# Effects of Dentin Debris on the Antimicrobial Properties of Sodium Hypochlorite and Etidronic Acid

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## Abstract

**Introduction:** The purpose of this study was to determine the influence of dentin powder on the concentration, pH, and antimicrobial activity of sodium hypochlorite (NaOCl) alone and combined with etidronic acid (HEBP). **Methods:** Biofilms of *Enterococcus faecalis* were grown on the surface of dentin blocks for 5 days and then exposed to 1% and 2.5% NaOCl alone or combined with 9% HEBP for 3 minutes in the absence and presence of dentin powder. The biovolumes of the biofilm were measured using confocal microscopy and the live/dead technique. The available chlorine and pH of the solutions were also measured. Nonparametric tests were used to determine statistical differences ($P < .05$). **Results:** The presence of dentin powder resulted in a reduction of the free available chlorine and pH in all the irrigating solutions; 1% NaOCl lost its antimicrobial activity completely in the presence of dentin powder. The antimicrobial activity was significantly reduced in the 2.5% NaOCl and 1% NaOCl/HEBP groups, and it was not affected in the 2.5% NaOCl/HEBP group. **Conclusions:** The presence of dentin powder significantly decreased the available chlorine and antimicrobial activity of 1% NaOCl, 2.5% NaOCl, and 1% NaOCl/HEBP irrigating solutions. The antimicrobial activity of 2.5% NaOCl/HEBP was not affected by the dentin powder after a 3-minute contact time against *E. faecalis* biofilms. *(J Endod 2016;42:771–775)*

## Key Words

Antimicrobial activity, dentin debris, etidronic acid, root canal irrigants, sodium hypochlorite

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The main goal of endodontic treatment is to reduce microorganisms from the infected root canal system to levels compatible with healing (1, 2). Mechanical instrumentation can remove the bulk of infected tissue as well as facilitate the delivery of irrigants throughout the root canal system (3). Sodium hypochlorite (NaOCl) remains the most widely used solution because of its antimicrobial activity and capacity to dissolve organic tissue (4). The chemical efficacy depends on its free chlorine form, which is influenced by factors such as its concentration, exposure time, pH, temperature, and interaction with other organic or inorganic substances present in the root canal space (5–7). Once delivered inside the root canal, NaOCl reacts with the organic matter, causing depletion of the free available chlorine and resulting in protein degradation, rise of temperature, and changes in pH (5, 7).

The accumulation of hard tissue debris during the cleaning and shaping process is a well-accepted phenomenon (8). This tridimensional smear layer can represent 6% of the total volume of the mesial root mandibular molar after instrumentation (8), and only 50% of these particles are removed by strong chelating agents such as EDTA used with NaOCl or conventional positive apical pressure techniques (9–11). An alternative strategy includes the prevention of hard tissue debris accumulation during chemomechanical preparation. For this purpose, etidronic acid (HEBP), a weak alkaline chelating agent with a pH around 10.7, has been proposed for incorporation in the 1% and 2.5% NaOCl solution with no significant short-term loss of the desired properties of either compound (6). The combination removes the smear layer similarly to EDTA and reduces the accumulation of hard tissue debris (10, 12, 13) while maintaining the dissolution activity and antimicrobial properties of NaOCl (14–16). Consequently, the NaOCl/HEBP solution could be used as a single irrigant during and after instrumentation, replacing the final rinse with a chelating agent (6).

The accumulation of dentin debris may influence the biological activity of irrigant solutions. Although many articles have investigated the effect of irrigants on organic and inorganic tissues and their reduction of bacterial loads, this study looks into how dentin debris may interfere with the physical characteristics and antimicrobial activity of NaOCl solutions. Previous studies using planktonic cultures show that dentin debris has an inhibitory effect on the antibacterial properties of different root canal irrigants such as chlorhexidine, BioPure MTAD (Dentsply Tulsa Dental Specialties, Tulsa, OK), and NaOCl (17, 18). To our knowledge, no studies to date explore the effect of dentin debris on the antimicrobial properties of NaOCl alone and combined with HEBP against microbial biofilms. Therefore, the aim of this study was to determine the influence of dentin powder on the chlorine concentration, pH levels, and antibiofilm activity of different NaOCl solutions. The tested null hypothesis was that the antibiofilm activity of NaOCl alone and combined with HEBP is not affected by the dentin powder.

## Materials and Methods

The irrigating solutions and the concentrations evaluated were 1% and 2.5% NaOCl (Panreac Química, Castellar del Vallès, Spain), 9% (wt/vol) HEBP (Cublen K8514 GR; Zschimmer & Schwarz, Mohsdorf, Germany), and combinations of 1% and 2.5% NaOCl

with 9% HEBP. For the NaOCl/HEBP association, both irrigants were prepared at a double concentration in sterile distilled water and mixed in a 1:1 ratio.

The dentin powder and specimens were obtained from human noncarious teeth (ethics committee UGR-438). For the dentin powder, the outer cementum was eliminated by polishing with silicon carbide papers, and the pulp tissue was removed using K-files. To avoid any contaminants such as metal particles that could modify the pH and chlorine measurements, the radicular dentin was ground using an agate ball mill (Spex 8000M Mixer Mill; Spex Certiprep Industries Inc, Metuchen, NJ) for 10 minutes. The resultant dentin powder was filtered in order to obtain a particle size ≤190 μm, sterilized in a glass flask using an autoclave (121°C for 15 minutes), and stored at 4°C until use. To evaluate the effect of dentin on the biological properties of the solutions, 10 mg dentin powder was suspended in 100 μL of the irrigating solutions (wt/vol).

## Determination of the Concentration and pH in NaOCl Solutions

The concentration of the NaOCl solutions in the presence or absence of dentin powder was determined by measuring the free available chlorine using a standard iodine/thiosulfate titration method (19). The pH values of the solutions were recorded with a pH meter (micropH 2001; Crison, Alella, Spain). Measurements were performed in triplicate immediately after the solutions were prepared as well as after 1, 3, and 10 minutes. Between measurements, the solutions were stored in darkness at 4°C.

## Antimicrobial Activity Test

Sixty dentin blocks (2 × 2 × 1.2 mm) were prepared from 12 noncarious freshly extracted teeth (20). The smear layer was removed using 17% EDTA for 5 minutes. After sterilization, they were kept in sterile saline solution until use.

For *Enterococcus faecalis* biofilm formation, a previous methodology was used (16). The dentin blocks were fixed with fluid resin to the tips of modified pegs of the MBEC-high-throughput (HTP) device (Innovotech, Edmonton, AB, Canada). The trough was then inoculated with approximately $1 \times 10^7$ CFU/mL *E. faecalis* ATCC 29212 suspended in 22 mL brain-heart infusion medium (Scharlau Chemie, Barcelona, Spain) supplemented with 1.3% glucose. The device was placed on a rocking table (Swing Sw 8 10000-00015; OVAN, Badalona, Spain) and incubated at 37°C for 5 days at 5 rocks per minute. The brain-heart infusion broth was refreshed every 2 days, and the purity of the inoculum in the trough was evaluated by Gram staining and colony morphology in agar plates after 5 days of incubation.

The infected dentin blocks were detached from the pegs and rinsed with 0.9% saline solution for 2 minutes. The specimens were randomly divided into 12 groups ($n$ = 5) according to the irrigating solutions and the presence or absence of dentin powder in the solution: group 1, 1% NaOCl; group 2, 1% NaOCl/9% HEBP; group 3, 2.5% NaOCl; group 4: 2.5% NaOCl/9% HEBP; group 5: 9% HEBP; and group 6: distilled water (control). Groups 7 through 12 consisted of the same solutions listed in groups 1 through 6 mixed with dentin powder (10 mg/100 μL). The dentin blocks were submerged in 100 μL of the irrigating solutions for 3 minutes. Then, the NaOCl of the solutions was inactivated by adding 5% sodium thiosulfate for 5 minutes to enhance the quality of the staining process for NaOCl-treated dentin (21). After exposure, the biofilms were rinsed with saline solution, stained, and observed under confocal laser scanning microscopy. Five samples per study group were used in 2 independent experiments.

For disinfection analysis, the SYTO 9/propidium iodide technique (Live/Dead BacLight; Invitrogen, Eugene, OR) was used (21). SYTO 9 is a green fluorescent stain, labeling both live and dead microorganisms;

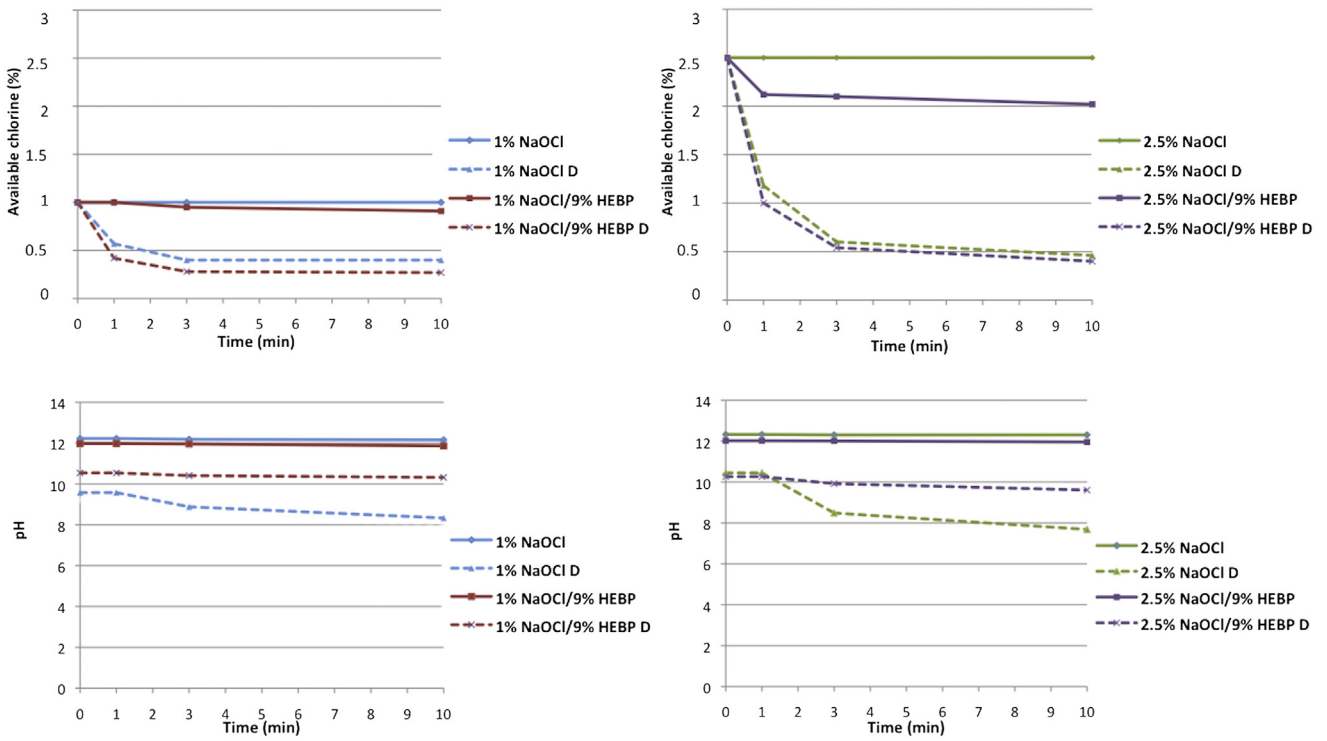


**Figure 1.** The mean values of the available chlorine and pH of the 1% and 2.5% NaOCl solutions alone and combined with 9% HEBP immediately after the solutions were prepared and after 1, 3, and 10 minutes. D, dentin powder.

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