Assessment of a gel-type chelating preparation containing 1-hydroxyethylidene-1, 1-bisphosphonate

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Abstract

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Aim To test an aqueous gel containing 1-hydroxyethylidene-1, 1-bisphosphonate (HEBP) regarding its interactions with sodium hypochlorite, its calcium binding capacity, and its potential in preventing the formation of a smear layer when used in conjunction with rotary root canal preparation.

Methodology The experimental aqueous gel consisted of (w/v) 2% alginate, 3% aerosil, 10% Tween 80 and 18% HEBP. Interactions of gel components with hypochlorite were assessed using iodometric titration and monochromatic ultraviolet spectrometry. Two commercial paste-type chelators containing ethylenediaminetetraacetic acid (EDTA) and peroxide (RC-Prep and Glyde) served as controls. Calcium-binding capacities were measured in mixtures with a Ca^{2+} standard solution buffered at pH 10 using a calcium-selective measuring chain. Finally, root canals of 16 extracted single-rooted premolars per group were instrumented using ProFile instruments dipped in the experimental gel, RC-Prep, or nothing. Additionally, canals were rinsed with 10 mL of a 1% NaOCl solution during/after preparation. Smear scores in instrumented teeth were monitored using scanning electron microscopy.

Results None of the experimental gel components showed short-term interactions with hypochlorite, whilst EDTA, peroxide, RC-Prep and Glyde immediately reduced the hypochlorite in solution. The experimental gel chelated 30 mg Ca^{2+} g^{-1}, compared with 16 mg Ca^{2+} g^{-1} and 11 mg Ca^{2+} g^{-1} chelated by RC-Prep and Glyde respectively. Smear scores obtained with the experimental gel were significantly ($P < 0.05$) lower than with RC-Prep in coronal and middle root thirds, whilst no differences were observed in apical root thirds.

Conclusions Under the conditions of this study, an HEBP gel appeared advantageous over currently available products.

Keywords: EDTA, Glyde, HEBP, NaOCl, phosphonate, RC-Prep.

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Introduction

Current therapies in root canal treatment rely on mechanical debridement of the root canal system. However, mechanical instrumentation alone cannot remove all viable micro-organisms from the infected root canal system (Byström & Sundqvist 1981), nor does it remove all organic tissue remnants from root canal walls (Gutierrez *et al.* 1990), which may serve as a source of nutrition for surviving bacteria (Love 2001). It was thus recognized that chemicals have to be employed in conjunction with instrumentation for optimal results (Walker 1936). Aqueous sodium hypochlorite (NaOCl) solutions, used as irrigants during root

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canal treatment, are the most frequently used agents in endodontics (Barbakow *et al.* 1995). Hypochlorite preparations are highly efficient antiseptics, and have a unique capacity to dissolve soft tissue remnants (Naenni *et al.* 2004).

Instrumentation of the root canal system produces a smear layer, which is composed of inorganic and organic material such as dentine filings and pulp tissue remnants (Gwinnett 1984). Removing this layer is advocated for optimal treatment results (Torabinejad *et al.* 2002). Sodium hypochlorite is unable to dissolve inorganic smear layer components (for review, see Hülsmann *et al.* 2003). Calcium complexing (chelating) agents are thus recommended as additional agents in root canal treatment, either in liquid form or in paste-type preparations (Hülsmann *et al.* 2003). Such paste-type products are advocated by instrument manufacturers to be used in conjunction with root canal instrumentation and sodium hypochlorite irrigation. The origin of these agents dates back to 1961, when Stewart devised a combination of urea peroxide with glycerol, which he termed Gly-Oxide (Stewart 1961). Later, based on the results of that first preliminary study and the successful introduction of ethylenediaminetetraacetic acid (EDTA) to endodontic practice (Nygaard Östby 1957), urea peroxide and EDTA were combined in a water-soluble carbowax (polyethylene glycol) vehicle (Stewart *et al.* 1969). This product has since been commercially available under the name of RC-Prep (Premier Dental, Philadelphia, PA, USA). Similar paste-type chelators containing EDTA and peroxide have later been marketed by other manufacturers (Hülsmann *et al.* 2003).

In the search for optimal strategies for root canal treatment, the basic chemical actions and interactions of the substances to be used have largely been neglected. For instance, the unique necrotic tissue-dissolving capacity and the antimicrobial action of sodium hypochlorite are functions of available chlorine in solution (Zehnder *et al.* 2002). The available chlorine in unbuffered NaOCl solutions (pH 12) is almost entirely in the form of hypochlorite (OCl^-), which is an extremely reactive agent that readily interacts with any compound that can be oxidized. EDTA and citric acid in aqueous solution immediately interfere with the soft tissue dissolving and antibacterial capacity of a hypochlorite solution (Grawehr *et al.* 2003, Zehnder *et al.* 2005). At the same time, the incorporation of peroxide into chelator pastes to be used in conjunction with hypochlorite also appears questionable, as hypochlorite is a stronger oxidizing agent than peroxide, and thus may be reduced by the latter agent and loose its efficacy (Harada 2002).

Recent studies identified 1-hydroxyethylidene-1, 1-bisphosphonate (HEBP) as a possible alternative to citric acid or EDTA, in that it showed minimal short-term interaction with sodium hypochlorite and appeared to have adequate calcium-chelating capacity (Zehnder *et al.* 2005). Bisphosphonates are highly biocompatible chelators systemically administered in patients suffering from osteoporosis or neoplastic diseases involving osteolytic bone destruction (Russell & Rogers 1999). It was the goal of this pilot study to devise a simple aqueous gel containing HEBP as an active ingredient, which could be used in combination with a sodium hypochlorite irrigant, without losing the action of hypochlorite.

We report the preparation of an experimental HEBP gel, its short-term impact on available chlorine in NaOCl solutions, and its calcium-complexing capacity in comparison with paste-type chelators. Finally, the extent to which the experimental gel could prevent the formation of a smear layer on root canal walls during rotary Ni-Ti preparation was monitored.

Materials and methods

Gel preparation

Based on previous results, HEBP was selected as the active gel ingredient. Sodium salt of HEBP (Na_4HEBP) was obtained from a commercial source (Cublen K 8514 P; Zschimmer & Schwarz, Burgstädt, Germany). Aqueous solutions of this salt have a pH in the range of 11–12. Consequently, a gelating agent had to be found, which was able to form a consistent gel at this pH level. There are three main groups of biologically compatible gelating agents used in food and cosmetic industries: sodium carboxymethyl cellulose, alginates and acrylic acid. Such gelating agents from different manufacturers were tested for their compatibility with HEBP. Test gels were prepared, containing different compositions of a gelating, a thixotropic (Aerosil 200; Degussa-Hüls, Bitterfeld, Germany), and a polysorbate wetting agent (Tween 80; Kantonsapotheke, Zürich, Switzerland), plus HEBP. Physical appearance of these gels, their adhering capacity to rotary instruments, their behaviour upon agitation, their dissolution rate in water and their shelf life were monitored. Based on these preliminary findings, an aqueous gel consisting of (w/v) 2% alginate (D2998; Red Carnation Gums Ltd, Basildon, UK), 3% aerosil, 10% Tween 80 and 18% HEBP was selected for further studies.

Commercial paste-type chelators

RC-Prep (Premier Dental) and Glyde (DeTrey Dentsply, Konstanz, Germany) were used as control paste-type chelators. The term 'paste' is used for these products because their basis is a carbowax (polyethylene glycol) vehicle (Stewart *et al.* 1969). According to the developers of RC-Prep, the carbowax protects EDTA from being oxidized by the peroxide compound. A w/w content of 15% EDTA and 10% urea peroxide is reported for both products (Hülsmann *et al.* 2003).

Interactions with sodium hypochlorite

Interactions of 2% alginate, aerosil, Tween 80 and 18% HEBP with sodium hypochlorite were studied separately by adding 100 mg of respective substances to 1 mL of a 1% NaOCl solution. Available chlorine in mixtures, which were vigorously vortexed, was determined after 5 min and 1 h using iodometric titration (Vogel 1962).

Because the commercial pastes under investigation contain peroxide (H_2O_2), interactions of these with hypochlorite could not be analysed using a simple iodine-thiosulphate titration assay. The latter method cannot discern between oxidizing agents such as H_2O_2 and NaOCl, and consequently, a spectroscopic method was applied (Harada 2002). Absorption patterns of the pure 1% NaOCl, 10% H_2O_2 and 15% EDTA solutions were collected using a monochromatic ultraviolet (UV) spectrometer (Ocean Optics, Orlando, FL, USA) as follows: 200 μL of each solution was diluted with a pure 0.131 mol L^{-1} NaOH solution to 25 mL. The diluting solution had the same alkaline buffer capacity as the NaOCl solution under investigation, so that reactions after diluting took place in a pH environment comparable to the one in 1% NaOCl. The spectrum was read 1 min after transferring 1 mL of diluted solution to a quartz cuvette of 1 cm light path. Interactions of pure solutions and the pastes under investigation with NaOCl were tested accordingly: 200 μL of 1% NaOCl, 10% H_2O_2, 15% EDTA or 200 mg of the experimental gel, RC-Prep, or Glyde was suspended in 2 mL of 1% NaOCl in a test tube, which was then shaken by hand for 5 min. Subsequently, this mixture was diluted and spectrometrically measured as described. Spectrometry raw data were transferred to a personal computer and analysed using a Mathematica (Wolfram Research Inc., Champaign, IL, USA) script, which was specifically written for that purpose. This script allowed subtracting spectra obtained with pure aqueous solutions or gels/pastes from spectra of their corresponding mixtures with NaOCl. Calculations were possible based on the Beer–Lambert law, which describes the linear relationship between absorbance and concentration of an absorbing species. In the current study, absorbance measurements were made at dilutions to which the Beer–Lambert law is applicable, i.e. at absorbance values below 0.4. The linear relationship of absorbance and concentrations was validated using dilution series of pure substances prior to measuring the interactions of the pastes under investigation with NaOCl.

Calcium chelating capacity

The capacity of pure 15% EDTA and 18% HEBP in aqueous solution, the experimental HEBP gel, RC-Prep and Glyde to chelate calcium ions in solution was monitored as follows: first, calcium standard solutions ($CaCO_3$ dissolved in water, buffered at pH 10 with KCl and ammonium buffer) from 50 p.p.m. to 200 p.p.m. were measured using a calcium-selective measuring chain (Orion Research, Inc., Beverly, MA, USA). On a semi-logarithmic scale, there was an ideal linear relationship between mV measurements and $\log_{10}$ p.p.m. Ca^{2+} in solution in the measuring range ($R^2 = 0.99$). A volume of 600 μL of pure 15% EDTA, 18% HEBP, or 0.6 g of the experimental HEBP gel, RC-Prep or Glyde was added to 200 mL of the 200 p.p.m. Ca^{2+} standard solution, and mV measurements were taken after 10 min of constant stirring. An equilibrium had been reached until that point in time, and the amount of chelated calcium could be computed. Experiments were performed in triplicate; mean values are reported.

Prevention of smear layer during root canal preparation

Forty-eight single-rooted human premolars containing a single root canal were selected from the department's collection of extracted teeth as described (Haenni *et al.* 2003). They were randomly assigned to three groups containing 16 teeth each. Root canals were accessed and instrumented using ProFile .04 instruments as described (Haenni *et al.* 2003), so that a size 45 instrument reached the apical stop. Each instrument was dipped in the experimental gel (test group) or RC-Prep (control group) prior to use, so that one-third of the cutting area was covered. The 16 remaining teeth (positive smear controls) were instrumented without the use of a chelating agent. In every treatment group, canals were rinsed with a total of 10 mL of a 1% NaOCl solution between instruments using an ISO-size 35 irrigating needle 1 mm short of working length (Hawe Neos, Bioggio, Switzerland).

After experimental irrigation, teeth were copiously rinsed with deionized water to avoid any further chemical irrigant actions. Longitudinal grooves, which did not penetrate into the canal, were placed in the facial and lingual surfaces of the roots to facilitate their fracture. Roots were fractured along these grooves by dipping them in water and then in liquid nitrogen. After critical point drying, one fractured half of each root was mounted on a stub and gold-sputtered (SCD 030; Balzers Union, Balzers, Liechtenstein). Prepared root canal surfaces were observed in a scanning electron microscope (AMRAY 1810, Bedford, MA, USA), and five photomicrographs were taken at ×500 magification from typical areas of the coronal, the middle, and the apical thirds of the prepared root canal. On each of these photomicrographs, the presence or absence of smear layer was semiquantitatively estimated by two calibrated, blinded observers as follows: '0': 90–100% of dentinal tubules visible; '1', 50–90%; '2', 10–50%; '3', 1–10%; and '4', 0% of dentinal tubules visible. A pilot study showed that, in view of the variability of inner root surface anatomy, this relatively simple scoring system gave reproducible results. In case the observers did not agree, the higher of their scores was assigned. Median scores per tooth were used for further calculations and comparisons between different irrigation regimes. These were performed using Kruskal–Wallis analysis of variance followed by Mann–Whitney *U*-test for individual comparisons. The level for the alpha-type error was set at 0.05.

Results

Interactions with sodium hypochlorite

As assessed using iodometric titration, none of the components of the experimental gel interfered with free available chlorine in hypochlorite solutions, except for HEBP, which caused a slight dose-dependent decrease in available chlorine after 1 h. In 1 : 10 (w/v) mixtures of the experimental 18% HEBP gel with a 1% NaOCl solution, 90% of the initially available chlorine was measured after 1 h. In contrast, in 1 : 10 mixtures, both RC-Prep and Glyde completely rid a 1% NaOCl solution of detectable hypochlorite after 5 min (Fig. 1). In UV light, pure hypochlorite (OCl^-) showed peak absorbance at 292 nm, which was clearly discernible from the absorption patterns of hydrogen peroxide and

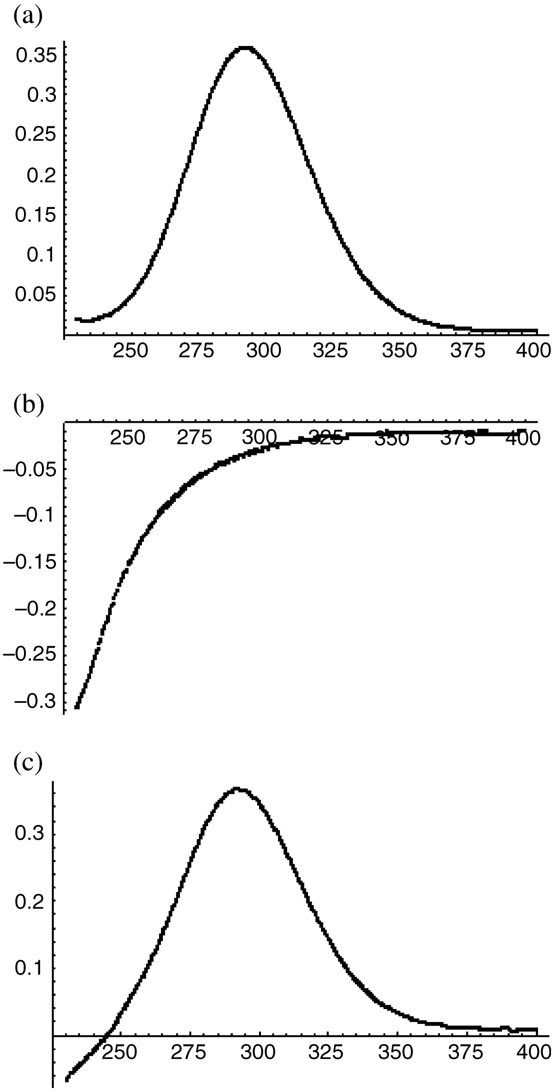

Figure 1 Absorption pattern (abscissa: wavelength in nm, ordinate: absorbance) of 1% NaOCl, showing the distinct hypochlorite (OCl^-) peak at 292 nm (a). Panel (b) depicts a difference spectrum of a 5-min 1 : 10 Glyde/NaOCl mixture from which the pure Glyde spectrum was subtracted. The resulting negative difference spectrum is related to the compounds that have vanished due to the reactions with NaOCl. There is obviously no trace of OCl^- in the mixture. In contrast, 100% of OCl^- was found in a 5-min 1 : 10 mixture of the experimental gel with 1% NaOCl, as can be seen in the corresponding subtraction spectrum on panel (c).

EDTA in aqueous solution with absorbance maxima below 250 nm. As observed in difference spectra, both EDTA and peroxide immediately reacted with the hypochlorite (data not shown).

Calcium chelating capacity

A pure 15% EDTA solution chelated 16 mg Ca^{2+} g^{-1} from the calcium standard solution. RC-Prep with a declared content of 15% EDTA also chelated 16 mg Ca^{2+} g^{-1}, whilst 1 g of Glyde only consumed 11 mg Ca^{2+}. An 18% solution of HEBP consumed 32 mg Ca^{2+} g^{-1}, compared with 30 mg Ca^{2+} g^{-1} chelated by the experimental 18% HEBP gel. To gain some preliminary information on the stability of the experimental gel, it was stored for 6 months in an air-tight container at 5 °C. Subsequently, its calcium complexing ability was reassessed and found to be identical to the one of a freshly prepared batch.

Prevention of smear layer during root canal preparation

Two root specimens, one in the RC-Prep and one in the positive control group, were lost because they could not be fractured in a manner so that the root canal space could be adequately monitored. Instrumented root canal walls irrigated with 1% NaOCl without the use of a chelator (positive controls) did not show any open tubules (Fig. 2). Counterparts that were prepared using RC-Prep-covered instruments showed some reduction in smear scores on few areas in coronal and middle root aspects. However, median smear scores in all three root portions were still '4', and smear values did not significantly differ between RC-Prep specimens and positive control roots. In contrast, the experimental HEBP gel significantly ($P < 0.05$) reduced smear scores in coronal and middle root aspects compared with positive control and RC-Prep. However, apical root areas were still mostly covered with a smear layer, and no significant differences between the three treatment groups were found.

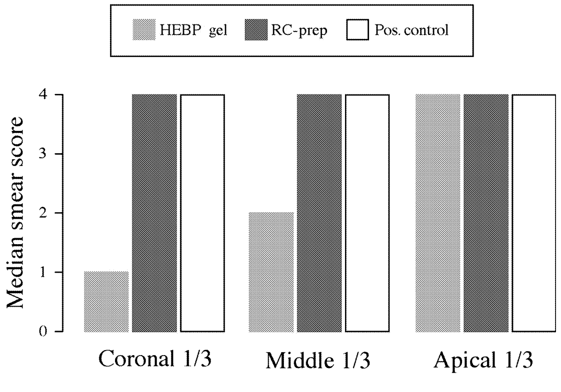

Figure 2 Median smear scores on coronal, middle and apical root canal walls of extracted teeth instrumented with ProFile .04 instruments ($n = 15/15/16$ per group/area, medians of median values obtained from five photomicrographs per area per tooth). Root canal walls were scored on SEM photomicrographs at 500× magnification by two calibrated observers blinded to the treatment regimen.

Discussion

The current study showed that, from a chemical perspective, the use of paste-type chelator preparations containing EDTA and peroxide is highly questionable if sodium hypochlorite is used as an irrigant. *In vitro* methods were employed to assess chemical actions and interactions of the substances under investigation. The experimental HEBP gel was then tested in an *ex vivo* environment in extracted human teeth for its ability to inhibit the formation of a smear layer during instrumentation. These results do not necessarily allow any definite conclusions on the actions of the tested substances *in situ*. Blood, tissue remnants and a multitude of other variables may affect the actions of the agents under investigation in the root canal system. Nevertheless, multiple variables of potential clinical significance were isolated and assessed in these experiments.

Relatively small amounts of RC-Prep or Glyde completely eliminated all the available chlorine in a 1% NaOCl solution. Mixtures of these pastes with 1% NaOCl down to a w/v ratio of 1 : 10 were free of OCl^{-} after 5 min. Clinically, higher paste-to-sodium hypochlorite ratios are expected. For instance, a palatal root canal of a maxillary molar has a volume of 3–10 mm^3 during/after instrumentation (Peters *et al.* 2003). This means that approximately 3–10 mg of a hypochlorite solution will fill such a canal. As determined using a precision balance, a ProFile instrument can hold at least 5 mg of RC-Prep or Glyde in its radial lands alone, without any excess material covering the instrument. When used in conjunction with rotary root canal preparation, paste-type chelators are stirred with the hypochlorite solution used between instruments, and an immediate interaction between chemicals is to be expected. Furthermore, paste remnants are likely to continue hampering the hypochlorite action in canals.

The rationale for the combined use of peroxide and hypochlorite in root canal treatment can be viewed as a classic example of how readers can be influenced by thoughts in the discussion section of a scientific correspondence without providing any evidence to support these statements. Grossman (1943) correctly observed that oxygen was liberated when H_2O_2 and NaOCl solutions were mixed together. This is supported by recent observations on hypochlorite–peroxide interactions in a high-pH environment (Harada 2002):

$$OCl^- + H_2O_2 \rightarrow O_2 + H_2O + Cl^-$$

In the Discussion section of his correspondence, Grossman (1943) speculated that (nascent) oxygen would be helpful in killing anaerobes, and the 'bubbling effect' would help to float debris from the root canal system (Grossman 1943). Later on, this thought was taken up by the developers of RC-Prep (Stewart *et al.* 1969), and the bubbling effect is still used to advertise for paste-type chelator products. However, Cl^- is the inert form of chlorine in solution, and peroxide–hypochlorite mixtures will thus not exert any of the desired hypochlorite actions such as killing of microbiota and dissolution of necrotic tissue. A greater effect was shown when H_2O_2 and NaOCl solutions were used alternately than when mixed together on dissolution of necrotic tissue (The 1979). A recent study showed that by reducing the hypochlorite in aqueous solution, EDTA and citric acid both hamper the antimicrobial effectiveness of NaOCl solutions (Zehnder *et al.* 2005). It may be argued that paste-type chelator products that do not contain peroxide, such as File-EZE (Ultradent Products, South Jordan, UT, USA) and Calcinase slide (lege artis, Dettenhausen, Germany), were not tested in the present study. However, these contain more than enough EDTA to arrest the hypochlorite action in the root canal.

It must be reiterated that chemical agents in endodontics should be used wisely, i.e. they should have additive effects rather than interfering with each other. If EDTA is used to remove the smear layer, it would probably be better to administer it as an aqueous solution after the cleaning and shaping procedure with mechanical instruments and sodium hypochlorite has been completed (Yamada *et al.* 1983).

Whilst the HEBP gel presented in the current communication was compatible with hypochlorite and showed better calcium binding and smear-preventing capacity than currently available products, it was not able to significantly inhibit the formation of a smear layer on apical root canal walls. Studies by research groups who did not have a commercial interest in advocating any product consistently showed the difficulty in achieving a smear-free apical root canal portion, especially when paste-type chelators were used in combination with rotary root canal instrumentation (Hülsmann *et al.* 2003). Rotary instruments may transport the chelator product away from the root apex, so that the calcium complexing agent does not have enough working time in that area. It may therefore still be advisable to additionally use a chelating agent in liquid form administered through a slim irrigating needle that can reach the apical preparation area after the shaping procedure has been completed.

Future studies should aim at the question whether a gel- or paste-type chelator product is helpful from a mechanical point of view, i.e. whether torque values on rotary instruments can be reduced. If not, HEBP powder could also be mixed immediately prior to use with an aqueous sodium hypochlorite solution to obtain an all-in-one irrigant/lubricant.

Conclusions

1. The negative effect of EDTA and H_2O_2 on hypochlorite in solution was confirmed in this study, whilst HEBP showed little short-term interference with OCl^-.
2. The HEBP gel under investigation had superior hypochlorite compatibility, calcium chelating capacity, and smear layer preventing ability compared with currently marketed paste-type chelator products containing EDTA and hydrogen peroxide.
3. Under the conditions of this study, the HEBP gel was not able to significantly inhibit the formation of a smear layer in apical portions of narrow root canals.

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