

Hypochlorite solution for root canal irrigation that lacks a chlorinated odor

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ABSTRACT

Objectives: This is an *in vitro* study to develop a formulation of a hypochlorite solution for root canal irrigation that lacks a chlorinated odor. The antibacterial effect, tissue dissolution efficacy, and the cytotoxicity of the solution were assessed in cell culture and were compared with those of commercial sodium hypochlorite (NaOCl) solutions. **Materials and Methods:** Trichloroisocyanuric acid (TCA) was used as the source of hypochlorite ions in solution. All required properties of the NaOCl irrigant were evaluated and compared with those of original 2.5% NaOCl solutions currently in use. **Results:** Our results revealed that a TCA 3.5% + 1/6 Buffer-1 solution passed the short-term stability test. Moreover, no odor of chlorine gas was detected by three independent observers. The hypochlorite ion content and pH were stable over an incubation period of 4 weeks. The new solution did not differ from commercial products in terms of the dissolution property on bovine pulpal tissue ($P > 0.05$). Moreover, the antibacterial effect of this solution on *Enterococcus faecalis* did not differ from that of the commercial products ($P > 0.05$). In addition, our biocompatibility analysis demonstrated no difference among the tested solutions ($P > 0.05$). **Conclusions:** According to the results of all properties tested, TCA 3.5% + 1/6 Buffer-1 could be considered an option for NaOCl irrigation with the benefit of no detectable chlorine odor.

Key words: Hypochlorite solution, lacks a chlorinated odor, root canal irrigant

INTRODUCTION

Elimination of inflammatory or necrotic pulpal tissue is the primary aim of root canal treatment. Sodium hypochlorite (NaOCl) is extensively used as a root canal irrigant for root canal disinfection. Antibacterial infection and necrotic pulpal tissue dissolution are the two main purposes of root canal irrigants.^[1]

Studies by Byström and Sundqvist^[2,3] and Byström and Sunqvist^[4] confirmed the use of NaOCl as an effective root canal irrigant. NaOCl can reduce bacterial infection better than normal saline solution (NSS). The efficiency of irrigation can be increased by adding ethylenediaminetetraacetic acid (EDTA) for smear layer elimination. NaOCl penetrates deeper

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along the canal wall and into the dentin after EDTA irrigation. Studies investigating the use of NaOCl with EDTA revealed less bacterial infection than in patients in whom only NaOCl was used. The optimal chemical concentration of NaOCl is between 1% and 6%.^[5-7] NaOCl and EDTA irrigation not only display antibacterial and tissue dissolution effects mentioned above but also show deep penetration in areas that are impossible for mechanical instruments to reach. Therefore, several irrigants have been recommended for canal disinfection in combination with canal preparation.

NaOCl is commonly used as a root canal irrigant in combination with 17% EDTA.^[8] EDTA dissolves inorganic material and allows NaOCl to penetrate the smear layer for organic tissue dissolution and bacterial elimination. The disadvantage of contemporary NaOCl is the chlorinated odor (chlorine odor) which can be unpleasant for both dental staff and patients. The chlorine odor is released from hypochlorous acid after the reaction of NaOCl with water. Chlorine is a strong oxidant that inhibits bacteria through the irreversible oxidation of the sulfhydryl groups of bacterial enzymes.^[9] Fatty acids and glycerol are generated after the interaction between NaOCl and organic tissue. Based on these properties, NaOCl is the irrigant of choice for root canal treatment.

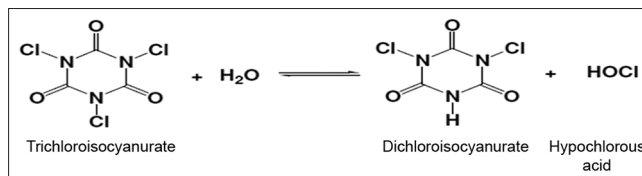
The disadvantage of contemporary NaOCl could be solved by preventing the evaporation of chlorine gas from NaOCl solutions. Adding a substance into the solution that reacts with chlorine gas is one possible strategy. However, these NaOCl solutions would still require the antibacterial and tissue dissolution effects to be effective for clinical use.

The aim of this study was to formulate an NaOCl solution with an unchlorinated odor that has similar tissue dissolution, antibacterial, and cytotoxic effects as NaOCl solutions already in use.

MATERIALS AND METHODS

- Formulation of a hypochlorite solution with an unchlorinated odor
- Our formulation consists of two main components.
 - Component 1: Ten milliliters of trichloroisocyanurate at a final concentration of 2.5% (w/v) was prepared from powder and was used to produce hypochlorite ions.
 - Component 2: Ten milliliters of vehicle solution composed of sulfonic acid, phosphate

buffer, sodium hydroxide, and deionized water was mixed with component 1. The chemical reaction is shown in the following equation.



In our solution, chlorine gas that disintegrates from hypochlorous acid would be captured by the solution and reconverted to hypochlorous acid. A pilot study revealed that the hypochlorite ion content and pH were stable in solution after an incubation period of 4 weeks. The new formulated NaOCl solution was called trichloroisocyanuric acid (TCA) 3.5% + 1/6 Buffer-1.

Pulpal tissue preparation

Bovine mandibular incisor teeth were collected under a protocol approved by the Ethics Committee of Rangsit University (No. RSEC 01/2557). Teeth were stored at -20°C and were thawed to room temperature before the experiment. Each tooth was split in half in the mesiodistal direction using a diamond bur. The pulpal tissue was removed, dissected, and weighed using a calibrated electronic balance (Precisa, Dietikon, Switzerland). The weight for each sample was approximately 0.1 ± 0.005 g.

Test irrigant

Two commercial 2.5% NaOCl solutions are available in Thailand: CU from Chulalongkorn University, Bangkok, Thailand and MU from Mahidol University, Bangkok, Thailand. These solutions were used for comparison with our 2.5% NaOCl solution. NSS was used as a negative control.

Tissue dissolution evaluation

Prepared bovine pulpal tissue weighing approximately 0.1 ± 0.005 g was placed in contact with 20 ml of each test irrigant in a test tube as follows:

- Group 1 TCA 3.5% + 1/6 Buffer-1
- Group 2 CU 2.5% NaOCl
- Group 3 MU 2.5% NaOCl
- Group 4 NSS.

Each test tube was incubated in a 37°C incubator (Mettler GmbH, Schwabach, Germany). After 20 min of incubation, the pulpal tissue was removed from the test tube, blotted dry with a paper towel, and then, the remaining tissue was weighed and

recorded. Ten parallel samples per group were incubated for all experiments. The percent pulpal tissue loss for each group was calculated using the following equation:

$$\% \text{ Tissue dissolution} = \frac{\text{Pretreatment tissue} - \text{Posttreatment tissue}}{\text{Pretreatment tissue}} \times 100$$

Antimicrobial activity evaluation

Pure cultures of *Enterococcus faecalis* (ATCC 19433) grown on a brain-heart infusion (BHI, BD - Difco, Franklin Lakes, NJ, USA) agar plate were suspended in sterile 0.9% NaCl. The cell suspension was adjusted spectrophotometrically to match the turbidity of a McFarland 0.5 scale (1.5×10^8 cfu/ml).

One milliliter of each test irrigant, including the control group (sterile saline), was placed on the bottom of the individual test tube. Two milliliters of the bacterial suspension was vortexed for 10 s with each test irrigant or sterile saline, and the suspension was incubated with the samples for 30 s, 1, 10, and 30 min with 6 tubes/each time period/test material. After each period of treatment time, 0.5 ml of each tube was transferred to a tube containing 1 ml of BHI and neutralizers (0.6% sodium thiosulfate) to prevent continued antimicrobial effects of the test irrigants. All tubes were incubated at 37°C for 7 days to check for additional bacterial growth. The tubes presented with medium turbidity similar to the turbidity of 4 on the McFarland scale (12×10^8 cfu/ml), which is considered positive bacterial growth.

After 7 days of incubation, 20 µl of each suspension was collected from the tube and placed on a BMI agar plate to determine whether any viable bacteria remained.

Cytotoxicity evaluation

The procedure for cytotoxicity evaluation was performed according to ISO 7405: 2008 Dentistry-Evaluation of biocompatibility of medical devices used in dentistry.^[10]

Test materials

TCA 3.5% + 1/6 Buffer-1, CU, and MU irrigants were diluted to 20%, 2%, 0.2%, and 0.02%.

As a positive control, ISO 10993-5^[11] recommends using polyvinyl chloride (Hatano Research Institute, Food and Drug Safety Center, Kanagawa, Japan) at 3 cm²/2 ml of media. The films were sterilized by

soaking in 70% alcohol for 1 min, washed in NSS for 1 min, and air-dried. Then, the films were inserted into Dulbecco's Modified Eagle's Medium (DMEM) and incubated at 37°C under 5% CO₂ for 24 h before testing.

As a negative control, ISO 10993-5^[11] recommends using Thermanox plastic coverslips (NUNC™ Naperville, IL, USA) at 6 cm²/2 ml of media. Thermanox plastic coverslips were cut into small pieces, soaked in DMEM, and incubated in 5% CO₂ at 37°C for 24 h before testing.

Cell culture

We used a transformed line of mouse fibroblast L929 cells (ATCC, cell line, ECACC No. 2869501, NCTC clone 929). Cells were maintained at 37°C under 5% CO₂ and 100% humidity in DMEM supplemented with 10% fetal calf serum and antibiotics (200 µg/ml penicillin G, 200 µg/ml streptomycin, 2 µg/ml fungizone). The medium was changed every other day. When cells reached confluency, they were detached using 0.2% (w/v) trypsin and transferred to new culture flasks.

After sufficient growth for experimentation, the cells were trypsinized and plated in 96-well culture plates at a concentration of 1×10^4 cells/well. Each well contained 100 µl of cell suspension, and the plates were incubated for 24 h at 37°C under 5% CO₂ to obtain a monolayer culture. After 24 h of incubation, the media were removed from each well. Then, 100 µl of eluate was collected from all test irrigants at concentrations of 20%, 2%, 0.2%, and 0.02%, and along with the positive control and negative control, the samples were added to 96-well culture plates (8 wells/test material). Following a 24-h incubation period at 37°C under 5% CO₂, cell viability was assessed using an 3-(4,5-Dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide (MTT) assay.

3 - (4 , 5 - D i m e t h y l t h i a z o l - 2 - y l) - 2 , 5 - diphenyltetrazolium bromide assay

The test materials were removed from each well. Then, 50 µl of MTT reagent (5 mg/ml) was added and incubated for 2 h at 37°C in a CO₂ incubator. The MTT solution was then discarded, and 100 µl of isopropanol was added to the wells. The plates were incubated with shaking to solubilize the formation of the purple crystal formazan. The absorbance was measured using a microplate reader at a wavelength of 570 nm.

RESULTS

Dissolution of bovine pulpal tissue

The percentage of tissue loss after contact with all test irrigants for 20 min is shown in Figure 1. We observed no differences in the percentage of tissue loss among samples treated with our NaOCl solution and the commercial irrigants CU and MU ($P > 0.05$). NSS showed less tissue dissolution than all tested NaOCl solutions ($P = 0.000$).

Antimicrobial activity evaluation

All irrigants were effective at killing 100% of *E. faecalis* for all tested times (30 s, 1 min, 10 min, and 30 min). No bacterial growth occurred on BHI agar plates after 7 days of incubation for all tested irrigants.

The control group, NSS, yielded positive cultures, and *E. faecalis* was recovered from all positive cultures as shown in Table 1.

Cytotoxicity evaluation

The cytotoxic effects of all tested irrigants and the controls are shown in Figure 2. Percentages of cell viability were similar after contact with 20% or 2%

Table 1: Antibacterial effects of tested solutions in direct contact with *Enterococcus faecalis* for different amounts of time

Tested solution	Contact time (n=6)			
	30 s	1 min	10 min	30 min
TCA 3.5%+1/6 Buffer-1	-	-	-	-
CU irrigant	-	-	-	-
MU irrigant	-	-	-	-
NSS	+	+	+	+

+: Bacterial growth, -: No bacterial growth, TCA: Trichloroisocyanuric acid, NSS: Normal saline solution

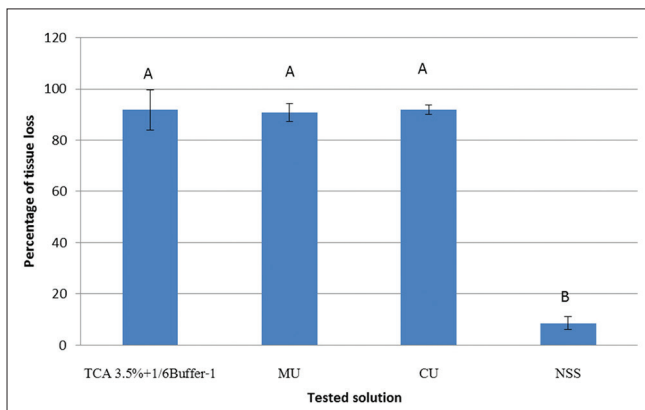


Figure 1: The percentage of tissue loss after 20 min of contact time (the same letter indicates $P > 0.05$)

solutions of our NaOCl formulation, commercial NaOCl (CU and MU irrigants), or positive control solution ($P > 0.05$). At the lower dilution of 0.2%, all tested irrigants were more toxic than the negative control ($P = 0.001$). The lowest concentration of all tested irrigants (0.02%) and the negative control showed similar levels of toxicity toward L929 cells ($P = 1.000$), which was less than that of the positive control solution ($P = 0.001$).

DISCUSSION

NaOCl is the most commonly used endodontic irrigant because of its tissue-dissolving and antibacterial activities. The new formulation of an NaOCl solution with an unchlorinated odor could be effectively used as a root canal irrigant to replace the original NaOCl solutions currently in use. Our formulation, TCA 3.5% + 1/6 Buffer-1, could be used in place of commercial 2.5% NaOCl that is available in Thailand, as the tested properties of the new formula did not differ from those of the original solutions.

For tissue dissolution, TCA 3.5% + 1/6 Buffer-1 displayed similar tissue-dissolving capacity to the original solutions ($P > 0.05$). Our study reveals that the percentage of tissue loss of all tested irrigants, including our formula and both CU and MU, was approximately 90%, whereas the control group, NSS, displayed much less tissue loss (8%, $P = 0.000$) Bovine pulpal tissue was selected because of its similarity to human pulpal tissue.^[12] Moore and Wesselink^[13] revealed that the amount of organic matter in relation to the amount of irrigant in the system affects the amount of tissue dissolution. Our study was standardized using a constant 20 ml of solution (both

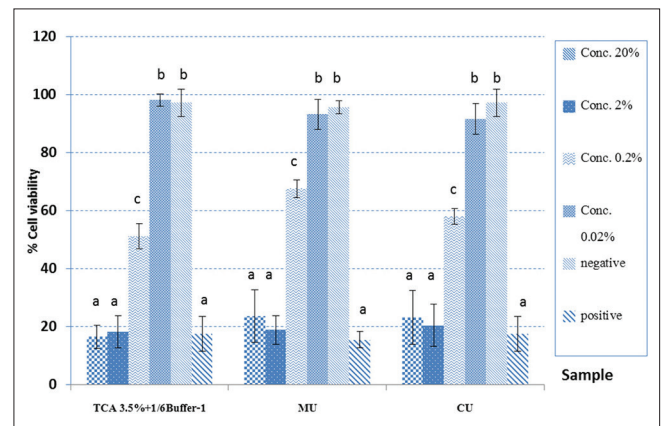


Figure 2: The percentage of viable cells after contact with the test solution at different concentrations (the same letter indicates $P > 0.05$)

test and control groups) in contact with 0.1 ± 0.005 g bovine pulpal tissue in every group.

Several methods exist to determine the capacity of tissue dissolution of different irrigants *in vitro*. One method visually measures the amount of time to completely dissolve the tissue sample. A major disadvantage of this method is the difficulty associated with determining the end-point of complete dissolution as a result of solution turbidity. Therefore, we used a different method by fixing the contact time to 20 min for tissue dissolution and then measuring the extent of dissolution for each solution. The percentage of tissue loss was calculated based on the weights before and after exposure to the test solutions.

In addition, the antibacterial effects of TCA 3.5% + 1/6 Buffer-1 through direct contact, which is a traditional method of measuring the antimicrobial effectiveness of endodontic irrigants with *E. faecalis*, demonstrated the same efficiency as commercial 2.5% NaOCl. All tested irrigants killed 100% of bacteria after 30 s of exposure. Although the direct contact test with planktonic bacteria does not simulate a clinical situation, these results have predictive value for ranking the effectiveness of the irrigation solutions. The biofilm model of bacterial growth may be considered in the future studies evaluating irrigation solutions. Nonetheless, our new formula and the original formulae could effectively eliminate *E. faecalis*, which is regularly found in endodontic cases.

The cytotoxicity evaluation of our new irrigant formula was performed according to ISO 7405:2008, dentistry evaluation of biocompatibility of medical device used in dentistry.^[10] Four different concentrations (20%, 2%, 0.2%, and 0.02%) were used for cytotoxicity testing. The 20%, 2%, and 0.2% concentrations for all tested irrigants resulted in high cytotoxicity after contact with the target cells. Approximately 20%–70% of cells remained viable after treatment at these concentrations. In contrast, the number of viable cells was >90% in response to 0.02% concentration of all irrigants; this survival percentage was not different from that of the negative control ($P = 1.000$) and was less cytotoxic than the positive control ($P = 0.001$). Ideally, the irrigant should not push out of the canal in clinical practice as pain and swelling could immediately occur due to extruding NaOCl solution from the apical foramen.^[14] The higher concentration of NaOCl solution is a more cytotoxic solution that affects the periapical tissue.

CONCLUSIONS

The results of this study report a new formulation of NaOCl solution that lacks a chlorinated odor but displays similar tissue dissolution, antibacterial, and cytotoxic effects to those of standard 2.5% NaOCl irrigants. Our formula represents another option for endodontic irrigation in clinical practice. The advantage of our formula is a lack of chlorine odor, which could be unpleasant to dental staff and patients.

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Conflicts of interest

There are no conflicts of interest.

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