

# Bactericidal and Cytotoxic Effects of Sodium Hypochlorite and Sodium Dichloroisocyanurate Solutions In Vitro

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**The antimicrobial and cytotoxic effects of sodium hypochlorite (NaOCl) and sodium dichloroisocyanurate (NaDCC) were evaluated and compared in vitro. The minimal inhibitory concentration and minimal bactericidal concentration of NaOCl and NaDCC were tested for *Streptococcus sobrinus*, *Streptococcus salivarius*, *Enterococcus faecalis*, and *Streptococcus mutans*. The cytotoxic effect was assessed by using human fibroblast tissue culture. Survival rate was assessed by a protein determination method. Results showed that the minimal inhibitory concentration and minimal bactericidal concentration values of NaOCl and NaDCC for the tested bacteria were in a similar range. NaDCC in concentrations higher than 0.02%, and NaOCl in concentrations higher than 0.01% were lethal to fibroblasts. In conclusion it seems that both agents were very effective in killing bacteria, and their cytotoxicity to fibroblasts in tissue culture was similar.**

Bacterial contamination of the root canal is of major concern in endodontics. Elimination of microorganisms and necrotic tissue from the root canal system is essential for successful outcome of treatment. Chlorine-releasing agents (CRA) are most commonly used for this purpose, particularly sodium hypochlorite (NaOCl) in concentrations ranging from 0.5% to 5.25% (1).

Another CRA is sodium dichloroisocyanurate (NaDCC) used generally for disinfection of contaminated surfaces and solutions. When dissolved, NaDCC is less prone to inactivation by serum than NaOCl (2).

Coates (2) reported that NaDCC solution containing 4,000 ppm available chlorine exhibited a similar bactericidal effect to NaOCl solution containing 17,000 ppm available chlorine. Additionally, when comparing commercial NaOCl and NaDCC products, it was found that solutions of NaOCl and NaDCC containing the same levels of available chlorine exhibited similar bactericidal action, despite significant differences in pH (3).

When antibacterial effects of unbuffered NaDCC (pH 6.6) and buffered NaOCl (pH 7.2 to 10.6) were compared in the presence of organic matter, it was found that NaDCC was significantly superior to NaOCl (4). Although NaOCl is a potent antimicrobial agent, it seems that NaDCC is as effective in the presence of organic tissue and may dissolve necrotic tissue more efficiently than NaOCl. Therefore, the aims of this study were: (i) to evaluate and compare the bactericidal effect of NaDCC and NaOCl on *Streptococcus sobrinus* (*S. sobrinus*), *Streptococcus salivarius* (*S. salivarius*), *Enterococcus faecalis* (*E. faecalis*), and *Streptococcus mutans* (*S. mutans*); and (ii) to assess the cytotoxicity of these agents in fibroblast tissue culture.

## MATERIALS AND METHODS

The study was conducted in two phases.

### Phase 1: Preparation of Antibacterial Agents

Fresh solutions of NaOCl and NaDCC were prepared. NaDCC tablets (Presept, Jonhson & Jonhson, Ascot, Berks, UK) were dissolved in Brain Heart Infusion Broth (BHIB) to a concentration of 2.5%, which was used as a fresh stock solution for the antibacterial assays. A fresh stock solution of 5% NaOCl was prepared and diluted in BHIB to the required concentrations for the antibacterial assay.

## ANTIBACTERIAL TESTS

The bacteria used for this study were *S. sobrinus*, *S. salivarius*, *E. faecalis* and *S. mutans* from the stocks of the Department of Oral Biology, Faculty of Dental Medicine in Jerusalem. The bacteria were cultured overnight at 37°C in air environment supplemented with 5% CO<sub>2</sub> in BHIB. After a microscopic inspection, sterile glycerol was added to a final concentration of 25%. The bacterial suspensions were dispensed into test tubes and stored at -70°C. This procedure ensured mid-log-stationary-phase cells in all of the experiments to follow.

The antibacterial effects of NaOCl and NaDCC solutions were examined separately. Minimal inhibitory concentration (MIC) of each agent was assessed using the broth dilution method, similar to the one described by Steinberg et al. (5). Briefly, bacterial suspen-

TABLE 1. MBC values (%) for the bacteria incubated in diluted solutions of NaDCC and NaOCl

	<i>S. sobrinus</i>	<i>S. mutans</i>	<i>E. faecalis</i>	<i>S. salivarius</i>
NaDCC	0.157	0.157	0.625	0.157
NaOCl	0.157	0.315	2.5	0.157

TABLE 2. MIC values (%) for the bacteria incubated in diluted solutions of NaDCC and NaOCl

	<i>S. sobrinus</i>	<i>S. mutans</i>	<i>E. faecalis</i>	<i>S. salivarius</i>
NaDCC	0.157	0.157	0.157	0.157
NaOCl	0.157	0.315	0.157	0.157

sions were supplemented with either NaOCl or NaDCC solutions in concentrations of 2.5%, 1.25%, 0.625%, 0.315%, 0.157%, 0.08%, 0.039%, and 0.0195%. After overnight incubation in air supplemented with 5% CO<sub>2</sub> at 37°C, bacterial growth was measured using a spectrophotometer (Compaspec, Cambridge, UK) at 540 nm. The MIC of NaOCl and NaDCC for the tested bacteria was determined as the end point where no bacterial growth was measured in comparison with the controls. Minimal bactericidal concentration (MBC) was determined by plating 0.1 ml from all incubated test tubes, where no visible bacterial growth was detected on brain heart agar plates. The agar plates were incubated for 24 hr at 37°C and air supplemented with 5% CO<sub>2</sub>. The MBC was determined as the lowest concentration of the tested agent at which a reduction of 99.9% in colony-forming units was detected. This was compared with controls, where no NaDCC or NaOCl was added. All experiments were conducted in triplicates.

To avoid false readings due to interactions between the growth media and active agents, control series of active agents in BHIB without bacteria were also included in this phase of the study.

### Phase 2: Cytotoxicity Tests

The cytotoxic effect of NaDCC and NaOCl was evaluated using human skin fibroblast tissue culture. The fibroblasts were maintained in Dulbecco's modified Eagle's medium supplemented with 10% fetal calf serum (both were obtained from Biological Industries (Kibbutz Beit Haemek, Israel)) and with glutamine, penicillin (100 units/ml), and streptomycin (100 mg/ml). NaDCC and NaOCl solutions were prepared by dilutions in distilled water to concentrations of 0.1%, 0.08%, 0.06%, 0.04%, 0.02%, and 0.01%. To the confluent fibroblasts grown in 6-well tissue culture dishes (2.7 ml medium + 0.3 ml cells), 5 ml of the antiseptic agents was added. The fibroblast tissue culture was then incubated at 37°C for 24 hr. At this time period, the fibroblasts were harvested with trypsin-EDTA and transferred into new tissue culture medium, incubated for 48 hr and harvested again using trypsin-EDTA solution. Fibroblasts were then washed three times with saline solution. The amount of fibroblasts growth in the presence of NaDCC or NaOCl was expressed by protein determination using the method described by Bradford (6).

## RESULTS

The results of the first phase are presented in Tables 1 and 2.

The MBC values of *S. sobrinus*, *S. mutans*, and *S. salivarius* were at a concentration of 0.157% NaDCC. The MBC values for *E. faecalis* were at 0.625% NaDCC. The MBC values for *S. sobrinus* and *S. salivarius* were at a 0.157% concentration of

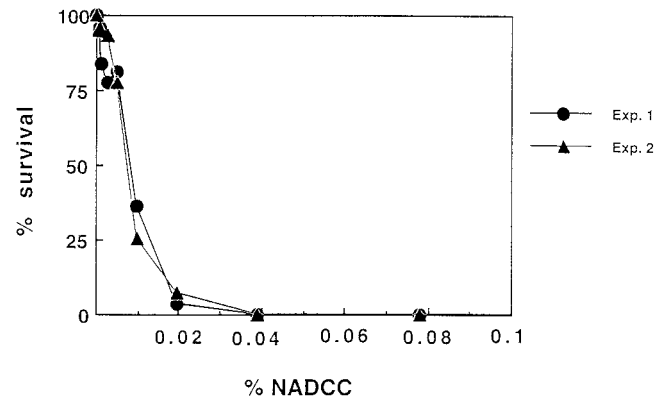


FIG 1. Survival rate of fibroblasts in the presence of various concentrations of NaDCC. Exp., experiment.

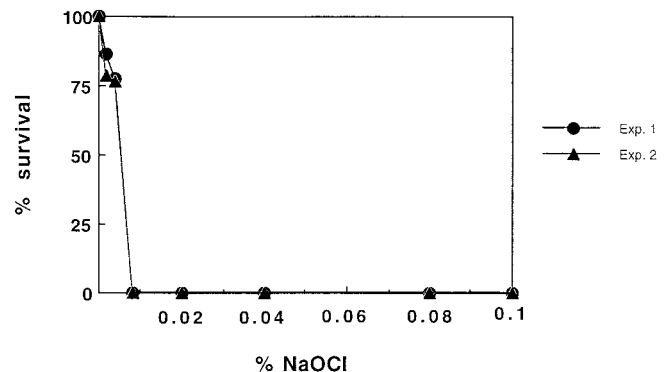


FIG 2. Survival rate of fibroblasts in the presence of various concentrations of NaOCl. Exp., experiment.

NaOCl and for *S. mutans* at a concentration of 0.315%. The highest concentration of 2.5% NaOCl was for *E. faecalis*.

The MIC values for *S. sobrinus*, *E. faecalis*, and *S. salivarius* were at a concentration of 0.157% for both disinfecting agents. The MIC for *S. mutans* incubated in NaOCl was at a higher concentration (0.135%) than for NaDCC (0.157%).

The results of the second phase are demonstrated in Figs. 1 and 2. Fibroblasts did not survive in the tissue culture after exposure to NaDCC solution in a concentration higher than 0.02% or in a NaOCl solution in concentrations higher than 0.01%.

## DISCUSSION

In the present study, the MIC and MBC of two CRAs were evaluated and compared against several oral bacteria. Generally,

the MIC and MBC values of NaOCl and NaDCC for the tested bacteria were found to be similar. The MIC and MBC of these agents against the tested bacteria were in the range of 0.157% to 0.315%. Only the MBC of NaOCl for *E. faecalis* was higher than that of the other bacteria. These results indicate that the bactericidal and bacteriostatic effects of these agents are similar. The bactericidal concentrations of NaOCl, found in this study, were lower than those reported by Shih et al. (7). The latter observed bacterial growth even in a 1:1,000 dilution and only a 5.25% solution was effective against *E. faecalis* and *Staphylococcus aureus* (7).

The antibacterial effect of NaOCl is time-dependent. Antibacterial effect was observed in the presence of 0.5% NaOCl only after 15 min (8). For shorter periods of time, the bactericidal effect of NaOCl was found to be lower (9).

Siqueira et al. (10), using the agar diffusion assay, found that the larger inhibition zone against black-pigmented Gram-negative anaerobes and four facultative anaerobic bacteria were demonstrated at concentrations of 2.5% to 4% NaOCl.

Using infected root canals in vitro, Vahdaty et al. (11) found that 0.5% and 2% NaOCl significantly reduced bacteria at a 100- $\mu$ m depth of the dentin tubules.

Heling et al. (1) found that 1% NaOCl was not sufficient to kill all bacteria in the tubules after 10 min. This concentration was higher than the MBC found in the present study. It therefore may be assumed that, inside the dentin tubules, extended duration or increased concentrations of NaOCl are required to achieve the full bactericidal effect.

Stability of the antiseptic agent is also of importance. Coates (3) demonstrated that tablets of NaDCC were stable for prolonged periods of time. However, the solutions were unstable and decomposed much faster than NaOCl of the same strength. This can be overcome by dissolving tablets and preparing small amounts of fresh solutions daily. Coates (3) also studied batch-to-batch variability of different NaOCl and NaDCC products. Whereas NaDCC products always presented minimal levels of available chlorine specified, concentrated NaOCl products sometimes did not, due to inherent instability. Therefore, for clinical use, the precise amount of available chlorine of NaOCl cannot be determined in commercial products with an unknown shelf period.

The antimicrobial potential of low concentrations of canal irrigating agents is an advantage when considering using fresh solu-

tions of NaDCC for endodontic therapy. Additionally, NaDCC was found to be more effective in killing *E. faecalis* than NaOCl—a bacteria known to be resistant to calcium hydroxide dressings (12). From the results of the second phase of our study, it seems that both agents were lethal to fibroblasts in tissue culture. NaOCl was toxic in a lower concentration than NaDCC.

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## You Might Be Interested

Differential diagnosis of pain is of interest to endodontists. A good review of current thinking on trigeminal neuralgia can be found in the *New England Journal of Medicine* (1996;334:1125).

Wallace Sturr