gargling did not reveal significant difference in all solutions tested. All test solutions did not affect the rate of saliva secretion.

The concentration of hypochlorous acid and chlorate ion in the strong acidic electrolyzed water is 1/20 to 1/30 the concentration of those used in clinical disinfection. However, a low pH value of the strong acidic electrolyzed water suggests the active species are mostly in a form of the hypochlorous acid demonstrating a strong microbicidal power. However, efficacy of the strong acidic electrolyzed water is likely to vary because of a limited amount of active oxygen and ozone. Activity of free chlorine could be further decreased in the presence of salivary protein. In the present study an amount of the salivary protein in the oral cavity was reduced by gargling twice in series and a decrease of the oxidation-reduction potential of the spitting fluid was kept at 40%, assuring the microbicidal activity.

The OF solution may be a potential mouthwash since gargling twice in series keeps the microbicidal power of the strong acidic electrolyzed water and provides the enamel caries resistance..

4. References

- (1) Issei Kimoto et al., Journal of Dental Health, 45, 662-663, 1995
- (2) Issei Kimoto et al., Journal of Dental Health, 47, 564-565, 1997.

Table 1 ORP and pH of Test Solution

Test solution	ORP (mV)	pH
OF solution, 0 ppm F	1122.4	2.51
OF solution, 250 ppm F	1008.3	4.05
OF solution, 500 ppm F	968.3	4.48
OF solution, 1000 ppm F	951.7	4.98
Sterile deionized water, 1000 ppm F	319.4	6.74
0.9% NaCl solution	378.0	6.38

Table 2 CFU Ratios (Subsequent Value/Previous Value) in Spitting Fluid after Second Gargling*

Test solution	Aerobic bacteria	Anaerobic bacteria
OF solution, 0 ppm F	0.42 ± 0.24**	0.38 ± 0.27**
OF solution, 250 ppm F	0.49 ± 0.22	0.56 ± 0.20
OF solution, 500 ppm F	0.67 ± 0.21	0.68 ± 0.21
OF solution, 1000 ppm F	0.53 ± 0.32	0.53 ± 0.30
Sterile deionized water, 1000 ppm F	0.81 ± 0.54	1.06 ± 1.02
0.9% NaCl solution	1.59 ± 0.51	1.00 ± 0.67

* CPU values in the spitting fluid of the control solution (previous values) is assumed to be 1.

** Mean ± standard deviation (n=7)

6th Workshop for Use of Strong Acidic Water in Dental Field
Abstract

March 1 (Saturday), 1997

Auditorium on 2th floor of Nippon University Hall

Chairman; Shodai Murai

Organizer; Study Group for Use of Strong Electrolyzed Water in Dental Field

Benefactor; Foundation for Study and Promotion of Functional Water

Program
March 1 (Saturday)

9.00-9.15

Opening speech ; Shodai Murai (Chairman of 6th Workshop for Use of Strong Acidic Water in Dental Field)

Speech by President; Akihiko Shiba (President of Study Group for Use of Strong Electrolyzed Water in Dental Field)

Speech by Guest of Honor; Jun-ichi Urata (Director of Foundation for Study and Promotion of Functional Water)

General Session (Morning)

9.15-9.35 Chair; Masahiko Ozaki (Third Section of Prosthodontics, School of Dentistry, Showa University)

(1) Determinants of Oxidation-reduction Potential of Oxidized Water

Takeo Igarashi*, Ko-ichi Ito, Nariaki Nomoto¹⁾, and Shodai Murai (Section of Conservative Dentistry and Periodontology, School of Dentistry, Nippon University, ¹⁾ Section of Chemistry, School of Dentistry, Nippon University)

(2) Effect of Various Strong Acidic Electrolyzed Waters on Prosthetic Alloys

Kazuhito Nimi*, Hiroyuki Nakane, Akihiko Shiba, Toshihiro Sakai, Hiroaki Tsukazaki, Azusa Kanaishi, Chika Kase, Tetsuji Suzuki, Yoko Kubota, Masahiko Ozeki, Kodo Tamaki¹⁾, and Takashi Miyazaki¹⁾, (Third Section of Prosthodontics, School of Dentistry, Showa University, ¹⁾ Section of Dental Materials and Devices, School of Dentistry, Showa University)

* Speaker

9.35-9.55 Chair; Takeshi Sueda (Second Section of Conservative Dentistry, School of Dentistry, Kagoshima University)

(3) Effect of Ultrasonic Washing on Acidic Functional Water

Atsushi Yamazaki* (Section of Dentistry for Disabled, School of Dentistry, Tokyo Medical and Dental University)

(4) Effect of Aqua Acidic Water on Nicotine-stained Root Surface

Hiroki Kawamura*, Hiroshi, Ito, Nobuyasu Asaki, Yo-ichi Saito, and Kyu-ichi Kamoï (Section of Periodontology, School of Dentistry, Nippon Dental University)

9.55-10.15 Chair; Mitsuo Amagasa (First Section of Oral Surgery, School of Dentistry, Tokyo Medical and Dental University)

(5) Use of Strong Acidic Electrolyzed Water in Repair of Dental Composite Resin

Masataka Inoue*, Atsushi Shiraishi, Ko-ichi Narikawa, Masayoshi Inoue, and Benji Fuji-i¹⁾ (Section of

Conservative Dentistry, Osaka Dental University, ¹⁾ Emeritus Professor of Osaka Dental University)

(6) Microbicidal and Sterilization Effects of Electrolyzed Water on Biofilms of Various Bacteria

Toshiya Kashiwara*, Tetsuo Ichikawa, Hideo Kanitani, Naeko Kawamoto, Katsuhiko Hirota¹⁾, Yo-ichiro Miyakae¹⁾, and Naoyuki Matsumoto (First Section of Prosthodontics, School of Dentistry, Tokushima University, ¹⁾ Section of Oral Bacteriology, School of Dentistry, Tokushima University)

Plenary Lecture 1

10.25-11.10 Chair; Ko-ichi Ito (Section of Conservative Dentistry and Periodontology, School of Dentistry, Nippon University)

Corrosion of Dental Metals and Strong Electrolyzed Water

Nariaki Nomoto* (Section of Chemistry, School of Dentistry, Nippon University)

Plenary Lecture 2

11.15-12.00 Chair; Kyu-ichi Kamoi (Section of Periodontology, School of Dentistry, Nippon Dental University)

Antiseptic Effect of Strong Acidic Electrolyzed Water on Various Bacteria Including Escherichia coli 157

Kazuko Saito* (Section of Oral Microorganisms, School of Life Dentistry at Nigata, Nippon Dental University)

12.00-13.00 Break

(12.00-12.45) Board Meeting)

12.45-13.00 General Meeting

Presentation of Commercial Products

13.00-13.30 Chair; Noriki Takahashi (Director in Science, Kanagawa Prefecture)

(1) Overview of Strong Electrolyzed Water Generator and Peripheral Devices

Kazunori Nakadegawa* (Division of Aqua System Products, Amano Co., Ltd.)

(2) Use of Strong Electrolyzed Water in Agricultural and Food Fields

Toshihiko Okada* (Sales and Development, Aiken Industries Co., Ltd.)

(3) Study on Antiseptic Effect of Strong Acidic Water with Low Chlorine Concentration from TOTO-Detail Study of Antiseptic Effect as Varying pH, Effective Chlorine Concentration, and Oxidation-reduction Potential

Sumi Takeshita*, Shigeru Ando (TOTO, Ltd.)

13.30-13.50 Chair; Atsushi Yamazaki (Section of Dentistry for Disabled, School of Dentistry, Tokyo Medical and Dental University)

(4) Overview of Strong Acidic Electrolyzed Water, “Aquapotential”, Approved as Use for Medical Device

Koji Narita, Hiroyuki Kagawa (Division of Functional Products, Asahi Glass Engineering Co., Ltd.)

(5) Acidic Electrolyzed Water and Setup of Infrastructure

Yasumitsu Koshino (Aqua Japan Co., Ltd.)

Plenary Lecture 3

13.55-14.40 Chair; Akihiko Shiba (Third Section of Prosthodontics, School of Dentistry, Showa University)

Effect of Strong Electrolyzed Water on Atopic Dermatitis

Shu-ichi Naito* (Kasumigaura Hospital, Tokyo Medical University)

General Session (Afternoon)

14.45-15.15 Chair; Tetsuo Ichikawa (Section of First Prosthodontics, School of Dentistry, Tokushima University)

(7) Use of Superacidic Water in Clinical Pediatric Dentistry and its Basic Research

Rie Yamaguchi*, Akio Ito, Eiki Ogasawara, Hisa-aki Shindo¹⁾, Wataru Motokawa, and Hidenori Uenishi²⁾ (Section of Pediatric Dentistry, Fukuoka Dental College, ¹⁾Section of Pediatric Dentistry, Kanagawa Dental College, ²⁾Section of Oral Bacteriology, Fukuoka Dental College)

(8) Effect of Acidic Electrolyzed Water on Oral Bacteria

Miho Machito*, Chiyuki Nyura-in, Hisashi Setoguchi, Yu-ichi Izumi, and Takeshi Suda (Second Section of Conservative Dentistry, School of Dentistry, Kagoshima University)

(9) Metal Corrosion by Neutral Electrolyzed Water-Comparison with Strong Electrolyzed Water

Takeo Maida*, Kazuhiko Endo¹⁾, Osamu Tanaka, Yu Yamada, Kazunobu Tanishi, Chikako Kato, and Makoto Tamura (Research Institute of Health Science, Health Science University of Hokkaido, ¹⁾Section of Dental Materials and Devices, Health Science University of Hokkaido)

15.15-15.45 Chair; Ikuo Takayanagi (Third Prosthodontics, School of Dentistry, Showa University)

(10) Disinfection Mechanism of Strong Acidic Water

Shu Ohnishi* (Ohnishi Dentistry in Hiratsuka)

(11) Clinical Use of Strong Mixed Electrolyzed Water in General Dentistry

Shuhei Mizogami* (Mizogami Dental Clinic)

(12) Clinical Use of Soft Oxidized Water at General Dentistry

Hiroshi Iwamoto*, Yo-ichiro Kitano (Iwamoto Dentistry)

15.45-16.15 Chair; Osamu Tanaka (Research Institute of Health Science, Health Science University of Hokkaido)

(13) Disinfection of Hands and Fingers with Strong Acidic Electrolyzed Water

Chika Kase*, Teruo Obana, Akihiko Shiba, Toshihiro Sakai, Hiroaki Tsukasaki, Hiroyuki Nakane, Azusa Kanaishi, Kazuhito Nimi, Tetsuji Suzuki, Yoko Kubota, and Masahiko Ozeki (Third Section of Prosthodontics, School of Dentistry, Showa University)

(14) Adsorption of Fibronectin by Decalcification of Root Surface Caused by Strong Acidic Water

Yoshiki Kanda*, Yo-ichi Saito, Nobuyasu Asaki, Mamoru Aoki, Naoya Iwasaki, Hiroshi Nakatani, and Kyu-ichi Kamoi (Section of Periodontology, School of Dentistry, Nippon Dental University)

(15) Mutagenicity and Cytotoxicity of Soft Oxidized Water and Strong Oxidized Water

Ko-ichi Shimada*, Hiromichi Ori-I, Yu-ichi Saito, Hajime Ebihara, Hisashi Ujiya, Ko-ichi Ito, and Shodai Murai (Section of Periodontology and Conservative Dentistry, School of Dentistry, Nippon University)

Lecture by Chairman of the Conference

16.20-16.50 Chair; Hisashi Hisamitsu (Second Section of Conservative Dentistry, School of Dentistry, Showa University)

Usefulness of Acidic Electrolyzed Water and its Issues

Shodai Murai* (Section of Conservative Dentistry and Periodontology School of Dentistry, Nippon University)

16.50

Closing Remarks

Ko-ichi Ito* (Chairman of Preparatory Committee for 6th Workshop for Use of Strong Electrolyzed Water in Dental Field)

Exhibition of Commercial Products 9.00-16.00 2nd Floor of Large Auditorium B, Nippon University Hall

“Strong Electrolyzed Water Generator and Materials”

Microbicidal and Sterilization Effects of Electrolyzed Water on Biofilm of Various Bacteria
Toshiya Kashiwara*, Tetsuo Ichikawa, Hideo Kanitani, Naeko Kawamoto, Katsuhiko Hirota¹⁾, Yo-ichiro Miyakae¹⁾, and Naoyuki Matsumoto (First Section of Prosthodontics, School of Dentistry, Tokushima University, ¹⁾ Section of Oral Bacteriology, School of Dentistry, Tokushima University)

1. Introduction

We investigated the effect of electrolyzed water on the health care of elderly people. At first we found the efficacy of the electrolyzed water similar to commercial denture cleaners in denture wearers staying at healthcare facilities for elderly people. The rate of sterilization was generally in a range of 60-70%, in some cases 0% and its microbicidal efficacy was not as high as we were previously told.¹⁾ This inefficiency was possibly due to the formation of a denture plaque film, into which the electrolyzed water could not penetrate deep. Electrolyzed water was previously evaluated only by its effect on the floating bacteria, but the effect on a bacterial biofilm such as a denture plaque biofilm was not investigated. In the present study, we investigated the resistance of biofilms against the electrolyzed water using various models of biofilms formed on the cell desk coated with type IV collagen.

2. Materials and Methods

1. Preparation of *Candida albicans* Biofilm

A clinically isolated strain, *Candida albicans* CAD, was used. A cell desk coated with type IV collagen derived from bovine placenta (MS-0013, Sumilon) was placed on flat bottom of a 24-well plate. After addition of 2.0 ml of L-broth, the above strain was inoculated at concentration of 4.0×10^6 CFU/ml ($OD_{600\text{ nm}} = 0.2$) into this medium and incubated at 37°C for 5 days under a static culture condition to yield a biofilm.

2. Preparation of Biofilms of *Staphylococcus aureus* and *Streptococcus constellates*

Smith strain of *Staphylococcus aureus* and *Streptococcus constellates* NCDO2226 strain were used. Each strain was inoculated into a brain heart infusion (BHI) liquid medium to incubate at 37°C for 18 hours, followed by centrifugal washing with PBS to adjust to 2.0×10^6 CFU/ml. The similar cell desk in the preparation of *Candida albicans* biofilm was immersed in 900 $\mu\ell$ of a BHI liquid medium in each well of a multiwell plate, followed by addition of 100 $\mu\ell$ of each bacterial culture to incubate at 37°C for 4 days under static incubation to yield their biofilms. 50 $\mu\ell$ each of Smith strain and NCDO2226 strain were inoculated into the same culture medium to incubate at 37°C for 4 days under static incubation to yield a biofilm with a mixture of *Staphylococcus* and *Streptococcus*.

3. Evaluation of Microbicidal Effect of Electrolyzed Water

(1) The cell disks, on which the *C. albicans* biofilm was formed were immersed in the electrolyzed water for 30 seconds, 1 and 5 minutes or 5 times in the fresh electrolyzed water in every minute to determine the OD values and viable cell counts. They were compared with the OD values and viable cell counts of the control, where the cell disks were immersed in a commercial denture cleaner, Pika for 5 and 10 minutes. Viable cell counts were determined as soon as possible. Strong acidic water and strong alkaline water purified by **Superoxide Lab (Janix Co., Ltd)** and neutral water purified by **Ameni Clean** (Matsushita

Electric Work, Ltd.) were used as the electrolyzed water.

(2) All biofilms were observed by a Hitachi scanning electron microscope to investigate a morphological change before and after immersion in the electrolyzed water.

(3) Results and Discussion

Resistance of the *Candida* biofilms to the electrolyzed water depended on the immersion time and was increased in the order of strong alkaline water, strong acidic water, and neutral water. Scanning electron microscopy (SEM) revealed that immersion of the biofilm in the strong acidic water resulted in loss of the majority of original yeast- and mycelium-like morphology in the residual thick biofilm, which differed from the control group. The biofilm immersed 5 times in the fresh strong acidic water in every minute lost most of glycocalyx and its layer structure was destroyed as compared with the biofilm immersed in the strong acidic water for 5 minutes. Similar change was observed in the morphology of the biofilms other than the *Candida* biofilm. It was also revealed that the *Staphylococcus* film was destroyed more extensively than the *Streptococcus* biofilm. SEM of the biofilm immersed in the strong alkaline water provided an image suggesting washout of the biofilm, quite different with the biofilm immersed in the strong acidic water.

These results suggest that to maximize the efficacy of the electrolyzed water as the denture cleaners the strong alkaline water is first used for cleaning of the denture, followed by immersing in the strong acidic water several times for a short period of time and then washing thoroughly with water.

(4) References

(1) Naeko Kawamoto et al., (1996), "Effect of Electrolyzed Water on Cleaning of Denture-Bacteriological Study-Journal of Prosthodontic Research, 40, 448-453.

Effect of Acidic Electrolyzed Water on Oral Bacteria

Miho Machito, Chiyuki Nyurain, Hisashi Setoguchi, Yu-ichi Izumi, and Takeshi Suda (Second Section of Conservative Dentistry, School of Dentistry, Kagoshima University)

1. Purpose

Acidic electrolyzed water has been reported to have a strong and fast-acting microbicidal power, but little adverse effect to living organisms. However, there has been little report on its clinical use in periodontal care. We investigated the effect of gargling with the acidic electrolyzed water on prevention of the plaque formation and cleaning of the periodontal pocket.

2. Method

In the present study the acidic electrolyzed water was manufactured by **Acitoron** (manufactured by MAC Japan, Inc.).

(1) Control of Plaque Formation by Gargling

Subjects in this study were seven healthy volunteers (4 males and 3 females, mean age; 28.7). After professional tooth cleaning, the subjects stopped tooth brushing and gargled four times a day for 1 minute with 160 ml of the acidic electrolyzed water. Subgingival plaques in buccal site were collected with Gracey scaler from the maxillary or submaxillary right and left first bicuspid two days later and the maxillary or submaxillary right and left second bicuspid four days later. The plaques were serially diluted with a prerduced Ringer's solution and inoculated on Trypticase soy blood agar, which was incubated anaerobically for 4 days to determine the colony forming unit (CFU). The gingival index (GI) and plaque index (PI) in all teeth, and an amount of gingival crevice fluid (GCF) from the maxillary or submaxillary right and left cuspids were determined four days later. A 0.35% isodine gel and physiological saline were used as a positive control and a negative control, respectively.

(2) Microbicidal Effect by Cleaning of Periodontal Pocket

Subjects were 15 patients (8 males and 7 females, mean age; 48.3), who visited the Section of Periodontitis Care, Kagoshima University Medical and Dental Hospital and diagnosed as adult periodontitis. Two single-root teeth with the periodontal pocket larger than 4 mm were selected as a subject tooth. After determining the probing depth (PD), GI, and PI, subgingival plaques were collected with a paper point (30 seconds), followed by irrigation of the periodontal pocket with 5 ml of the acidic electrolyzed water twice (30 seconds). One hour later the subgingival plaques were collected to determine CFU. Similar measurement for CFU, PC, GI, and PI was repeated 7 days later. Physiological saline was used as a control.

3. Results and Discussion

(1) Control of Plaque Formation by Gargling

Increase of the CFU value in the tooth surface means the adhesion of bacteria to the tooth surface and the growth of bacteria. The CFU value in the plaques in the 2nd day of gargling with the acidic electrolyzed water was lower than that in the control, but higher than that in the isodine gel. The CFU value

in the iodine gel in the 4th day of gargling was also high. The PI value in gargling with the acidic electrolyzed water was clearly lower than that in gargling with physiological saline, but higher than that in the iodine gel. The GI value was increased in some cases after stopping teeth brushing. There was no difference in an amount of GCF among all test specimens.

Contact with organic substances diminishes the microbicidal power of the acidic electrolyzed water, resulting in the increase in frequency or an amount used for gargling to ensure its efficacy.

(2) Microbicidal Effect by Cleaning of Periodontal Pocket

CPU values one hour after cleaning of the periodontal pockets were decreased in gargling with physiological saline to some extent, but its decrease became significant in gargling with the acidic electrolyzed water. The CPU value after 1 week was lower in case of the acidic electrolyzed water than that of physiological saline, suggesting the microbicidal efficacy of the acidic electrolyzed water. These results confirm the acidic electrolyzed water has the microbicidal power against bacteria in the gingival crevice. There was no difference in values of PF, GI, and PI among different gargles.

The acidic electrolyzed water has low side effect on living organisms like other drugs and low injurious properties to the tissues. These results confirm the *in vivo* microbicidal effect of the acidic electrolyzed water, suggesting possible use in clinical practice.

4. References

- (1) Tetsuya Nishida et al., "Control of Plaque Formation Aqua by Oxidized Water", *Journal of Japanese Society of Periodontology*, 35, 692-697, 1993
- (2) Naoki Tanabe, "Examples and Caution in Use of Functional Water (Clinical Use)-Effect of Gargling", *Journal of Dental Engineering*, 115, 13-16, 1995.

Usefulness and Issues of Acidic Electrolyzed Water

Shodai Murai (Section of Periodontology, Department of Conservative Dentistry, School of Dentistry, Nippon University)

Strong acidic electrolyzed water is obtained from an anode chamber in electrolysis of tap water containing a small amount of salt (0.05%) through a diaphragm between an anode and a cathode. It has a pH value lower than 2.7, an oxidation-reduction potential (ORP) higher than 1100 mV, and a chlorine concentration (Cl) of 10-50 ppm. The strong acidic water is known to have a fast-acting microbicidal effect, but pointed out to have disadvantage such as corrosiveness to metals.

This has led to the development of weak acidic electrolyzed water with a pH value of 5.0-5.5, an ORP value of 800-1100 mV, and Cl of 50-80 ppm. The weak acidic electrolyzed water is prepared by diaphragm-free electrolysis of brine, to which hydrochloric acid is added to adjust pH closer to the alkaline region of around pH 5.6, thus yielding the water with a higher content of hypochlorous acid. It has been confirmed that microbicidal activity of this water is similar to that of the strong acidic electrolyzed water.

Both acidic waters have excellent microbicidal effect to bacterial pathogen and little adverse effect to living organisms and the environment, resulting in wider use in preventive disinfection for nosocomial infection, disinfection of medical devices or treatment of various infectious diseases in the medical field. Meanwhile in the dental field, in addition to disinfection of the inside of hospital and hands and fingers, its usefulness is proved in disinfection before and after the extraction of tooth and disinfection of a wound site as application to the oral surgery and gargling, irrigation of the periodontal pocket, and disinfection of stomatitis as application to the periodontal care.

Although excellent microbicidal effect has been recognized, there are problems such as decrease of its activity depending on how to use, store, and transport and decrease of the microbicidal activity in the presence of organic substances. A desirable basic research includes the exploration of the underlying mechanism of microbicidal effect as well as a study of safety-related topics such as mutagenicity and possible formation of allergens. User-related problems include the corrosion of metals in the oral cavity and the possible decalcification of teeth by the acidic electrolyzed water. I will discuss these issues.

5th Workshop for Use of Strong Acidic Electrolyzed Water in Dental Field
March 16 (Saturday), 1996

Auditorium on 4th floor of Building #5, School of Dentistry, Tokyo Medical and Dental University

Organizer; Third Section of Prosthodontics, School of Dentistry, Showa University

Benefactor; Foundation for Study and Promotion of Functional Water

Program of Workshop

9.00 Opening speech; Akihiko Shiba (President of Study Group for Use of Strong Acidic Electrolyzed Water in Dental Field)

Speech by Guest of Honor; Jun-ichi Urata (President of Foundation for Study and Promotion of Functional Water)

General Session (Morning)

9.10-9.40 Chair; Atsushi Yamazaki (Section of Dentistry for Disabled, School of Dentistry, Tokyo Medical and Dental University)

(1) Effect of Dilution of Strong Acidic Electrolyzed Water on Microbicidal Power Affected

Tetsushi Suzuki*, Yuka Suzuki, Akihiko Shiba, Chika Kase, Hiroyuki Nakane, Hiroaki Tsukazaki, Toshihiro Sakai, Toshio Senda¹⁾, and Noboru Okamura¹⁾ (Third Section of Prosthodontics, School of Dentistry, Showa University, ¹⁾ Section of Healthcare and Hygiene, School of Medicine, Tokyo Medical and Dental University)

(2) Effect of Toothpaste on Activity of Electrolyzed Oxidized Water

Takeo Igarashi*, Ko-ichi Shimada, Tetsuya Nishida, Naoto Yoshinuma, Ko-ichi Itoh, and Shodai Murai (Section of Conservative Dentistry and Periodontology, School of Dentistry, Nippon University)

(3) Effect of Electrolyzed Water on the in vivo Candida Biofilm

Tetsuo Ichikawa*, Toshio Kashiwara, Naeko Kawamoto, Hideo Kanitani, Naoyuki Matsumoto, Yo-ichiro Miyake¹⁾, and Katsuhiko Hirota¹⁾ (First Section of Prosthodontics, School of Dentistry, Tokushima University, ¹⁾ Section of Oral Bacteriology, School of Dentistry, Tokushima University)

* Speaker

9.40-10.10 Chair; Ko-ichi Itoh (Section of Conservative Dentistry and Periodontology, Nippon University)

(4) Clinical Sign and Bacterial Flora Affected by Subgingival Pocket Irrigation with Aqua Oxidation Water
Naoya Iwasaki*, Yukihiro Numabe, Akiko Itoh, Akira Naeshiro, and Kyu-ichi Kamoi (Section of Periodontology, School of Dentistry, Nippon Dental University)

(5) Use of Strong Acidic Water for Plaque Control

Shu Ohnishi* (Section of Dental Anatomy, Osaka Dental University)

(6) Effect of Strong Acidic Electrolyzed Water on Early Plaque Formation on Titanium Surface

Chizuru Nagashima*, Katsutoshi Deguchi, Hajime Miyashita, and Genji Hasegawa, Atsuo Iwasawa¹⁾, and Yoshiko Nakamura¹⁾, (Section of Periodontology, School of Dentistry, Showa University, ¹⁾ Laboratory for Clinical Pathology, Fujioka Hospital of Showa University)

Lecture by the President

10.15-10.45 Chair; Sadashi Anzai (Director of Foundation for Study and Promotion of Functional Water)
Function and Use of Strong Acidic Electrolyzed Water
Akihiko Shiba* (Third Section of Prosthodontics, School of Dentistry, Showa University)

Presentation of Commercial Products

10.50-12.00 Chair; Hajime Miyashita (Section of Periodontology, School of Dentistry, Showa University)

- (1) Strong Acidic Electrolyzed Water Generator-**Aquarex**
Kazuo Ii*, Yukiko Tochika, Isao Asaka (Life Science Center, Iwaki Glass Co., Ltd.)
- (2) Strong Acidic Electrolyzed Water Generator from TOTO
Shigeru Ando*, Hiroaki Tate (Development Center for Living-related Products, TOTO Inc.)
- (3) Proper Selection of Strong Electrolyzed Water Generator
Takashi Unno*, Toru Takao (Tokyo Sales, Shibata Chemicals Co., Ltd.)
- (4) Overview of Strong Acidic Electrolyzed Water Generator, “**Itec**”, for Use in Healthcare
Toshihiko Okada, Yoshikazu Hamazaki, Hiromasa Kawaguchi (Sales and Development, Aiken Industry Co., Ltd.)
- (5) Soft Oxidized Water
Jun Masuoka* (**Omuko** OMC Co., Ltd.)
- (6) Comments on Acidic Electrolyzed Water from Aqua Japan
Yasumitsu Koshino* (Aqua Japan Co., Ltd.)
- (7) Overview of Strong Acidic Electrolyzed Water Generator, “**Oasis Bio**”
Tomohiko Hisano*, Hiroyuki Kagawa, Koji Narita (Group of Membrane System, Department of Functional Products, Asahi Glass Engineering Co., Ltd.)

12.00-13.00 Break)

(12.00-12.30 Board Meeting)

(12.45-13.00 General Meeting)

Plenary Lecture (Afternoon)

13.00-13.30 Chair; Akihiko Shiba (Third Section of Prosthodontics, School of Dentistry, Showa University)

Principle and Application of Strong Acidic Electrolyzed Water

Toshio Ogawa* (Emeritus Professor of Kochi University)

General Session (Afternoon)

13.40-14.10 Chair; Masahiko Ozeki (Third Section of Prosthodontics, School of Dentistry, Showa

University)

(7) Effect of Strong Acidic Electrolyzed Water on Denture Base Resin

Masatoshi Iwahori*, Hayae Kawasaki, Makoto Sakai, Mutsuo Yamauchi¹⁾, and Susumu Nagasawa (First Section of Prosthodontics, School of Dentistry, Asahi University, ¹⁾ Dental Clinic, Research Institute of Clinical Dentistry, School of Dentistry, Asahi University)

(8) Effect of Strong Acidic Electrolyzed Water on Medicaments for Mucosal Membrane

Makoto Sakai*, Masatoshi Iwahori, Hayae Kawasaki, Mutsuo Yamauchi¹⁾, and Susumu Nagasawa (First Section of Prosthodontics, School of Dentistry, Asahi University, ¹⁾ Dental Clinic, Research Institute of Clinical Dentistry, School of Dentistry, Asahi University)

(9) Use of Strong Acidic Water in Treatment of Denture Stomatitis

Yu Yamada*, Hisae Baba¹⁾, Osamu Tanaka, Takeo Maida, Chikako Kato, Kazunobu Tasai, and Makoto Tamura (Research Institute of Health Science, Health Science University of Hokkaido, ¹⁾ Section of Oral Bacteriology, Health Science University of Hokkaido)

14.10-14.50 Chair; Noriki Takahashi (Director in Science, Kanagawa Prefectural Government)

(10) Disinfection of Dental Unit Waterlines by Circulation of Strong Acidic Electrolyzed Water

Shuhei Mizogami* (Mizogami Dental Clinic)

(11) Use of Strong Electrolyzed Water in Dental Field

Satoru Tanizu* (Tanizu Dentistry)

(12) Use of High Oxidation Potential Water in Endodontic Care, Particularly in Root Canal Irrigation

Kazuaki Mizunuma:, Toshitake Furusawa (Furusawa Dental Clinic in Sendai)

(13) Use of Strong Acidic Electrolyzed Water in General Clinical Dentistry

Jun Tateishi*, Katsuhiko Nakazawa (Nakazawa Dental Clinic)

14.50-15.20 Chair; Osamu Tanaka (Department of Medical Science, Medical Science University of Hokkaido)

(14) Study of Topical Fluoride Solution Using High Oxidation Potential Water

Issei Kimoto*, Katsumi Yamaguchi, Yukio Hirata, Mariko Komiyama, Hirohisa Arakawa, and Ki-ichi Ihzuka (Section of Oral Hygiene, Kanagawa Dental College)

(15) Use of Strong Acidic Electrolyzed Water in Treatment of Chronic Periodontal Inflammation-Preliminary Study

Ken-ichi Saito*, Toshiyuki Nemoto (Section of Dental Surgery, Postal Agency Service Hospital of Kanto)

(16) Antimicrobial Effect of Soft Oxidized Water and Strong Oxidation Potential Water

Ko-ichi Shimada*, Yoshitomo Moritani, Kazunari Ando, Jun-ichi Negoto, Ko-ichi Itoh, and Shodai Murai (Section of Conservative Dentistry and Periodontology, School of Dentistry, Nippon University)

Symposium

15.25-16.55 Chair; Shodai Murai (Section of Conservative Dentistry and Periodontology, School of Dentistry, Nippon University)

Use of Strong Acidic Electrolyzed Water in Dental Field

Part 1; Current Status and Future

Panel Member; Rei-ichi Okuda (Second Section of Conservative Dentistry, School of Dentistry, Tohoku University)

Kyu-ichi Kamoi (Section of Periodontology, School of Dentistry, Nippon Dental University)

Susumu Nagasawa (First Section of Prosthodontics, School of Dentistry, Asahi University)

Mitsuo Amagasa (First Section of Dental Surgery, School of Dentistry, Tokyo Medical and Dental University)

17.00 Closing Remark Shodai Murai (Director of Study Group for Use of Strong Acidic Electrolyzed Water in Dental Field)

Exhibition 10.00-17.00 Conference Room #1, 3rd Floor
“Strong Electrolyzed Water Generator and Materials”

Clinical Sign and Bacterial Flora Affected by Periodontal Pocket Irrigation with Aqua Oxidation Water
Naoya Iwasaki*, Yukihiro Numabe, Akiko Itoh, Akira Naeshiro, and Kyu-ichi Kamoi (Section of
Periodontology, School of Dentistry, Nippon Dental University)

1. Purpose

The purpose of the present study is to investigate the effect of aqua oxidation water on clinical parameters of the infected sites and the subgingival bacterial flora when used as an irrigant for periodontal pocket irrigation of patients diagnosed with periodontitis.

2. Method

Subjects were 13 patients (8 males and 5 females, mean age; 46.6), who visited the Section of Periodontal Care, Dental Hospital, Nippon Dental University and diagnosed with adult periodontitis without a medical history of serious systemic diseases. They were also the patients, to whom neither antimicrobial agents nor anti-inflammatory agents were administered last three months after first medical examination. The subject teeth were the maxillary and submaxillary single-rooted teeth, to which dental restoration products had not been attached and which had the probing depth greater than 4 mm. Informed consent was obtained from each participant by thorough explanation of the purpose of the present study and patient's understanding and agreement of the risks and the benefits of the procedure prior to the start of the research.

For the preparation of test specimen, the patients were first trained to control the plaque formation under sufficient supervision. The subject teeth were two single-rooted teeth except the adjacent dentition. On day 1 of the test (0W) after various laboratory and bacteriological tests, scaling and root planing were performed on the subject teeth, which were reexamined two (2W) and four weeks (4W) later. Using Peiro Pik™, the periodontal pocket of the subject teeth was additionally irrigated with aqua oxidation water at one week (1W), two (2W) and three weeks (3W) after initiation of the test, forming the test group. Only plaque control was performed for the control group.

Plaque index (PII), gingival index (GI), probing depth (PD), attachment level (AL), and bleeding on probing (PD) were recorded in the laboratory test. In bacteriological tests, Periochek^{TR}, a detection kit specific to the enzyme activity of periodontal pathogens, was used to examine the presence or absence of the pathogens. The bacteriological activity was rated to score 0 for negative results, 1 for positive results, and 2 for strongly positive results, respectively, which were represented by a mean value.

3. Results and Discussion

In PII there was significant improvement in the control group after 4 weeks (4W) ($p < 0.01$) and the test groups after 2 weeks (2W) ($p < 0.01$) and after 4 weeks (4W) ($p < 0.01$), respectively, as compared with PII at the start of the test (0W).

In GI there was significant improvement in the control group of 4W ($p < 0.05$) and the test groups of 2W ($p < 0.01$) and 4W ($p < 0.01$), respectively, as compared with GI of 0W.

In PD there was significant improvement in the control group of 4W ($p < 0.01$) and the test groups of 2W

($p < 0.01$) and 4W ($p < 0.01$), respectively, as compared with PD of 0W.

In AL there was no significant difference in the control group, but significant improvement was seen in the test group of 4W ($p < 0.05$) as compared with AL of 0W.

In BOP there was no significant difference in the control group, but significant improvement was seen in the test groups of 2W ($p < 0.05$) and 4W ($p < 0.01$), respectively, as compared with BOP of 0W.

In the test with Periochek^{TR} there was significant improvement in the control groups of 2W ($p < 0.05$) and 4W ($p < 0.01$) and in the test groups of 2W ($p < 0.01$) and 4W ($p < 0.01$), respectively, as compared with the test with Periochek^{TR} of 0W.

4. Discussion

Bacteria in subgingival plaque are considered to be the most important source for causing periodontitis and their removal and control are critical to determine potential consequences in the periodontal therapy. Mechanical plaque control such as teeth brushing or scaling and root planing has been used in the past, but the device used in this treatment limits the distance it can reach. This has recently led to use of the regional chemotherapy for the deep periodontal pockets. The agents used in this treatment are a disinfectant such as povidone-iodine solutions and cetylpyridinium chloride solutions and an antimicrobial agent such as minocycline hydrochloride. However, these agents have issues such as a side effect to living organisms, injurious properties against tissues and resistance to bacteria.

It is said that the aqua oxidation water used in this study has a pH value lower than 2.6, an oxidation-reduction potential higher than 1100 mV, a strong and fast-acting microbicidal power, and a broad antimicrobial spectrum with little irritation to the oral cavity and superior safety. The present test demonstrated that the test group using the aqua oxidation water for the periodontal pocket irrigation resulted in improved results in all clinical tests at early stage as compared with the control group. These results indicate that use of the aqua oxidation water in the periodontal pocket irrigation improves local clinical signs, suggesting it has a potential to control the periodontal pathogens in subgingival bacterial flora.

Use of Strong Acidic Water in Treatment of Denture Stomatitis

Minoru Yamada*, Hisae Baba¹⁾, Osamu Tanaka, Takeo Maida, Chikako Kato, Kazunobu Tanishi, and Makoto Tamura (Research Institute of Health Science, Health Science University of Hokkaido, ¹⁾ Section of Oral Bacteriology, Health Science University of Hokkaido)

1. Purpose

Denture stomatitis is the disease, in which the mucous membrane beneath the denture base appears red and swollen. One of the possible causes of this disease is the infection with the fungi mainly composed of *Candida albicans* adhered to the mucosal membrane of a filthy denture base. Control of the denture plaque is one of the preventive approach for this disease. It is therefore particularly important in this country to train the wearers to fit the denture comfortably without excessive friction and an adverse effect to the surrounding tissue since the number of the denture wearers will be increased in the aging society.

In the past, mechanical cleaning of the denture by denture brushes and chemical cleaning by denture cleaners are mainly used as the therapy of denture stomatitis. However, these methods take a longer time to heal.

As strong acidic water has a strong bactericidal power to various bacteria and a superior safety, we explored whether it can be used for the therapy of denture stomatitis using the bacteriological examination. We also investigated the biological terms of the bactericidal activity.

2. Method

(1) Bacteriological Examination

Subjects were the patients diagnosed with the denture stomatitis. Assays were collected with a sterile cotton swab from the mucosal surface of the denture base in use before and after cleaning with brushes and after immersion in strong acidic water. They were suspended in distilled water and 0.2 ml of this bacterial suspension was inoculated into a *Candida* GE culture medium to incubate at 37°C for 24 hours. The colony count on the culture medium was then determined. An immersion time in the strong acidic water was one minute. The subjects were asked to immerse the dentures in the strong acidic water after every meal and before bedtime. Similar experiments were performed 3, 5, 7, and 10 days later while observing the degree of healing in denture stomatitis.

(2) Biological Terms of Bactericidal Activity

Assays were collected with a sterile cotton swab from **the mucosa above and under the denture base** used by the same patient as one who had completed the microbiological examination. The cotton swab was added to **Stomastat** (Sankin Industries, Inc.) of a simple test solution for the detection of *Candida*. This mixture was incubated at 37°C for 24 hours and a change of pH was followed by a Toyo pH paper strip (manufactured by Toyo Filter Paper Co., Ltd.). Similar experiments were performed 3, 5, 7, and 10 days later while observing the degree of healing in denture stomatitis similarly as the bacteriological examination.

3. Results and Discussion

In the bacteriological examination, many colonies were observed before brushing the denture. The number of colonies was decreased slightly after brushing the denture, but stayed fairly high. However, little colonies were found after immersion of the denture in the strong acidic water. As the strong acidic water was used for cleaning the denture after every meal and before bedtime, the number of *Candida* tends to decrease even before cleaning with brushes, suggesting the bactericidal efficacy of strong acidic water. In biological terms, prior to immersion in the strong acidic water, the pH value was lower in the mucosa above the denture base as compared to the mucosa under denture base. However, immersion of the denture in the strong acidic water resulted in **the increased pH of** the mucosa above the denture base at early stage, followed by **the increased pH** of the mucosa under the denture base. At the same time, the inflammatory symptom such as reddening of the mucosal membrane tends to decrease slightly. Other factor to contribute to the denture stomatitis is the mechanical irritation due to the ill-fitting of denture. In the present study, the dental occlusion and the goodness of fit were not adjusted, but the inflammation tends to heal in a relatively short period of time. The results above suggest the strong acidic water is effective in healing the denture stomatitis. We plan to investigate in future a quick denture cleaning method using the strong bactericidal power of the strong acidic acid in combination with the ability of alkaline water to dissolve proteins.

4. References

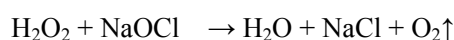
(1) Taizo Hamada, "Clinical Values of Denture Cleaners, Denture Plaque Control", the Quintessence, Special Edition, 67-72, 1984.

Use of High Oxidation Potential Water in Endodontic Care, Particularly in Root Canal Irrigation

Kazuaki Mizunuma, Toshitake Furusawa (Furusawa Dental Clinic in Sendai)

Purpose

Presently alternate irrigation of hydrogen peroxide and sodium hypochlorite is preferably used in the root canal irrigation during a course of the endodontic care. Two chemicals are believed to react in the root canal to release oxygen according to the following equation, thus promoting the disinfection action.



However, use of sodium hypochlorite certainly in the endodontic care has problems, because their contact with gingiva and skin is corrosive. Operation of the alternate irrigation has to end with irrigation with sodium hypochlorite, because use of hydrogen peroxide in the final step of the irrigation releases oxygen increasing the internal pressure of the root canal, possibly causing toothache. It is also known that there are bacteria and virus, which cannot be inactivated by irrigation with these chemicals.

We tried to use high oxidation potential water as an alternative in the root canal irrigation. The high oxidation potential water is obtained from the anode in electrolysis of water containing a trace amount of a salt and has pH lower than 2.6, an oxidation-reduction potential (ORP) higher than 1,100 mV, and the chlorine concentration in a range of 10 ppm. Instantaneously nonspecific bactericidal and virucidal actions of the high oxidation potential have been reported in many experiments.

Method

- (1) Root canal substances in the infected teeth were mechanically removed. By this treatment the dental pulp, contaminants, and infected dentin should not remain in the root canals. Their presence substantially reduced the cleaning efficacy of the high oxidation potential water. This dramatic loss of the cleaning efficacy was not limited to the treatment only with the high oxidation potential water.
- (2) A syringe was filled with the high oxidation potential water to irrigate the root canals. Approximately 10 to 20 ml of the high oxidation potential water was injected gradually over about 30 seconds depending on the kind of infected tooth. The high oxidation potential water spilled was suctioned under vacuum to remove. In case of the fistula formation, a syringe needle was inserted not only from the side of the dental pulp cavity but also into the passage of fistula to irrigate the root canal. This assured an increase of the cleaning efficacy.
- (3) The root canal was sufficiently dried with sterile cotton plugs, medicated, and temporarily sealed.
- (4) Assays collected were inoculated into a Pladia medium to incubate at 37°C, determining if the bacteria is present in the root canal in a course of 24, 48, and 72 hours.
- (5) After confirming the root canals to be bacteria-free, they were filled according to a routine procedure.

Materials

Subject teeth used for the root canal irrigation with the high oxidation potential water were 400 infected teeth, of which some required the pulpectomy for caries and the treatment of the infected root canal for the periapical lesion. They consisted of 69 maxillary anterior teeth, 142 maxillary molar

teeth, 28 submaxillary anterior teeth, and 161 submaxillary molar teeth. There were 277 teeth diagnosed only with caries and pulpitis, but without inflammatory symptoms in the apical and marginal regions and 123 teeth diagnosed with inflammation in the apical and marginal regions.

The high oxidation potential water was produced by an ND-002 high oxidation potential water generator from [Remodeling 21 Inc.](#) and had pH lower than 2.7, an OPR higher than 1,100 mV, and the residual chlorine concentration lower than 10 ppm.

Results

In examination of the assays it was found that all root canals received the pulpectomy were able to be bacteria-free after the root canal irrigation once or twice. Particularly the root canals, of which the periapical lesion was recognized and so-called the treatment of the infected root canal was required were also rendered bacteria-free after the repetition of the irrigation several times. There were some cases, in which the root canal were not rendered bacteria-free because of the very large periapical lesion and there were 6 cases (1.5%), which received the apicoectomy.

The patients were followed up clinically. All of them were free of symptoms, suggesting there is no problem.

Discussion

The high oxidation potential water is known to instantaneously exert the nonspecific bacterial and virucidal actions. In this study we used it for the root canal irrigation and found it appears to have fairly high efficacy as an irrigant. It has no injurious action to a human body and may provide advantages such as simpler operation and shorter treatment times.

Use of Strong Acidic Electrolyzed Water in Treatment of Chronic Periodontal Inflammation-Preliminary Study

Ken-ichi Saito*, Toshiyuki Nemoto (Section of Dental Surgery, Postal Agency Service Hospital of Kanto)

1. Purpose

In recent years use of strong acidic electrolyzed water (hereinafter referred to as acidic water) has been increased because it has a broad-spectrum bactericidal activity to the pathogenic bacteria.

This time we used the strong acidic water in the treatment of chronic marginal periodontal inflammation (so-called alveolar pyorrhea), which is found in many middle-aged to elderly people in this country, but for which the effective treatment is unavailable yet. Preliminary results will be reported below.

2. Subjects and Method

Subjects were four males and three females with the age of 38 to 56 diagnosed with chronic periodontal inflammation, which included the lesions developed locally in the periapical region or developed up to the root apex (referred to as a marginal type) and those locally developed at the root apex (referred to as a root apex type). The present cases had the marginal type in four cases and the root apex type in three cases. Three latter cases had the fistula in gingiva, among which two cases had a history of apicoectomy.

A syringe equipped with a small dental irrigation needle was filled with approximately 20 ml of the strong acidic electrolyzed water, and the tip of needle was inserted into the gingival crevice or fistula in the gingiva to irrigate intralesionally under high pressure. In every case the gingival crevice was curetted before cleaning. As a general rule, this procedure was performed once a week to compare a change of the inflammatory symptoms such as redness, swelling, toothache, pus discharge, and the disappearance of the fistula at one month after initiation of the treatment with those before the treatment. The efficacy of the present method was then evaluated. Antimicrobial agents or other bactericidal mouthwashes were not administered before or after treatment.

3. Results

In three out of four of the marginal type cases, the redness and swelling of the gingiva were reduced or disappeared and the quantity of pus discharged was substantially reduced. In another case symptoms of the disease were slightly reduced.

In two out of three of the root apex type cases, the disappearance of the fistula was observed. However, there were the cases where use of antimicrobial agents or the root canals therapy previously had little effect on the fistula, but this time the fistula disappeared after irrigation once or twice with the strongly acidic electrolyzed water. In another case, the fistula did not completely disappear, but a decrease of the swelling and redness was observed.

4. Conclusion

The inflammation symptom was clearly improved in five out of seven cases diagnosed with the

chronic periodontal inflammation by the treatment with the strong acidic water, confirming its efficacy to this disease. While the number of cases studied was small, the strong acidic water was also effective in the cases where the fistula was formed after receiving the apicoectomy, suggesting that the present method may have a potential of improving the prognostic indicator for the patients, who received the apicoectomy.

We plan to investigate the efficacy of the strong acidic electrolyzed water by increasing the number of cases as well as using the microbiological study together with a control study.

High Oxidation Potential Water-its Use in Conservative Treatment of Teeth

Rei-ichi Okuda (Section Section of Conservative Dentistry, School of Dentistry, Tohoku University)

It has been demonstrated that water produced in the anode chamber in an electrolytic cell by electrolysis of tap water containing a trace quantity of salt (around 0.01%) exhibits a low pH value (less than 2.7) and a high oxidation-reduction potential (higher than 1,000 mV) and contains a large quantity of dissolved oxygen (a little more than 20 ppm). This water is called super-oxidized water, strong acidic water, aqua oxidation water, and others.

In the past I called this water **strong acidic water**, but recently changed the name as the high oxidation potential water since a broad spectrum of the microbicidal activity was revealed and the oxidative properties associated with a high value of the oxidation potential are responsible for its activity..

It has been demonstrated that the microbicidal activity of this water is effective against not only bacteria, but also fungi, molds, and virus as well as protozoa. We may say this activity is a new discovery.

It has been also revealed that although this water has the high microbicidal activity, it is readily neutralized with a trace of contaminants, resulting in loss of the activity.

Other findings include that it is non-irritant, non-toxic, non-corrosive non-, carcinogenic, and non-bioaccumulative as contacted with skin, mucosa, and fresh and old wounds.

The reason why this water having such advantages and disadvantages has been appreciated is the development of a method to remove the majority of contaminants (decontamination) in the endodontic region before its use, thus resulting in easy and safe utilization of the full power of disinfection and sterilizing effects by the high oxidation potential water.

Of course use of this water has started after development of a simple equipment to generate this kind of water and it has a big advantage of not generating pollution by effluents.

These properties enable the water to be used in a broad area, which is not limited to the medical and dental fields.

Effective use of the high oxidation potential water has been tried in the field of a daily dental care. However, unless properties of the water are fully understood, its use has minimal advantage but more harm.

This water has been tried in the disinfection of a dental treatment room, furnitures, dental units, surgical dressings, medical clothes, non-corrosive dental gizmos and hands and fingers as well as fresh and old wounds in the medical treatment and endodontic regions of patients with periodontitis, resulting in some positive results.

At the same time, we cannot deny the situation, where expectation of this water was stretched far beyond its ability without real understanding of the properties, sometime resulting in use of a wrong method or application in the wrong field not serving the purpose.

In this lecture, I will explain use of the high oxidation potential water in the conservatory dentistry with examples. I hope my presentation will help you fully understand the properties of this water for useful application.

Current Status of Strong Acidic Electrolyzed Water and its Future-From the Angle of Periodontology

Kyu-ichi Kamoi (Section of Periodontology, School of Dentistry, Nippon Dental University)

In recent years drugs with more reliable microbicidal effect to virus and bacteria is required due to the diversified disinfection and sterilization utilized secondary to the emergence of HIV or MRSA, the latter of which may be an important cause of nosocomial infection. As one of the potential drugs, strong acidic electrolyzed water was identified as functional water with the microbicidal and disinfection effects and has become topics of conversation.

In the periodontal field the strong acidic electrolyzed water has been tried for gargling and the periodontal pocket irrigation to control the plaque formation and improve the clinical symptoms by its microbicidal activity. My presentation will be focused on a current study in our lab about the efficacy of strong acidic electrolyzed water for the control of the plaque formation and the improvement of clinical symptoms.

1. Effect of Strong Acidic Electrolyzed Water on Oral Immunological Mechanism

We investigated the effect of gargling with the strong acidic electrolyzed water on the phagocytic capacity of polymorphonuclear leucocytes (S-PMNs) in saliva, which control the nonspecific cellular immunological mechanism. It was then demonstrated that S-PMNs has the high phagocytic capacity after gargling. We also investigated the kinetics of actin filaments (F-actin), which play an important role as the cytoskeleton of the polymorphonuclear leucocytes in the saliva before and after gargling with the strong acidic electrolyzed water. It was then demonstrated that the content of the F-actin formed in PMN was low in saliva after gargling, suggesting PMN chemotaxis was decreased. At the same time we demonstrated that the stimulation enhances the formation of F-actin in PMN after gargling with the strong acidic electrolyzed water.

2. Effect of Strong Acidic Electrolyzed Water on Periodontal Pocket Irrigation as Chemical Plaque Controlling Agent

We generated an experimental model of the periodontitis, which was treated by gargling separately with the strong acidic electrolyzed water, a cetylpyridinium chloride solution, and distilled water. Examination with various clinical parameters indicated the microbicidal efficacy of the strong acidic electrolyzed water. The periodontal pockets of the patients diagnosed with the moderate adult periodontitis were irrigated separately with the strong acidic electrolyzed water or distilled water. As compared to the cases treated with distilled water, the irrigation with the strong acidic electrolyzed water demonstrated the improvement in the clinical parameters and the reduction of the bacterial flora in the periodontal pockets at the early stage. The strong acidic electrolyzed water has been applied in the periodontal region leading to the activation of the host defense in the oral cavity, control of the plaque formation by gargling, and control of the formation of the bacterial pathogens for the periodontitis in the periodontal pockets. Wider use of it is anticipated:

Use of High Oxidation Potential Water in Endodontic Treatment

Rei-ichi Okuda

Introduction

I will herein explain what properties of high oxidation potential water can be utilized for the root canal therapy and then discuss when this water can be used for the therapy.

Characteristics of High Oxidation Potential Water

The high oxidation potential water can respond a broad spectrum of microorganisms including bacteria, fungi, molds, and virus to inactivate. It is non-toxic and non-irritant to skin, mucosa, and fresh and old wounds

It was demonstrated that the high oxidation potential water used in our study contains a trace quantity of hypochlorous acid (HClO, 10-20 ppm) and dissolved oxygen (O₂, a little more than 20 ppm) and has high hydrogen ion concentration to exhibit a pH value of about 2.2-2.7. The disinfecting power of this water presumably comes mainly from hypochlorous acid. A concentration of a sodium hypochlorite solution used in the root canal therapy is very high **in terms of the hypochlorite** to reach to a range of 5,000 to 10,000 times higher than that of the high oxidation potential water. The former solution is strongly corrosive and irritant to skin.

An aqueous hypochlorous acid solution is distinctly characterized by the fact that the high reactivity is still kept even at the concentration decreased to a few ppm and the disinfecting activity in an acidic solution with a pH lower than 5.5 is particularly high. The dissolved oxygen also has the disinfecting and bleaching actions.

The presence of a hydroxyl radical, OH•, is also confirmed in this aqueous solution obtained by the electrolysis.

Another important fact about the properties of the high oxidation potential water we have to know is that the presence of a trace of contaminants causes a fast reaction, resulting a loss of the disinfecting activity within a short period of time. This fact means that we cannot expect “the capability of the high oxidation potential water to treat a large volume of contaminants with a small volume of the water”.

This kind of water appears to be totally worthless as a disinfectant, but clinicians can use this water as a useful disinfectant so far as they understand the properties of the high oxidation potential water with some restriction and properly utilize the extremely high disinfecting activity of it.

What really clinicians want for the function of the disinfectant in daily clinical use is not treating a large volume of contaminants with a small volume of a chemical, but rather treating and killing reliably, safely, and effectively the microbial pathogens with it. What they want is whether the treatment can reduce the bacterial count or their activity to create conditions where infection does not occur any more or in the infected wounds whether the treatment of the wounded sites can reduce the bacterial count to create conditions where natural healing is promoted.

In the disinfection treatment, the majority of contaminants such as blood, body fluid, pus, and others can be removed easily by thorough washing, a basic process in the principle of disinfection, but rather

important problems still remain after this process.

The high oxidation potential water should come out exactly in this timing as a safe disinfectant with the strong disinfecting activity.

Unless the process described above cannot create the conditions described above in this timing, the number of bacteria is increased within a short period of time, failing the disinfection of the microbial pathogens and resulting in the meaningless treatment.

Meanwhile use of excellent disinfectants may not sometime achieve the desired effect. In many cases, this failure is caused by the aggregation of objects to be disinfected, the formation of thick layer adhered to the surface of the treating area or the deposition of the contaminants on a tight space, making the disinfectant difficult to reach the objects. Other case includes one where contamination takes place in the cornified layer of skin and the deep part of dentin.

Root Canal Therapy and High Oxidation Potential Water

In the root canal therapy, importance of the preliminary examination and treatment such as proper diagnosis and anti-inflammatory treatment should be emphasized. I do not hesitate to say that the most important matter after this procedure is completing the root canal therapy in the early stage and maintaining the disinfected condition for a long period of time.

The complex shape of the root canals and the presence of root collaterals as well as the presence of the softened dentins or the contaminated dentins by the invasion of bacteria through the root collaterals and dental tubules make the disinfection and sterilization more difficult.

The pulp cavity in an avulsed anterior tooth (A) and an avulsed molar (B) in the infected root canal, which had been stocked in water was perforated and stained by dropwise addition of India ink. 24 hours later photographs of these teeth were taken and demonstrated in Figures 1A and 1B, respectively. These figures demonstrate India ink was penetrated into the surrounding of the root canal. The extent of ink diffusion in the root canals was varied significantly among each tooth as well as with the sites of root canals. The contamination level is varied substantially in the deep area of the inner walls of the root canal depending on the tooth and the site of root canals, suggesting the variations in contamination as well as variations in difficulty of the treatment depending on the teeth.

In the disinfection of such root canals, it is most important to remove the contaminants in the root canals as much as possible. The contaminated dentin in the inner walls of the root canals is generally removed by the action of endodontic files. Mechanical filing of the contaminated dentin is particularly important for disinfection of this site. Neglect of this treatment results in very poor efficiency of the disinfection treatment.

Treatment of Infected Root Canal with High Oxidation Potential Water

The root canals, which were demonstrated in Figure 1 as the "root canal stained with India ink" were cleaned with the high oxidation potential water and enlarged with an H-type file to observe the cross-section of the root canal wall. It is known that the high oxidation potential water possesses the bleaching action. A 1000-fold dilution of India ink with the high oxidation potential water discolors the ink

as demonstrated in Figure 2.

Figure 3 demonstrates the cross-section of the dental root after completing the root canal enlargement according to this procedure. The ink penetrated from the inner wall into the deep area of the root canal was little discolored. Upon contact the high oxidation potential water underwent the chemical reaction with a calcium ion or proteins contained in dentin in the course of diffusion into the deep area, resulting in the loss of the disinfection activity.

Summary

The high oxidation potential water can be anticipated to be very effective so far as the purpose of use is the removal of, by irrigation, foreign materials, exudates, blood, bacteria, bacterial byproducts, soft tissue fragments, and dentin shavings in the root canals or on the surface of its inner wall or is the disinfection of the surface layer of the inner wall of the root canals.

**Question and Answer on High Oxidation Potential Water
Special Edition**

Frontier in Disinfection, Part 2

**Member of Editorial Board; Rei-ichi Okuda (School of Dentistry, Tohoku University)
Somei Sakoda (Dental practitioner, Tokyo)**

Questions on use of electrolyzed oxidized water for treatment

1. What is the effect of gargling?

Jun Negishi, Atsushi Kurino, and Ki Kato (Second Section of Conservative Dentistry, School of Dentistry, Hokkaido University)

The purpose of “gargling” with electrolyzed oxidized water is two-fold of (1) dental plaque control and (2) deodorization of halitosis.

1. Plaque Control

A most important cause for periodontal diseases and ----- is bacteria in the plaque and the plaque control is a must for their treatment and prevention.

Obviously a “physical method” using a cleaning device such as a toothbrush is a major approach for the plaque control. Maintaining the effective plaque control by teeth brushing for a long period of time is not an easy job even for the patients, who are skillful of teeth brushing. The patients have to be trained repeatedly on tooth brushing. On the other hand, a chemical plaque control was developed as a supplemental method for the physical plaque control and various agents starting with a 0.2 aqueous chlorhexidine solution have been tried.

The key to know whether the electrolyzed oxidized water can be used for this purpose depends on whether it has the following effects; (1) removal of the plaque, (2) prevention of the plaque formation, and (3) bactericidal effect on bacteria in the plaque attached.

(1) Removal of Plaque

It is well known that gargling with water cannot remove the plaque attached to the tooth surface. How about gargling with the electrolyzed oxidized water? The plaques on the tooth surface of patients were stained with a dye to gargle 10 times with the electrolyzed oxidized water for comparison of the appearance of the plaque attached to the tooth surface before and after gargling (Figures 1 and 2). There was little change in the amount of plaque attached regardless of the gargling, suggesting the gargling with the electrolyzed oxidized water cannot be anticipated to have a positive effect on the removal of plaque.

(2) Control of Plaque Formation

There is a report by Nishida et al.¹⁾ on this topic. In this report subjects were loaded for 7 days with a resin-made device, of which the root dentin was implanted on the site corresponding to the buccal gingiva of molar. During this period of time, the device with the root dentin was cleaned twice a day in the morning and the evening by immersing for 30 seconds in either one of physiological saline, electrolyzed oxidized water or 0.2% chlorhexidine solution in a beaker. The root dentins were observed by a scanning electron microscope. The amount of plaque formed was clearly less in the case cleaned with the electrolyzed oxidized water than that cleaned with physiological saline, although the efficacy of the electrolyzed oxidized water is slightly less efficient than the case cleaned with 0.2% chlorohexadine. This experiment did not use the condition where the subjects wearing the device gargled, but the results suggest the gargling

with the electrolyzed oxidized water has the controlling effect on the plaque formation.

(3) Bactericidal Effect on Bacteria in the Plaque Attached

On this topic we did experiments to investigate whether the electrolyzed oxidized water has a certain level of the bactericidal efficacy against bacteria in plaque. Teeth were brushed with a sterile toothbrush with bristles, which was immersed with stirring for 1 minute in 2 liters of the electrolyzed oxidized water (generated in JED-020 manufactured by Japnix Inc., pH; lower than 2.6, oxidation-reduction potential (ORP); higher than 1,000 mV) to treat the plaques attached to the bristles. The treated suspension was inoculated into a thioglycolate medium to incubate at 37°C for 8 hours. At the same time, as a control after brushing the teeth, the toothbrush not treated with the electrolyzed oxidized water was directly placed on the medium to incubate for comparison. Comparison of a change of the medium by the growth of bacteria demonstrated that the bacteria was actively grown in both groups with or without cleaning with the electrolyzed oxidized water and there was no difference between both groups in the control of the bacterial growth.

In this experiment the electrolyzed oxidized water had a possibility not demonstrate the bactericidal effect because of improper selection of the medium in spite of observing the potential bactericidal effect in (2). However, the electrolyzed oxidized water did not certainly demonstrate the strong bactericidal effect on the plaque attached to the toothbrush. The plaque is an aggregate of a number of bacteria, which exist not only on the surface of the plaque, but also its inside. The electrolyzed oxidized water may affect the plaque on the surface, but cannot kill the bacteria inside the plaque because the bactericidal activity is lost quickly upon contact with proteins. A similar inefficiency of the electrolyzed oxidized water may be anticipated in the cleaning of the plaques attached to the tooth surface. Similar to commercial gargles, the gargling with the electrolyzed oxidized water alone cannot be anticipated to be effective in killing the bacteria inside the plaque attached to the teeth.

(4) Summary

The above results indicate that use of the electrolyzed oxidized water by gargling for the patients, who do not brush the teeth sufficiently to accumulate many plaques cannot remove the plaques attached, leaving the bacterial pathogen intact. Even if a dentist once removes thoroughly the plaques in these patients and encourage them gargling, the disinfection effect of the electrolyzed oxidized water disappears to form the plaques immediately. Gargling in this kind of patients has little effect on the plaque control and instead we better train them first a way of thorough teeth brushing to control the plaque formation. Namely, the physical removal of the plaque by brushing should be naturally a first choice for the plaque control.

On the other hand, the electrolyzed oxidized water is likely to exert the disinfection effect on the patients, who regularly practice teeth brushing to control the plaque formation leaving little plaque on the teeth surface, resulting in a decrease of the quantity of the plaque reattached to the teeth surface to demonstrate a potential of the better plaque control.

The periodontal surgery can remove completely the plaques on the surgery site once, but the site cannot be brushed a while after surgery. During this period of time, gargling with the electrolyzed oxidized

water may control the amount of the plaque attached and prevents the plaque maturation to some degree. There is a report²⁾, in which although the presence of proteins reduces the bactericidal activity of the electrolyzed oxidized water, an amount of salivary proteins in the electrolyzed oxidized water is decreased with increasing the frequency of gargling, thus resulting in an increase of the bactericidal efficacy. Gargling several times with the electrolyzed oxidized water is anticipated to have a positive effect on the patients, but its effectiveness is not clarified yet and will be an issue for a future study.

2. Deodorization of Halitosis

Halitosis is caused by various reasons and often a very serious problem for the patients. As one of trials to investigate whether the halitosis can be deodorized by gargling with the electrolyzed oxidized water, we investigated its deodorant effect using a periodontal dressing loaded in the oral cavity, where the “smell” was generated. The patients who had the curettage or periodontal surgery in the Second Section of Conservative Dentistry, Center of Dental Clinics, Hokkaido University were loaded with the periodontal dressing for a few days. Immediately after removal of the periodontal dressing from the patients, the smell of the dressing was evaluated by single assessor to rate it in three scales of “weak”, “moderate”, and “strong”. The dressing was then immersed for 1 minute in the electrolyzed oxidized water (generated by an ND-002R generator manufactured by Remodeling 21, Inc., pH; lower than 2.6, ORP; higher than 1,100 mV) and then taken out to evaluate and rate the smell for comparison according to the same rating scale above. After immersion in the electrolyzed oxidized water, the smell became weaker in three out of six cases, which had been rated “moderate” immediately after removal of the dressing from the surgery site. At the same time, immersion in the electrolyzed oxidized water demonstrated no change of the smell in three cases, which had been rated “strong” immediately after removal the dressing.

We believe the electrolyzed oxidized water has the deodorization effect on the halitosis to some degree depending on the strength of the smell, although our cases are not sufficient to conclude the deodorant effect on the halitosis. A further study on the frequency and method of cleaning may lead to better results in deodorization.

Conclusion

The plaque control cannot be achieved only by gargling with the electrolyzed oxidized water and rather thorough oral brushing is considered to have the higher efficacy in the plaque control. However, use of the electrolyzed oxidized water may be effective in the sites where the brushing cannot be used in cases immediately after the surgery. The electrolyzed oxidized water may have the deodorization effect on halitosis to some degrees, but a further study is required to know its detail.

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Questions on use of electrolyzed oxidized water for treatment

2. Can we use it in the periodontal pocket irrigation?

Jun Negishi, Gakuo Endo, Masamitsu Kawanami, Ki Kato (Second Section of Conservative Dentistry, School of Dentistry, Hokkaido University)

Plaque control in the periodontal pocket, namely, the subgingival plaque control determines whether the periodontal treatment succeeds. An ordinary periodontal treatment includes not only the supragingival plaque control, but also the subgingival scaling and root planing for the control of the subgingival plaque. Effectiveness of the subgingival scaling and root planing largely depends on the degree of the lesion progression and the morphology of the dental root as well as the skill level of a clinician to operate. There were many cases, where the effects of scaling and root planing were not reflected as expected. Agents such as chlorhexadine and others have been tried in the periodontal pocket irrigation. Many reports¹⁻⁴ described some positive effects of these agents in the periodontal pocket irrigation, but no clear evidence of the effects by the agents themselves was available yet. Furthermore, these agents (antibiotics, bactericides, and others) are chemical agents and required to understand the injurious and side effects on living organisms.

On the other hand, it is said that electrolyzed oxidized water is a disinfectant, which exerts the disinfection effect on various virus, bacteria, and fungi and which can be widely used to human skin and in mouth without an adverse effect. It is also said to be inexpensive, pollution-free, and nonallergic.⁵ We investigated the effect of the electrolyzed oxidized water in the periodontal pocket irrigation.

Periodontal Pocket Irrigation

Subjects were 8 patients, who had been diagnosed with periodontitis but good plaque control and have attended the Second Section of Conservative Dentistry, Dental Clinical Center, Hokkaido University Hospital. Two teeth kept with the periodontal pocket of deeper than 4 mm were selected from each patient, who received more than a month ago the initial treatment, periodontal surgery, or scaling and root planing for the maintenance therapy. The periodontal pocket of one tooth was cleaned with the electrolyzed oxidized water, while that of other tooth was with physiological saline. The electrolyzed oxidized water was generated using a JED-020 generator manufactured by Janix Inc. (pH; lower than 2.6, oxidation-reduction potential (ORP); higher than 1,000 mV) and used immediately in the experiment. A tip of the cleaning needle (Clean Washing Needle^{TR} 27G manufactured by NIPRO Co., Ltd.) attached to a syringe was inserted into the deepest part of the periodontal pocket to irrigate for 1 minute with 5 ml of the electrolyzed oxidized water or physiological saline. The degrees of plaque adhesion to the tooth (plaque index (PII)), gingival inflammation (gingival index (GI)), and bleeding on probing (BOP), and the probing depth (PD) were determined immediately before irrigation and one week after irrigation.

Comparing the results in the cases before irrigation and one week after irrigation, the severity of gingivitis (GI) and PD were improved somewhat in some cases in both groups, in which the periodontal pocket was irrigated with either the electrolyzed oxidized water or physiological saline. However, there was no significant difference in all laboratory parameters in both groups before and after irrigation and between

two groups. In four kinds of laboratory parameters above, difference was calculated by subtracting the values determined one week after irrigation from those determined before irrigation. The difference was used as the index of improvement by the irrigation with the electrolyzed oxidized water, but there was no significant difference in both groups in four different laboratory parameters (Table 1).

On the other hand, among six positive (+) cases before irrigation three cases were improved to become negative (-) in the number of teeth bled on probing (BOP) after irrigation with the electrolyzed oxidized water, whereas among 5 positive cases before irrigation only one case was improved to become negative in BOP after irrigation with physiological saline.

Table 1 Laboratory Data before Irrigation and One Week after Irrigation of Periodontal Pocket and the Amount of Improvement (n=8 in Both Groups)

Periodontal Pocket Cleaner		PII	GI	PD (mm)	BOP (+), number of teeth
Electrolyzed oxidized water group (n=8)	Before irrigation	0.6 ± 0.518	1.0 ± 0	4.1 ± 0.354	6
	After irrigation	0.8 ± 0.463	0.6 ± 0.518	3.5 ± 1.069	3
	Amount of improvement	-0.2	0.4	0.6	3
Physiological saline group (n=8)	Before irrigation	1.0 ± 0	1.0 ± 0	4.0 ± 0	5
	After irrigation	0.9 ± 0.354	0.5 ± 0.535	3.5 ± 0.535	4
	Amount of irrigation	0.1	0.5	0.5	1

PII; mean of plaque index (Loe & Silness) ± standard deviation

GI; mean of gingival index (Loe & Silness) ± standard deviation

PD; mean of probing depth ± standard deviation

BOP; number of bleeding teeth on probing ± standard deviation

Discussion

The cases used in the present study were limited to the patients, who had the periodontal pocket depth in a range of 4 mm and received the treatment of scaling and root planing more than a month ago. The results in the present study suggest that meaningful effects cannot be anticipated by the periodontal pocket irrigation once a week at the hospital with the electrolyzed oxidized water. However, there was no case, in which the periodontitis was deteriorated after irrigation. Improvement of BOP was observed in 3 out of 8 cases.

A main reason why the periodontitis was not significantly improved in the present study is probably due to the adhesion of a large amount of plaques to the subgingival root surface. This indicates that the

periodontal pocket irrigation alone can remove a loose part of plaques, but not the plaques adhered to the pocket. The electrolyzed ionized water appears not to penetrate deep inside the plaque (refer to p. 16-18 in Section 1 of the article entitled with “What is the effect of gargling?”). The pathogenic plaques thus remain in the subgingival region, resulting in no significant improvement of the periodontitis.

There is a report⁵ to describe a significant decrease of the bacterial count in the periodontal pockets immediately after irrigation with the electrolyzed oxidized water. Removal of the loose plaques by the irrigation certainly reduces the bacterial count and the bactericidal activity may be effective against some bacteria in the plaques adhered in the periodontal pocket. However, efficacy of the subgingival pocket irrigation against the periodontitis is limited so far as a large amount of the plaques are adhered to the root surface in the periodontal pocket. The present results suggest it is doubtful that the electrolyzed oxidized water can be effective to kill this type of the plaques.

Scaling and root planing in the subgingival region are a basic dental care and a sloppy work in this care naturally results in the low effectiveness of the periodontal pockets irrigation. The periodontal pocket irrigation can be anticipated to be effective in the plaque control to some degree if used in combination with a basic care such as the scaling and root planing in the subgingival region or after the periodontal pocket irrigation is performed thoroughly. However, as similar to many results reported on other agents, there is no direct evidence to prove the efficacy of the electrolyzed oxidized water, which demonstrates a similar level of the efficacy to the periodontal pocket irrigation with physiological saline. Namely, water itself is effective in the periodontal pocket irrigation and use of the electrolyzed oxidized water as a periodontal pocket irrigant cannot be anticipated to have significant effects. However, as an electrolyzed oxidized water generator had been installed in a hospital for various purposes including the prevention of nosocomial infection, an inexpensive irrigant can be readily obtained and used advantageously to reduce the bacteria in the periodontal pocket.

However, the periodontal pocket irrigation has to be performed in combination with the debridement combined with a scaler (including an ultrasonic scaler) at all times and with the removal of the subgingival plaques adhered and the dental calculus.

Conclusion

Use of the periodontal pocket irrigation with the electrolyzed oxidized water alone as a treatment method for the periodontitis cannot be anticipated much in terms of a clinical effect. Namely, in the oral care it is important to perform thoroughly a basic periodontal care such as the oral hygiene instruction, scaling, root planing, and others to remove the subgingival plaque adhered and calculus. As the periodontal pocket irrigation is performed additionally after these treatments, reattachment of the plaques can be prevented to yield possibly a positive effect to some degree. More effective methods including these effects have to be investigated in future.

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Questions on use of electrolyzed oxidized water for treatment

5. Can electrolyzed oxidized water irrigate a root canal?

Rintaro Terada and Minoru Kubota (First Section of Conservative Dentistry, School of Dentistry, Iwate Medical University)

Mechanical enlargement of root canals, so-called root canal enlargement, is considered to be very important in the root canal therapy. However, it is presently difficult to obtain bacteria-free conditions in the root canal by the root canal enlargement, because dental pulps in the root canals, dentin fragments, and other tissues cannot be removed completely by its operation alone leaving some in the root canals and providing the perfect biotope for bacteria. In addition, the root canal enlargement is not effective in the cleaning of the convex and concave walls of the root canals, the accessory canals, root collaterals, and root branches. Therefore, cleaning of these areas relies 100% on liquid agents to act for the dissolution of organic substances and disinfection.

In general clinical practice, a so-called alternate irrigation method has been widely employed using a syringe filled alternately with a sodium hypochlorite solution and a hydrogen peroxide solution. As a more effective method, a suction irrigator¹ and a system using water jet irrigation² or an ultrasonic wave^{3,4} have been proposed, but not used widely yet because of some disadvantage.

On the other hand, in the dental business there has been an interest in the pharmacological action of electrolyzed oxidized water, which is one of functional waters. The electrolyzed oxidized water is functional water with the virucidal and bactericidal activities. Its usefulness has been reported in many healthcare front lines. The electrolyzed oxidized water exhibits a high oxidation-reduction potential and a low pH value and its effects and mechanism of action have been studied.

We used the electrolyzed oxidized water in the root canal irrigation for disinfection. As the properties of this water to be low in pH may be possibly useful for removal of smear layers associated with the root canals, we investigated using the avulsed teeth the cleaning effect of the electrolyzed oxidized water on the smear layer of the root canal wall generated by the root canal enlargement.

Materials and Method

Subject teeth in this study were twenty avulsed maxillary anterior teeth with the untreated root canals, which were stored in water. The pulp cavity of the subject teeth was perforated according to a routine procedure. After confirming a #15 K-file reached the apical foramen, the root canal length was measured. The working length was then established 1 mm shorter than root canal length. The root canal was immersed in physiological saline to perforate using #15 to #60 K-files according to a circumferential filing technique, providing the specimen for the following experiment.

Experimental group 1; a group using physiological saline for irrigation

A tip of an Endodontic syringe^{TR} (manufactured by Neo Inc.) was inserted into the site 1 mm shorter than the working length, followed by injection of physiological saline under mild pressure into the root canals to irrigate for 2 minutes under continuous flow.

Experimental group 2; a group using alternate irrigation with 5% NaClO and 3% H₂O₂

Similar to the operation performed in Group 1, both agents were alternately injected to the root canals to irrigate for 2 minutes.

Experimental group 3; a group using Morphonine^{TR} (15% EDTA, manufactured by Showa Yakuhin Kako Co. Ltd.) for irrigation

Using an Endodontic syringe^{TR} the root canal was fully filled with Morphonine^{TR} to allow to stand for 2 minutes.

Experimental group 4; a group using electrolyzed oxidized water for irrigation

Similar to the operation performed in Group 1, the electrolyzed oxidized water was injected into the root canal to irrigate for 2 minutes under continuous flow.

The electrolyzed oxidized water used in the present study was prepared using an electrolyzed oxidized water generator JAW-035 (manufactured by Nippon Intec Co. Ltd.) and a pH and an oxidation-reduction potential (ORP) were adjusted to less than 2.6 and higher than 1,100 mV, respectively, to use it immediately after preparation.

After end of each experiment, a cotton plug was placed in the root canals of the subject tooth to dry, followed by drawing of a line cleavage buccolingually by a disc to dissect it by a mallet and a chisel to expose the root canal. The specimen were then dried, on which platinum was vacuum-deposited (Ion Sputter E-1030, manufactured by Hitachi, Ltd.) according to a routine procedure. The root canal walls of the orifice and the middle section of root canal, and the apical region was observed separately by a scanning electron microscope (S-2300, manufactured by Hitachi, Ltd.)

Results

Characteristic images of the root canal walls observed by scanning electron microscopy are described below.

Experimental group 1

There were clearly the smear layers in every site on the root canal wall with an irregular rough surface. Streaks like an abrasion mark by filing were observed on the smear layer (Figure 1).

Experimental group 2

There were clearly the smear layers in every site on the root canal wall with streaks like an abrasion mark, but less concave and convex surface (Figure 2).

Experimental group 3

The smear layer on the root canal wall was removed in both the orifice and the middle section of the root canal and the opening of dentinal tubules exhibited a clear contour. In the apical region the smear layer in the root canal was mostly, but not completely removed to leave part of it. An opening of the dentinal tubules exposed exhibited a clear contour (Figure 3).

Experimental group 4

In the root canal orifice, the smear layer on the root canal wall was removed to exhibit a clear contour of an opening of the dentinal tubules. In the middle section of the root canal and the apical region, the smear layer on the root canal wall was mostly, but not completely removed to leave part of it. An

opening of the dentinal tubules exposed exhibited a clear contour in the middle section of root canal, but a blurred contour in the apical region (Figure 4).

Discussion

At present there is no consensus of opinion on the clinical significance of the smear layer in the endodontic care. However, the smear layer starts the inclusion of organic substances and bacteria in an early stage of development and its presence is considered to be undesirable. In root canal filling the removal of the smear layer may improve the adhesion of an oral canal sealant to dentin.

The electrolyzed oxidized water used in the present study for the root canal irrigation demonstrated a similar level of the effectiveness in removal of the smear layer as the irrigation with an ETDA solution. This is due to a low level of the decalcification at low pH of the electrolyzed oxidized water.

In a preliminary study, we confirmed that the root canal irrigation for two minutes under continuous flow is important for removal the smear layer as reported above after studying an effect of the electrolyzed oxidized water by varying a water content and a duration of its action. We also confirmed the efficacy is affected by the properties, a water content, and a duration of action of the electrolyzed ionized water. This might be due to the properties of the electrolyzed ionized water, which is readily denatured on contact with organic and inorganic substances⁵. Therefore, we need to thoroughly understand these characteristics in use of the electrolyzed ionized water.

Use of the electrolyzed ionized water in the root canal treatment is equal to use of an ideal root canal irrigant because of its ability to remove the smear layer in addition to the pharmacological action described in Chapter 2 of the present Special Edition. Therefore, its use is good news for the endodontic care.

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