Int J Clin Exp Med 2016;9(11):21304-21311
www.ijcem.com /ISSN:1940-5901/IJCEM0035224

Original Article

Development of a mouthwash alternative using a low-level hypochlorous acid solution with macroporous platinum electrodes and its application to oral health

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Received July 6, 2016; Accepted September 30, 2016; Epub November 15, 2016; Published November 30, 2016

Abstract: Multiple studies have reported the side-effects of alcohol in mouthwash. The use of alcohol in mouthwash has remained controversial and is a challenging issue. The aim of this study was to propose a low-level hypochlorous acid solution of electrolyzed water as an alternative to mouthwash by monitoring oral bacteria though a bactericidal activity experiment. To determine the solution's applicability in daily life, we developed an electrolysis device with macroporous electrodes for quickly reaching relevant free chlorine concentrations (3 to 5 mg L^{-1}). We tested the generally used electrode and macroporous electrode for their production efficiency of low-level hypochlorous acid solution of electrolyzed water. The free chlorine on the macroporous Pt film electrode at 9.20 s was significantly higher than on the electrolysis electrode at 22.7 s. Moreover, our study showed that a low-level hypochlorous acid solution exhibited strong bactericidal activity on four anaerobic bacteria (*P. gingivalls, P. intermedius, P. nigrescens, F. nucleatum)* responsible for periodontitis and five facultative anaerobic bacteria *(S. mutans, S. sobrinus, S. godonii, S. oralis, S. salivarius*). Thus, we conclude that the low-level hypochlorous acid solution has potential for application to mouthwash after further research via *in vivo* and *in vitro* experiments.

Keywords: Electrolysis, electrolyzed water, macroporous electrode, oral hygiene, self-directed oral health

Introduction

Interest in oral hygiene and self-directed oral health has been growing considerably. Consequently, the number of people using mouthwash has been steadily increasing [1]. Mouthwash is readily available without a doctor's prescription, and has the attributed of decreasing plague, minimizing inflammation of gingivitis, and reducing cavities due to tooth oxidation [1, 2]. Particularly, fluoride and xylitol in mouthwash provide assistance in preventing cavities. Since certain bacteria exposed to xylitol start dying, xylitol in mouthwash is a significant component [2, 3]. Due to the sweetness of xylitol, the salivary secretion rate is increased by salivary gland stimulation. Sweetness of xylitol also helps restore the pH to neutral [2].

Although mouthwash is important for routine oral hygiene and is helpful to overall dental care, it is not a necessity as it has some deficiencies [2-6]. Recently, mouthwash has been criticized for poor removal of halitosis. Most mouthwash contains alcohol. The oral environment may become dry through the evaporation of the alcohol following mouthwash usage. Hence, halitosis becomes more severe over time by spreading bacteria in a drier environment. Moreover, there have been many papers on adverse effects of the alcohol including oral cavities, pharynx damage and larynx damage [2-6]. Therefore, the frequent use of mouthwash may be a risk factor for oral cancer. However, the evidence for risks related to alcohol in mouthwashes is still limited. Recently, meta-analysis indicated that there was no significant relationship between mouthwash-use and oral cancer [4]. Nevertheless, some experts have recommended the alternative use of nonalcoholic mouthwash [5, 6]. Clearly, this issue has proven to be controversial. Yet, despite these controversial issues, most mouthwashes approved by the American Dental Association (ADA) contain alcohol.

We aim to propose a novel alternative to mouthwash. This study proposed electrolyzed water

Table 1. Characteristics of electrolyzed water

Classification	Electrolyzed water		
	Strongly acidic electrolyzed water	Slightly acidic electrolyzed water	Neutral electrolyzed water
pH	2.2 to 2.7	5.0 to 6.5	6.5 to 7.5

as the alternative method. As shown in **Table 1**, electrolyzed water is classified into three groups: strongly acidic electrolyzed water, slightly acidic electrolyzed water, and neutral electrolyzed water [7, 8]. Since strongly acidic electrolyzed water stimulates oral mucosa, it is not fit for mouthwash application.

On the other hand, both slightly acidic electrolyzed water and neutral electrolyzed water are used in various fields by its weak acidity, and may contain hypochlorous acid (HOCl) [7-9]. HOCl is well known to have strong bactericidal ability, eradicating various microorganisms [10]. Hence, slightly acidic electrolyzed water and neutral electrolyzed water have been utilized in many different fields including biological applications, domestic homes, and long-term care facilities [7-10].

Current electrolysis devices can produce the low free chlorine concentrations (less than 100 mg L^{-1}) [7, 11]. Based on related free chlorine concentrations 0.25 mg L^{-1} for drinkable water and 4 mg L^{-1} for tap water, the free chlorine concentrations of produced electrolyzed water must be lower than 5 mg L^{-1} for mouthwash application. Accordingly, conventional electrolysis devices can produce sufficient free chlorine, but they are not yet available for the mouthwash application.

Here, we developed the appropriate electrolysis device and electrodes. Our electrolysis device aims to produce electrolyzed water within the pH range of 5 to 7, as well as relevant free chlorine concentrations (from 3 to 5 mg L^{-1}). We named the resulting electrolyzed water 'low-level HOCL acid solution'. Moreover, much effort was spent to reduce the production time of low-level hypochlorous acid solution for shortening user waiting time. Previous studies reported that most chemical reactions occur at the electrode surface. An enlarged electrode surface area can therefore facilitate the chemical reaction [12, 13].

In the present study, we designed an electrode with a macroporous structure at platinum (Pt) films for enlarging the electrode surface area. After performing the system evaluation, we conducted a feasibility study following bactericidal activity of oral bacteria.

Materials and methods

Electrolysis electrode design

Figure 1 shows the designed electrolysis electrode which is 14.9 mm in height, 15.4 mm in width, and 2.8 mm in thickness. The tetragonal shape at core center was processed as 11.4 mm in width and 5.5 mm in height. The interhole distance was set at 2.0 mm. The electrolysis electrode was made of titanium and coated with a thin layer of Pt with a thickness of approximately 3.0 μm.

Macroporous structure at Pt (platinum) films

Previous studies had demonstrated that the macroporous electrode containing free-standing macroporous film with a pore size of a few millimeters facilitates the chemical reaction [14-16].

Reagents

Without further purification, all the following chemicals were used: Octaethylene glycol monohexadecyl ether C16EO8 (Fluka), hydrogen hexachloroplatinate hydrate (Aldrich), sulfuric acid, D-(+)-glucose (Sigma), L-ascorbic acid (Aldrich), and 4-acetamidophenol (Sigma).

Instruments

Five pairs of electrolysis electrodes were employed for all the electrochemical measurements. All experiments were analyzed by electrochemical analyzer (model CH660, CH Instruments Inc., Austin, TX78733). Ag/AgCl (3 M KCl) and a Pt wire were utilized for reference and counter electrodes, respectively. A Pt rod electrode (0.020 cm^2) was used for substrating the Pt film. The Pt disk electrode was polished with alumina (0.3 μm) and then utilized for smoothing the Pt electrode.

Preparation of liquid crystals

A mixture was made of C16EO8 (0.42 g), distilled water (0.29 g), and hydrogen hexachloroplatinate hydrate (0.29 g). The temperature of the mixture was raised to 80°C, at which point the mixture was homogeneous. Electrolysis electrodes were inserted into the homoge-

Figure 1. Electrolysis Electrode Design.

Figure 2. DR3000; Water Quaility Analyzer.

neous mixture, and then the mixture temperature was reduced to room temperature (23-26°C), at which point it became a highly viscous liquid crystalline material that was subsequently used.

Electrodeposition of macroporous platinum films

Pt deposition was processed on a polished Pt rod electrode in applying a constant potential (-0.06 V vs Ag/AgCl). The processed electrodes were placed in distilled water for 1 h to extract the C16EO8. This procedure was repeated 3-4 times. Chemical cleaning of the electrolysis electrode was performed using a cycling potential between +1.2 and -0.22 V versus Ag/AgCl in 0.5 N sulfuric acid until reproducible cyclic voltammograms were confirmed.

Electrochemical experiments

The surface roughness of the macroscopic dimensions of the electrode were determined by measuring the areas under the hydrogen adsorption/desorption peaks of the cyclic voltammograms (scan rate, 0.2 V s^{-1}) in 0.5 N sulfuric acid solution. A conversion factor of 210 μC cm^{-2} was used to determine the electrode area [17, 18]. The number of hydrogen adsorption/desorption peaks was presented as the surface roughness factor: where. Q_H is the area of hydrogen adsorption peaks, A_{geo} is the geometric area [17-19].

Electrolysis experiment

To confirm the free chlorine concentrations of electrolyzed water, we used the DR 3000 (HACH, USA) (**Figure 2**). The electrolysis device generates a 49.5% duty rate of 0.2 Hz biphasic rectangular wave pulse. The supply voltage rectangular wave pulse was 9.0 V. The volume of electrolyzed water was as 43 mL. We carried out a comparative experiment for the electrolysis electrode and the macroporous structure Pt films electrode. To make a 0.9% Sodium Chloride Solution, 0.387 g NaCl was dissolved in water. The pH of water was 6.5. The free chlorine concentration of electrolyzed water was measured at 4 different intervals (5, 10, 15 and 20). The measurements were repeated 10 times to measure the current flow.

Required time for reaching a free chlorine concentration of 3.5 mg L^{-1} was analyzed by a regression analysis. We set up the electrolysis system's operation time as the estimated time from the regression analysis results with the highest R-square. After modifying the operation time of the electrolysis system, the electrolysis experiment was repeated 10 times to measure the current flow.

Bactericidal activity

To review the applicability of the alternative to mouthwash, four anaerobic bacteria (*P. gingivalls* CCARM0145 (kindly provided by the Cul-

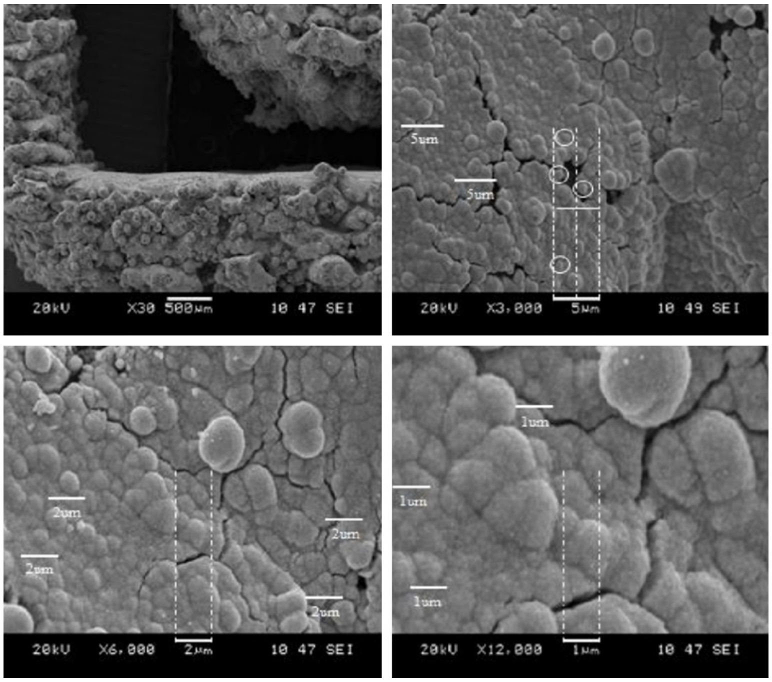

Figure 3. Morphologies of the electroplated films determined with an electric flux density of 0.64 C cm^{-2}.

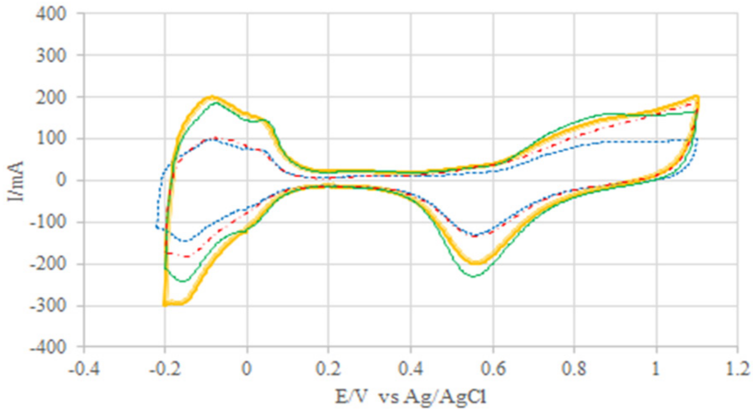

Figure 4. Cyclic voltammograms of macroporous platinum electrodes in in 0.5 N sulfuric acid solution (scan rate, 0.2 V s^{-1}) electroplated by passing 2.55 C cm^{-2}.

ture collection of antimicrobial resistant microbes), *P. intermedius* KCOM 1101, *P. nigrescens* KCOM 1108, *F. nucleatum* KCOM 1001 (kindly provided by the Korean collection for oral microbiology, Gwangju, Korea)) responsible for periodontitis and five facultative anaerobic bacteria (*S. mutans* KCOM 1054, *S. sobrinus* KCOM 1157, *S. godonii* KCOM 1967, *S. oralis* KCOM 1507 (kindly provided by the Korean collection for oral microbiology, Gwangju, Korea), *S. salivarius* 5-B9059 (kindly provided by the Sinchon Severance Hospital Seoul, Korea) associated with cavity development were cultivated on blood agar plates (BAP) [20-23]. Four anaerobic bacteria were incubated in an anaerobic chamber for 2 days. Five facultative anaerobic bacteria were grown in a 10% CO_2 incubator for 1 day. After picking up nine strains of bacteria in BAP, we made a bacterial suspension by adding 1 mL of phosphate buffered saline (PBS). After moving the sample to an Eppendorf tubes, the bacterial suspension (100 μL) was mixed with the low-level hypochlorous acid solution (900 μL). The control group was mixed with PBS (900 μL). The samples were incubated at room temperature for 1 min and 100 μL of each mixture was diluted with PBS (900 μL). Thereafter, the mixtures were serially diluted 10-fold, plated on either BAP, and viable colonies were enumerated. After plating in BAP, the bacteria colonies were incubated in a CO_2 incubator at 37°C for 24-48 h. The bactericidal activity was calculated according to Equation 1. All experiments were repeated twice.

$$\text{Bactericidal activity (\%)} = (\text{PBS} - \text{HOCl}) / \text{PBS} \times 100 \quad (1)$$

CFU: Colony-forming unit.

PBS: CFU/mL of viable bacteria treated with PBS.

HOCL: CFU/mL of viable bacteria treated with low-level hypochlorous acid solution.

Results

The roughness of macroporous structure at Pt (platinum) films

The morphology of the macroporous structure of the Pt film at the electrolysis electrode was detected by field emission scanning electron microscopy (FESEM) using JSM-7401F (JEOL).

Table 2. Surface roughness factors

	Case 1	Case 2	Case 3	Case 4	Case 5	Average ± STD
Division 1						
Surface Area (cm^2) at electrolysis electrode	7.71	7.72	7.75	7.74	7.76	7.73 ± 0.02
Division 2						
Surface Area (cm^2) at macroporous structure Pt films electrode	1225.24	1033.81	1139.04	843.33	822.86	1012.86 ± 158.94
Roughness factor = Division 2/Division 1	159	134	147	109	106	131 ± 20.77

Table 3. Free chlorine measurements

Time	5 s	10 s	15 s	20 s
Electroysis electroe [mg L^{-1}]	1.60 ± 0.30	2.20 ± 0.20	2.30 ± 0.40	3.50 ± 0.70
Macroporous structure Pt films electrode [mg L^{-1}]	2.90 ± 0.10	4.30 ± 0.20	4.50 ± 0.30	6.40 ± 0.50

Table 4. Measured free chlorine and amount of current flow

Classification	Electrolysis electrode	Macroporous structure Pt films electrode
PPM [mg L^{-1}]	3.40 ± 0.30	3.50 ± 0.20
Current [mA]	120 ± 15	260 ± 20

The FESEM image (**Figure 3**) shows a microscale surface area. The conspicuous cracks and obvious macroscopic morphology were observed. Thin macroporous metal film containing free-standing Pt microparticles with a minimal of 1 μm in diameter were detected. Pt macroparticles are interconnected with others and distributed capriciously. Partially, the interstitial macropores width among the merged Pt macroparticles was between 2 and 5 μm. As a result, the interstitial macropores and macroparticles had a 3D structure.

Comparing the results from the recorded cyclic voltammograms (**Figure 4**), the apparent voltammetric behavior of the five pairs of electrodeposited electrolysis electrodes was similar. Owing to the macroporous structure, the electrode surface area was enlarged as the 131 ± 20.77 roughness factor (**Table 2**).

Results of regression analysis

As shown **Table 3**, both electrodes increased steadily up to 20 s. The free chlorine at the electrolysis electrode was significantly lower than at the macroporous structure Pt films electrode for all time intervals.

The results of the regression analysis indicated that the quadratic regression results had the highest $R^{\wedge 2}$ square of 0.917 at the electrolysis electrode and 0.975 at the macroporous structure Pt films electrode. The quadratic regression equation of the electrolysis electrode is Equation 2. The result of the quadratic regression equation for the macroporous structure Pt films electrode is Equation 3.

$$\text{PPM [mg L}^{-1}] = 0.004 \times \text{Time}^2 + 1.446 \quad (2)$$

$$\text{PPM [mg L}^{-1}] = 0.009 \times \text{Time}^2 + 0.012 \times \text{Time} + 2.627 \quad (3)$$

The results of the quadratic regressions indicate that the electrolysis electrode at 22.7 s had a free chlorine concentration of approximately 3.5 mg L^{-1} and the macroporous structure Pt films electrode had a free chlorine concentration close to 3.5 mg L^{-1} at 9.20 s.

The characteristic of electrolysis electrodes

Table 4 indicates the results for free chlorine and of the current flow when the electrodes were operated at the estimated times (respectively, 9.20 s and 22.7 s). The free chlorine of both electrolysis electrodes were within the range of 3 to 5 mg L^{-1}. The free chlorine for the macroporous structure Pt films electrode at 9.20 s was significantly higher than that for the electrolysis electrode at 22.7 s. We confirmed that the amount of current at the macroporous structure Pt films electrode was also higher.

Results of bactericidal activity

We determined bactericidal activity of the free chlorine generated from saline on several oral bacterial strains involved in the formation of dental cavities and periodontitis. The non-treated saline solution served as a control. Bacteria suspensions were treated with the low-level hypochlorous acid solution or non-treated saline controls for 1 min and then serially plated on appropriate bacterial culture plates (**Figure 5**). The number of bacterial colonies found in

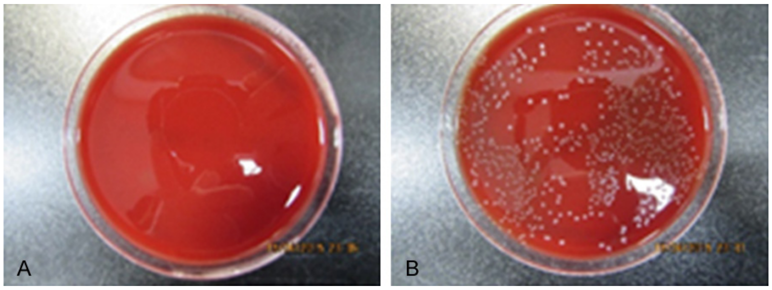

Figure 5. Representative gross image of *S. salivarius* colonies on blood agar plates following free chlorine treatment. *S. salivarius* was treated with saline alone (A) or with free chlorine (B) for 1 min, diluted and plated on blood agar blood plates.

Table 5. Bactericidal activity of hypochlorous acid on oral bacteria strains

Bacterial colony	Gram stain reactivity	Bactericidal activity (%)
Porphyromonas gingivalis	Negative	≥ 99.999%
Prevotella intermedia	Negative	≥ 99.999%
Prevotella nigrescens	Negative	≥ 99.9999%
Fusobacterium nucleatum	Negative	≥ 99.9999%
Streptococcus mutans	Positive	≥ 99.9999%
Streptococcus sobrinus	Positive	≥ 99.999%
Streptococcus gordonii	Positive	≥ 99.99%
Streptococcus oralis	Positive	≥ 99.99999%
Streptococcus salivarius	Positive	≥ 99.9999%

the low-level hypochlorous acid solution group was compared with the non-treated saline group and then the bactericidal activity was calculated as a percentage (**Table 5**). We found that the low-level hypochlorous acid solution exhibited ≥ 99.99% bactericidal activity for all strains tested. The bactericidal activity was likely approximately 100% for most strains as we did not detect bacterial growth due to the limit of detection. Both Gram-positive and Gram-negative bacteria were equally susceptible to the free chlorine treatment. These results demonstrate the rapid and complete eradication of the most common bacteria that promote dental diseases.

Discussion

Both a lack of oral hygiene and poor oral health contribute to the risk factors for cancers of the oral cavity, larynx, oropharynx, and hypopharynx. At the same time, the number of mouthwash users is increasing at an alarming rate. However, previous studies have warned that the frequent use of mouthwash may be a risk factor as well due to the alcohol included in many formulations [1-5].

Our study proposed an alternative to alcohol containing mouthwash, and showed the practicality of usage in daily life. The strengths of our study include the application review in daily life and the feasibility review based on bacterial activity.

According to previous reports, slightly acidic electrolyzed water and neutral electrolyzed water showed the possibility of various uses such as sterilization of equipment, utensils, and surgery instruments. Consequently, many studies have reported on a new electrolysis device that can produce sufficient free chlorine [11-13]. The previously reported electrolysis device consists of four sections: an electrolyte containing an ionic substance, electrodes (an anode and a cathode), a diaphragm, and a direct electric current supply [10, 19].

Previous studies also reported on a significant difference in the technical capabilities of electrolysis devices depending on their electrode materials [15]. Pt among different electrode materials has been used as a catalyst in many different fields including electrochemical sensors [24-27]. Moreover, the size of the electrode surface is a crucial factor for improving the production efficiency of electrolyzed water. According to previous reports, most electrochemical reactions occur on the electrode surface. Therefore, a larger electrode can facilitate more electrochemical reactions [20-23]. Additionally, some studies reported that faradaic currents associated with electrochemical reactions are influenced more by an enlarged electrode surface area rather than its geometric area [14-17]. Among methods of enlarged electrode surface, the metal film for producing a freestanding macroporous film with a pore size of a few micrometers enlarged the electrode surface area and facilitated the chemical reaction. Accordingly, the macroscopic dimensions of Pt films have received much attention for their potential application [29, 30]. Hence, we used a macroporous electrode with a macroscale surface area. We developed a macroporous structure at Pt films electrode and an electrolysis device for reducing user waiting times through the faster generation of low-level hypochlorous acid solution.

The FESEM image (**Figure 3**) shows an extensive macroporous structure at the electrode surface. A free-standing Pt macroparticles with a 5 μm thickness was shown. As a result, the electrode surface area was enlarged as indicated by the 131 ± 20.77 roughness factor. The roughness of the macroporous structure Pt films electrode facilitates the chemical reaction by enhancing the faradaic current of a sluggish reaction as indicated by the free chlorine and current flow results. In conclusion, our study provides a method of directly producing a low-level hypochlorous acid solution for 9.20 s. Moreover, the results of bactericidal activity indicated that a low-level hypochlorous acid solution within the range of 3 to 5 mg L^{-1} has strong bactericidal activity within 1 min. This study successfully demonstrated that low-level hypochlorous acid solution has bactericidal activity against nine oral bacteria.

In general, our results indicate that low-level hypochlorous acid solution for the range of bacteria tested exhibits greater bactericidal activity for four anaerobic bacteria (*P. gingivalls, P. intermedius, P. nigrescens, F. nucleatum)* responsible for periodontitis and five facultative anaerobic bacteria *(S. mutans, S. sobrinus, S. godonii, S. oralis, S. salivarius*) associated with cavity development [20-23]. Therefore, we conclude that the developed macroporous structure Pt films electrode system has the potential to replace alcohol containing mouthwash through its production of low-level hypochlorous acid solution.

Acknowledgements

This work was performed under the commercialization of production technologies in the industrial cluster program funded by the Korea Industrial Complex Corporation (KICOX), Grant 1415135930.

Disclosure of conflict of interest

None.

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