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Ex vivo antimicrobial activity of several bleaching agents used during the walking bleach technique

D. P. Oliveira[1,2,3,4], B. P. F. A. Gomes[1], A. A. Zaia[1], F. J. Souza-Filho[1] & C. C. R. Ferraz[1]

[1]Department of Restorative Dentistry, Piracicaba Dental School, State University of Campinas, Piracicaba, SP, Brazil; [2]University of North Carolina at Chapel Hill, Chapel Hill, NC, USA; [3]Center of Superior Studies of Maceió (CESMAC), Maceió, AL, Brazil; and [4]Federal University of Alagoas, Maceió, AL, Brazil

Abstract

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Aim To investigate *ex vivo* the antimicrobial activity of a paste of sodium perborate associated with various vehicles comparing it with 37% carbamide peroxide and 35% hydrogen peroxide.

Methodology The antimicrobial activity of these agents was evaluated against three microorganisms: *Enterococcus faecalis*, *Streptococcus mutans* and *Candida albicans*. One millilitre of each tested substance was placed on the bottom of wells of 24-well cell culture plates. Six wells were used for each time period and group. Two millilitres of the microbial suspension was ultrasonically mixed for 10 s with the bleaching pastes and placed in contact with them for 10, 30, 45 s; 1, 3, 5, 10, 20, 30 min; and 1 and 2 h. After each period of time, 1 mL from each well was transferred to tubes containing 2 mL of freshly prepared brain heart infusion agar + neutralizers. Agar plates were inoculated in appropriate gaseous conditions. Data were analysed statistically by the Kruskal–Wallis test with the level of significance set at $P < 0.05$.

Results In all groups containing chlorhexidine (groups 3, 5 and 7), the antimicrobial activity of the bleaching paste was significantly increased when compared with groups with other kinds of vehicle (groups 1, 2, 4, 6 and 8). For all tested groups, the most resistant microorganism was *E. faecalis.*

Conclusions Chlorhexidine when used as a vehicle for sodium perborate enhanced its antimicrobial activity.

Keywords: antimicrobial activity, bleaching agents, 2% chlorhexidine.

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Introduction

Bleaching is a conservative aesthetic treatment for root filled discoloured teeth, as it offers advantages over full-coverage restorations; it is simple, effective and is of a relatively low cost (Haywood 1992, Rotstein *et al.* 1992, Baratieri *et al.* 1995).

Several techniques for bleaching root filled teeth have been proposed. Spasser (1961) was the first to use a paste of sodium perborate (SP) and water sealed into the crown. Nutting & Poe (1967) used SP with superoxol and suggested the term 'walking bleach' be used to refer to the technique.

Sodium perborate as an oxidizer decomposes into sodium metaborate and hydrogen peroxides, releasing nascent oxygen (Ari & Ungor 2002). The gas released may increase the pressure inside the pulp chamber increasing the potential to loosen or displace the temporary restoration, with the possibility of contamination within the root canal system through saliva and bacteria (Naoum & Chandler 2002).

During the walking bleach technique, the antimicrobial activity of the bleaching agent and/or its vehicle complement the coronal seal (Walton & Rotstein 1996). Recently, a gel base containing 2% chlorhexidine (CHX) was introduced as a vehicle for SP in place of water or hydrogen peroxide (Oliveira *et al.* 2006).

Correspondence: Daniel Pinto de Oliveira, Department of Restorative Dentistry, University of Campinas, Av. Limeira, 901, Piracicaba, SP, CEP:13414-018, Brazil
(e-mail: danifop@hotmail.com).

Due to its antibacterial activity and substantivity, CHX has potential to increase the antimicrobial properties of the bleaching agents and act as an adjunct to destroy bacteria that may reach the root canal system through a defective coronal seal during the walking bleach technique (Rolla & Melsen 1975, Lenet *et al.* 2000, Gomes *et al.* 2003, Oliveira *et al.* 2006).

The aim of this study was to compare the antimicrobial activity of several bleaching agents mixed with or without a gel base containing 2% CHX.

Material and methods

The bleaching agents and controls tested are shown in Table 1. All microorganisms tested: (i) *Enterococcus faecalis* ATCC 29212, (ii) *Candida albicans* NTCC 3736 and (iii) *Streptococcus aureus* ATCC 25923 were subcultured onto appropriate culture media under gaseous conditions for 48 h on 5% sheep blood–brain heart infusion (BHI) agar plates (Lab M, Bury, UK) at 37 °C. Pure cultures of these microorganisms were suspended in sterile 0.85% NaCl solution. The cell suspension was adjusted spectrophotometrically to match the turbidity of a McFarland 0.5 scale (1.5×10^8 CFU mL^{-1}).

One millilitre of each tested substance was placed at the bottom of each well of 24-well cell culture plates (Corning, New York, NY, USA, ref. No. 3524, well Vol. 3.2 mL), including the control groups (CHX, natrosol and water). Six wells were used for each time period, microorganism and agent tested. Overall, 3366 wells were used, comprising 2448 for all the test groups and 918 for the control groups. Two millilitres of the microbial suspension was placed into the well and ultrasonically mixed for 10 s with the agents. The combinations of tested substance + microbial suspension were left together for 15, 30 s; 1, 3, 5, 10, 15 and 30 min; 1, 2, 4, 6, 8, 12, 24 and 48 h; and 7 days. After each period of time, 1 mL from each well was transferred to tubes containing 3 mL of freshly prepared broth medium to which the neutralizer was added in order to avoid a residual action. The neutralizer for groups with SP or carbamide peroxide was 0.6% sodium thiosulphate, whilst 0.5% Tween 80 + 0.07% lecithin was used for CHX (Gomes *et al.* 2001). All tubes were left at 37 °C for 7 days in appropriate gaseous conditions. Agar plates were inoculated with 10 μL from each tube and the plates were incubated at 24–48 h in appropriate gaseous conditions. The purity of the positive cultures was confirmed by Gram staining, by colony morphology on blood agar plates and by the use of biochemical identification kits (API 20 Strep, API C AUX, API 20 Staph, BioMérieux SA, Marcy-l'Etoile, France; Rapid ANA II System, Remel Inc., Lenexa, KS, USA). MINIAPI software (BioMérieux SA) was used to automatically read ID 32 tests and visually read API range tests from BioMérieux.

Table 1 Bleaching agents and control groups evaluated

Groups	Bleaching agents
1	Sodium perborate (Proderma, Piracicaba, Brazil) + water (2 g : 1 mL)
2	Sodium perborate + hydrogen peroxide (H_2O_2) 30% (Proderma) (2 g : 1 mL)
3	Sodium perborate + 2% chlorhexidine gel (Proderma) (2 g : 1 mL)
4	Sodium perborate + natrosol gel (Proderma) (2 g : 1 mL)
5	Sodium perborate + hydrogen peroxide (H_2O_2) 30% + chlorhexidine gel (4 g : 1 mL : 1 mL)
6	Carbamide peroxide gel 37% (Super Endo; Whiteness, Porto Alegre, Brazil)
7	Carbamide peroxide gel 37% + chlorhexidine gel 2% (2 : 1)
8	Hydrogen peroxide (H_2O_2) 30%
Control 1	Chlorhexidine gel 2%
Control 2	Natrosol gel
Control 3	Water

The time required for each group to produce total microbial growth inhibition was recorded, transformed into seconds and analysed statistically using the Kruskal–Wallis test, with significance level set at $P < 0.05$.

Results

The adherence and normality of samples were tested using the GMC program (USP, Ribeirão Preto, SP, Brazil), demonstrating that the data were nonparametric. The samples were then compared using the Kruskal–Wallis test (BIOESTAT program; CNpQ, 2000, Brasília, DF, Brazil), with significance levels at $P < 0.05$. Table 2 shows the contact time required by the bleaching agents and control groups to produce negative cultures for the microorganisms. All microorganisms in the control group 1 produced negative cultures in 1 min or less. Control groups 2 and 3 yielded positive cultures during the maximum time tested (7 days). In all groups containing CHX as the vehicle (groups 3, 5 and 7), the antimicrobial activity of the bleaching paste was significantly increased when compared with groups with other vehicles (groups 1, 2, 4, 6 and 8). For all tested groups, the most resistant microorganism was *E. faecalis.*

Table 2 Contact time required for bleaching agents and control groups to produce negative cultures (i.e. 100% inhibition of growth) for the tested microorganisms

Microorganisms	*Streptococcus mutans*	*Candida albicans*	*Enterococcus faecalis*
1: SP + water	10 min (c, B)	5 min (b, A)	20 min (b, C)
2: SP + 30% HP	5 min (b, A)	3 min (b, A)	20 min (b, B)
3: SP + 2% CHX	3 min (a, AB)	1 min (a, A)	5 min (a, B)
4: SP + NA	10 min (c, B)	5 min (b, A)	20 min (b, C)
5: SP + 30% HP + 2%CHX	3 min (a, AB)	1 min (a, A)	5 min (a, B)
6: 37% CP	15 min (d, B)	10 min (c, A)	30 min (c, C)
7: 37% CP + 2%CHX	3 min (a, AB)	1 min (a, A)	5 min (a, B)
8: 30% HP	5 min (b, A)	3 min (a, A)	20 min (b, B)
Control 1: 2% CHX	30 s (a, A)	15 s (a, A)	1 min (a, B)
Control 2: NA	7 days (e, A)	7 days (d, A)	7 days (d, A)
Control 3: water	7 days (e, A)	7 days (d, A)	7 days (d, A)

SP, sodium perborate; HP, hydrogen peroxide; CHX, chlorhexidine; CP, carbamide peroxide; NA, natrosol gel.
Different letters (from a to e and A to C) indicate significant difference (Kruskall–Wallis $P < 0.05$). Capital letters indicate differences in horizontal direction. Lower-case letters indicate differences in vertical direction.

Discussion

Previous investigations have noted the importance of coronal microleakage in the failure of root canal treatment (Saunders & Saunders 1990, Magura *et al.* 1991, Uranga *et al.* 1999). There are many factors that can affect coronal leakage including the thickness of the sealer cement (Magura *et al.* 1991), presence of voids within the root filling (Magura *et al.* 1991), solubility of sealer (Saunders & Saunders 1990), smear layer removal, mastication force, penetration of bacteria and the effect of saliva (Uranga *et al.* 1999), use of an additional barrier over root filling (Chailertvanitkul *et al.* 1997). The literature related to microleakage during the walking bleach technique is limited.

A paste containing SP when used in the walking bleach technique will break down into sodium metaborate, oxygen and water (Ari & Ungor 2002). Consequently, these components make it difficult for the canal wall to remain dry, a phenomenon that may affect the sealing ability of any resin or cement used to restore the tooth. As a result, it is important that the bleaching agent has good antimicrobial properties. Hosoya *et al.* (2000) comparing *in vitro* the sealing capacity of five materials used as a temporary sealing agent for the walking bleach technique demonstrated that coronal sealing is a critical procedure during the walking bleach technique.

A recent literature review (Attin *et al.* 2003) recommended the use of SP powder and distilled water for intracoronal bleaching, and specifically contraindicated the use of 30% hydrogen peroxide or the application of heat, which increases the risk of external cervical resorption. Some authors described the successful clinical use of external bleaching of root filled teeth with carbamide peroxide gels (Putter & Jordan 1989, Swift 1992, Frazier 1998, Teixeira *et al.* 2004).

The literature related to vehicles used with SP vehicle is controversial. Some studies report better results for hydrogen peroxide (Ho & Goerig 1989, Warren *et al.* 1990) compared with distilled water. Other studies report a similar bleaching ability for both vehicles (Rotstein *et al.* 1992, Weiger *et al.* 1994, Ari & Ungor 2002). A previous study concluded that CHX gel did not reduce the bleaching efficacy of SP paste when this antimicrobial agent was used as vehicle and as a supplement for carbamide peroxide gel (Oliveira *et al.* 2006). The natrosol gel (hydroxyethyl cellulose, pH 5.5) used as a base for CHX gluconate is soluble in water and used widely to thicken shampoos, gels and soaps. This base allows the dissociation of SP and carbamide peroxide into oxygen peroxide that has the potential to effectively bleach teeth. The properties of CHX gel, such as broad spectrum of antimicrobial activity, substantivity, low toxicity and water solubility, have increased the interest in its use as an endodontic irrigant (Gomes *et al.* 2001). In fact, it has been used in endodontics either as an irrigant solution or as an intracanal medication, with good results (Gomes *et al.* 2003). In the present study, the addition of CHX (groups 3, 5 and 8) increased the antimicrobial action of the bleaching paste when compared with groups with the same bleaching agent, but without CHX (groups 1, 4 and 7). Comparing the microorganisms tested, *C. albicans* was the most sensitive microorganism to the antimicrobial action of the bleaching agents. The most resistant was the *E. faecalis* with *S. mutans* in an intermediate position. However, when

CHX was added all microorganisms were sensitive to this agent. These results reflect those reported previously (Gomes *et al.* 2001, Vianna *et al.* 2004).

The microorganisms used in the present study were in a planktonic culture. These free microorganisms serve as the primary source for the organization of biofilms (Bowden & Hamilton 1998). Biofilms will provide protection and increase the resistance to adverse external influences (Lewis 2001). Clearly, this study should be repeated using a biofilm model to evaluate its resistance to antimicrobial agents such as CHX (Zaura-Arite *et al.* 2001).

As the addiction of CHX doses does not affect the bleaching ability of these agents (SP and carbamide peroxide) (Oliveira *et al.* 2006), the antimicrobial activity of CHX should be considered as an adjunct to kill bacteria that may leak via a defective coronal seal during the walking bleach technique.

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