Wetting Properties and Critical Micellar Concentration of Benzalkonium Chloride Mixed in Sodium Hypochlorite

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Abstract

Introduction: The purposes of the present study were to (1) assess the effect of the addition of benzalkonium chloride to sodium hypochlorite on its wetting properties, contact angle, and surface energy; (2) determine the critical micellar concentration of benzalkonium chloride in sodium hypochlorite; and (3) investigate the influence of addition of benzalkonium chloride on the free chlorine level, cytotoxicity, and antiseptic properties of the mixture. **Methods:** Solutions of benzalkonium chloride, with concentrations ranging from 0%–1%, were mixed in 2.4% sodium hypochlorite and tested as follows. The wetting properties were investigated by measuring the contact angle of the solutions on a nondehydrated dentin surface by using the static sessile drop method. The pending drop technique was subsequently used to determine the surface energy of the solutions. The critical micellar concentration of benzalkonium chloride mixed in sodium hypochlorite was calculated from the data. When 2.4% NaOCl was mixed with benzalkonium chloride at the critical micellar concentration, 3 parameters were tested: free chloride content, cytotoxicity, and antibacterial effects against *Enterococcus faecalis*. **Results:** The contact angle ($P < .001$) as well as the surface energy ($P < .001$) significantly decreased with increasing benzalkonium chloride concentrations. The critical micellar concentration of benzalkonium chloride in sodium hypochlorite was 0.008%. At this concentration, the addition of benzalkonium chloride had no effect on the free chlorine content, cytotoxicity, or antibacterial efficiency of the mixture. **Conclusions:** The addition of benzalkonium chloride to sodium hypochlorite at the critical micellar concentration reduced the contact angle by 51.2% and the surface energy by 53.4%, without affecting the free chloride content, cytotoxicity, or antibacterial properties of the mixture. *(J Endod 2012;38:1525–1529)*

Key Words

Antibacterial effect, benzalkonium chloride, contact angle, critical micellar concentration, irrigation, nondehydrated dentin surface, sodium hypochlorite, surface energy, surfactant, wetting properties

The success of endodontic treatment relies heavily on the efficiency of the chemomechanical cleaning of root canals (1). Endodontic instruments cannot mechanically act on the entire pulp canal anatomy (2). The canal can still contain pulpal tissues, bacteria, and hard tissue debris after mechanical cleaning (3). Consequently, an antiseptic and proteolytic irrigant is necessary to clean and disinfect these untouched areas. Although many studies have focused on fluid mechanics (4–6), there is still a gap of knowledge concerning the penetration depth and the spreading of irrigants in the root canal system.

To compensate for the lack of mechanical debridement in nonaccessible spaces, several techniques have been proposed to activate root canal irrigants and improve their efficiency, including passive ultrasonic techniques (7), sonic activation (8), laser and photodynamic activation (9), and master cone motion (8).

In addition to these techniques, the irrigant itself may be improved by enhancing its wetting properties (10). Surfactants diffuse in water and adsorb at interfaces between air and water, thus reducing the surface energy of water and increasing its wetting on a surface. Studies that examined the addition of surfactants to irrigants have reported encouraging results, observing a net improvement in irrigant penetration depth (11), a higher dentin permeability (12), improved cleaning and disinfection of canal walls (13, 14), and better pulp tissue dissolution (15–17).

The surface tension of any liquid decreases according to the surfactant concentration. The surfactant molecules move toward the liquid/air interface until the latter becomes saturated. This concentration of surfactant leading to saturation is called the critical micellar concentration (CMC). Above the CMC, the addition of surfactant alone contributes to the formation of micelles in the liquid, and the surface tension remains relatively constant; the best wetting properties are achieved at this concentration (18). To our knowledge, no study has established the CMC of surfactant in sodium hypochlorite or determined the corresponding wetting properties.

Benzalkonium chloride is the cationic detergent most commonly used in medicine. In ophthalmology, it is the most common preservative to avoid contamination of eye solutions (19). In dentistry, it is frequently used in dentin bonding agents (20), orthodontic resins (21), and in commercial ethylenediaminetetraacetic acid solution (Salvizol; Pierre Rolland, Merignac, France). It also may be used in recent commercial root canal irrigants whose composition remains undisclosed.

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Benzalkonium chloride may chemically react with sodium hypochlorite. This reaction may modify the free chlorine content and therefore alter the solvent ability of the irrigant. This action also may affect the antibacterial and cytotoxic properties of the mixture, leading to a loss of efficiency and future complications (22).

The purposes of the present study were as follows:

1. To assess the effects of the addition of benzalkonium chloride to sodium hypochlorite on wettability
2. To determine the CMC of benzalkonium chloride in sodium hypochlorite
3. To investigate the influence of the addition of benzalkonium chloride on the free chlorine level, cytotoxicity, and antibacterial activity of the mixture

Materials and Methods

Dentin Specimens

Human third molars without fractures or carious lesions were used after gaining informed consent from patients. The teeth were thoroughly cleaned and submerged horizontally in self-curing resin until half of the tooth was covered. The nonembedded areas were longitudinally polished under ultrapure water (Milli-Q; Millipore, Billerica, MA) with grit #80 to grit #180 carbon paper to obtain flat and wide dentin surfaces. To maintain their hydration, the teeth were stored in distilled water at 4°C until usage.

Irrigant Solutions

The investigated solutions were 9 different mixtures of 2.4% sodium hypochlorite (NaOCl) with benzalkonium chloride (BAK) (B1383 - semisolid; Sigma-Aldrich, St Louis, MO) at concentrations (C_{BAK}) ranging from 0%–1%. All solutions were freshly prepared before each experiment and homogenized by using a magnetic stirrer immediately before measurements were taken. A 2.4% NaOCl solution without BAK was used as the control (0%).

Contact Angle Measurement

Contact angles (θ_{BAK}) were measured by using the static sessile drop method. A homemade system was used, which consisted of 3 X, Y, and Z manipulators to move the dentin surfaces and a peristaltic pump to circulate the irrigant from a crystallizer to a needle. Each 2-μL drop was deposited slowly on the root dentin surface, and an image was captured with a high-resolution camera. Contact angles were determined by using a program written in LabVIEW (National Instruments, Austin, TX). Just before the measurements were taken, an absorbent paper was smoothly applied to each dentin surface to remove excess water without drying the specimen. The dentin was therefore assumed to be nondehydrated and slightly moist. Three to 6 drops could be deposited on each root dentin specimen, depending on the available surface. An average was taken and used as a single value for each specimen for further statistical analysis. Thirty teeth were used for each concentration, providing a powerful statistical analysis.

Surface Tension Measurement

The surface energy (g_{BAK}) of each solution was assessed with the pendant drop method. The same setup as previously described was used, and drops were formed continuously at a low flow rate of 18 μL/min. When a drop detached from the apex of the needle, the capillary force was overcome by the gravity force, given by $F_g = m \times g$, where m = the mass of the droplet and g = the acceleration due to gravity. Therefore, g_{BAK} could be determined at the moment of the drop detachment, according to Tate's equation: $g_{BAK} = mg/2 \times p \times r$, where r = the radius at the end of the nozzle. In the present study, the mass of 10 droplets was measured with a precision balance to calculate g_{BAK}. The experiment was carried out 3 times for each BAK concentration. The results of the first study allowed us to limit the number of surfactant concentrations to be tested to 4 values: 0.001%, 0.006%, 0.1%, and 1%, with 2.4% NaOCl used as a control.

Free Chlorine Level Assessment

Free chloride content of 2.4% NaOCl with or without BAK at the CMC was recorded by titration with sodium thiosulfate (23). Ten milliliters of the solution was added to 50 mL of water, 1 g potassium iodide, 12.5 mL of 5 mol/L acetic acid, and 1 mL of 1% starch solution (Sigma Chemical Co, St Louis, MO). The uncolored solution turned yellow, and the volume of 0.1 mol/L sodium thiosulfate necessary to obtain a transparent liquid was recorded. This permitted the calculation of the chlorine content of the solution. The experiment was carried out 10 times for each solution.

Cytotoxicity

The tests were performed according to ISO standards (24). A total of 929 fibroblasts were cultivated in minimum essential medium supplemented with 10% fetal bovine serum, 100 IU/mL penicillin, 100 μg/mL streptomycin, and 0.25 μg/mL amphotericin B (Bio-Science, Walkersville, MD) and were plated at 30,000 cells/cm^{-2} in 96-well plates (Falcon 3072; Becton Dickinson, Oxford, UK). The 96-well dishes were then placed into a humid incubator with an atmosphere of 5% CO_2 and 95% air before use. After 24 hours, the medium from the 96-well plates was removed and replaced by 100 μL of the test medium. The test medium was obtained by serial dilutions (v/v) of 2 root canal irrigants in culture medium, 2.4% NaOCl and 2.4% NaOCl + 0.008% BAK.

The 2 liquids were tested either undiluted or diluted to 1:10, 1:100, 1:1000, or 1:10,000. The medium was left in contact with the target cells for 10 minutes before performing the test. Each dilution was tested in a separate plate, because chloride is volatile and may evaporate, modifying the outcome of the study. A succinyl dehydrogenase assay (methyl-thiazol-diphenyltetrazolium) was performed on the 96-well plates after 10 minutes of incubation (ie, 24 hours + 10 minutes after the beginning of the experiment). The medium was removed and immediately replaced with 100 μL/well of 0.5 mg/mL 3-(4,5-dimethylthiazol-2-yl)-2,(-diphenyl tetrazolium bromide) dissolved in the medium (Sigma Chemical Company). After incubation for 2 hours at 37°C, the supernatants were discarded, and the formazan crystals were solubilized with dimethyl sulfoxide (100 μL/well) (Sigma Chemical Co). The absorbance of each 96-well dish was determined by using an automatic microplate spectrophotometer (E 960; Bioblock, Strasbourg, France) at 550 nm. The absorbances of the wells containing the same medium were compared with those of the control medium to determine the percentage of cell viability. The positive control was phenol (0.64 mg/mL), and the negative control was the medium itself. Experiments were performed in triplicate.

Antibacterial Effect against *Enterococcus faecalis*

Pure cultures of *Enterococcus faecalis* (CIP 103214; Institut Pasteur, Lyon, France) were prepared according to the Institut Pasteur instructions, and the turbidity was adjusted to 1 McFarland. The standard solutions were obtained by mixing the *E. faecalis* suspension with distilled water from 1:30–1:30,000 dilutions. The test solutions (2.4% NaOCl and 2.4% NaOCl + BAK at the CMC) were diluted with distilled water from 1:2.5–1:25,000 dilutions. Then, 200 μL of each solution was mixed with 200 μL of the *E. faecalis* suspension for 5 minutes. Subsequently, 200 μL of a sodium thiosulfate solution