Inhibition of Sodium Hypochlorite Antimicrobial Activity in the Presence of Bovine Serum Albumin

Fernanda Geraldes Pappen, DDS, Wei Qian, DDS, PhD,‡ Jolanta Aleksejūnienė, DDS, PhD,‡ Renato de Toledo Leonardo, DDS, PhD,† Mario Roberto Leonardo, DDS, PhD,§ and Markus Haapasalo, DDS, PhD‡*

Abstract

Introduction: This study investigated the inhibition of the antimicrobial activity of sodium hypochlorite (NaOCl) by bovine serum albumin (BSA). The killing of *Enterococcus faecalis, Candida albicans, Staphylococcus epidermidis*, and *Escherichia coli* by NaOCl in concentrations from 2% to 0.03% was measured in the presence of BSA in concentrations between 6.7% and 0.1%. **Methods:** NaOCl, BSA, and microorganism suspensions were mixed, and, after 30 seconds, 6 minutes, and 30 minutes, samples were taken and NaOCl was inactivated by 5% sodium thiosulphate. The microbes were incubated in tryptic soy broth broth for up to 7 days for the detection of growth. **Results:** All microorganisms were killed within 30 seconds by 0.03% NaOCl when BSA was not present. High concentrations of BSA significantly reduced the antimicrobial activity of NaOCl against the four species. **Conclusions:** The inhibition of sodium hypochlorite by BSA was directly dependent on their quantitative relationships. The result partly explains the poorer performance *in vivo* of NaOCl as compared to *in vitro* experiments. *(J Endod 2010;36:268–271)*

Key Words

Antimicrobial effect, bovine serum albumin, sodium hypochlorite

From the *Department of Semiology and Clinics, Faculty of Dentistry, Federal University of Pelotas, Pelotas, Brazil; †Department of Restorative Dentistry, Faculty of Dentistry of Araraquara, State University of São Paulo, Araraquara, São Paulo, Brazil; ‡Department of Oral Biological and Medical Sciences, Faculty of Dentistry, University of British Columbia, British Columbia, Vancouver, Canada; and §Department of Pediatric, Preventive and Community Dentistry, Ribeirão Preto Faculty of Dentistry, University of São Paulo, Ribeirão Preto, São Paulo, Brazil.

Supported by MEC/CAPES (Foundation for the Coordination of Higher Education and Graduate Training in Brazil) as an exchange Scholarship (BEX 4146-06/9).

Address requests for reprints to Dr Fernanda Geraldes Pappen, Division of Endodontics, UFPel- Faculty of Dentistry, Federal University of Pelotas, Rua Sete de Setembro, 43/1101, Pelotas, RS, CEP 96015-300. E-mail address: ferpappen@yahoo.com.br.

0099-2399/$0 - see front matter

doi:10.1016/j.joen.2009.09.025

The etiology of apical periodontitis is the presence of infection in the root canal system; therefore, the elimination of microorganisms and the prevention of reinfection are the main goals of successful endodontic therapy of necrotic teeth. The success rate of the treatment of primary apical periodontitis is between 75% and 90%, whereas in retreatment cases the success rate is somewhat lower, between 50% and 80% (1–3). One of the reasons for the difference in success rate may be the presence of resistant microbes in persistent apical periodontitis. *Enterococcus faecalis* and yeasts have been isolated more frequently from root canals of teeth with resistant apical periodontitis than from primary infections (4–6). Both are known for their relatively high resistance to calcium hydroxide; however, data of their sensitivity to sodium hypochlorite (NaOCl) are contradictory (7–13).

Sodium hypochlorite (NaOCl) is the most commonly used irrigating solution in endodontic treatment. When used in concentrations between 0.5% and 6%, it presents high antimicrobial activity and dissolves vital and necrotic pulpal tissue and organic components of dentin such as collagen (11). NaOCl kills bacteria quickly even at low concentrations. According to Waltimo et al (8), 0.5% NaOCl killed *Candida albicans* in 30 seconds. However, when diluted further to 0.05%, NaOCl did not kill *C. albicans* even after 24 hours of incubation (8).

Zehnder et al (11) reported eradication of *E. faecalis* after 3 minutes of exposition to 0.0005%. Portenier et al (14) showed almost instantaneous killing of *E. faecalis* in planktonic culture by 0.0001% NaOCl. Contrary to these results, Vianna et al (12) reported that 0.5% and 1.0% NaOCl required 30 and 20 minutes, respectively, to completely kill *E. faecalis*, *Staphylococcus aureus*, and *C. albicans.* In addition, Radcliffe et al (13) showed that 0.5% NaOCl eliminated *E. faecalis* only after 30 minutes of incubation. Differences between studies can often be explained by differences in the methods applied and by the presence of confounding factors during the testing. Many root canal disinfectants present excellent antimicrobial activity *in vitro* whereas *in vivo* they often fail to completely kill all microbes. Several factors can weaken the effectiveness of the disinfectants in the *in vivo* conditions, such as localization of microbes in the root canal system, poor penetration of the substance, low concentration, short exposure time, and low overall volume of the irrigant.

One potential factor reducing the activity of the disinfecting agents is the chemical environment of the root canal. The root canal system contains a complex mixture of organic and inorganic compounds. Haapasalo et al (9) showed that dentin inhibits the antimicrobial effect of 1% NaOCl against *E. faecalis*. Portenier et al (15) showed that a number of organic compounds, including bovine serum albumin (BSA), reduced the antimicrobial effectiveness of chlorhexidine, calcium hydroxide, and iodine potassium iodide.

The complex reaction of solutions of NaOCl on proteins was undertaken in studies beginning from the 20s. Wright (16, 17) established that the protein was both oxidized and chlorinated in alkaline solution by NaOCl. In 1947, Baker (18) had shown that the reaction between egg albumin and NaOCl results in the degradation of protein and the reduction of hypochlorite. Additionally, studies on the mechanism of inflammation have shown that albumin can reduce the inactivation of alpha 1-antiproteinase by hypochlorous acid (HOCl) (19, 20).

However, the effect of organic material on the ability of NaOCl to kill endodontic microbes has not been studied in any detail. Sassone et al (21) reported no inhibition in bacterial killing by 1% and 5% NaOCl in the presence of 0.5% BSA, but other concentrations were not studied. Albumin is the main protein in human serum, and it is also a major component of inflammatory exudate (22, 23). Its presence in gingival crevicular fluid and in the fluid from dentinal tubules has also been reported (24–26). In this study, the effect of bovine serum albumin on the antibacterial activity of NaOCl against four different microorganisms was investigated.

Material and Methods

Solutions

NaOCl was obtained as a 6% stock solution by the manufacturer (VWR, West Chester, PA). BSA (Fisher Scientific, Fair Lawn, NJ) was prepared into a 20% stock solution in distilled water. The stock solutions were twofold serially diluted in sterilized water immediately before use, obtaining NaOCl solutions in the following concentrations: 6%, 3%, 1.5%, 0.75%, 0.36%, 0.18%, and 0.09%. BSA solutions were obtained in the following concentrations: 20%, 10%, 5%, 2.5%, 1.25%, 0.64%, and 0.32%. In the experiments, the final BSA and NaOCl concentrations were one third of the original concentrations after mixing the three solutions (BSA, NaOCl, and microorganism suspension).

Microorganisms

Microorganisms used in this study were *E. faecalis* strains VP3-181, GEL 31, and GEL 32 (27, 28); *Escherichia coli* C498 (29); *Staphylococcus epidermidis* C621; and *C. albicans* C627 (clinical strains, UBC Microbiology Department collection). All microbes were grown at 37°C in air overnight on tryptic-soy-agar (TSA) plates (Difco, Detroit, MI), checked for purity, suspended in sterilized water, and adjusted spectrophotometrically to a cell density of 4×10^7 cfu/mL for the bacteria and 4×10^6 colony-forming units (cfu)/mL for *C. albicans*, allowing the same microbial biomass for the bacterial and yeast solutions.

Effect of BSA on the Antimicrobial Activity of NaOCl

Equal volumes of 50 μL of BSA solution and microorganism suspension were mixed in a 96-well microtiter plate (Sarstedt, Newton, NC). Using a serial pipette, 50 μL of NaOCl in concentrations from 6% to 0.08% was added to the BSA/microorganism mixture. Two control groups were included; in one, sterile water was used instead of NaOCl with all BSA concentrations (and with the microbes). In the other control group, water was used instead of BSA with all NaOCl concentrations to measure the killing by NaOCl without the presence of any potential inhibitor.

After 30 seconds, 6 minutes, and 30 minutes, in room temperature, 20 μL of the test mixture was transferred into a second microtiter plate containing 180 μL of 5% sodium thiosulphate to inactivate NaOCl. Immediately, 20 μL of the content of the wells from the second plate was transferred into 180 μL of tryptic soy broth (TSB, Difco) in a third microtiter plate well.

The plates were sealed and incubated at 37°C for 7 days and checked for the presence of growth (turbidity) after 24, 48, and 72 hours and after 7 days. At the end of the incubation, 7 μL TSB from each well was cultured in TSA to confirm the visual reading results and to check for purity of the growth.

Evaluation of Carryover Effect

To verify the effectiveness of the inactivation of NaOCl by 5% sodium thiosulphate, the following controls were performed: in parallel tests, 5 μL of microorganism suspension (4×10^7 cfu/mL for the bacteria and 4×10^6 cfu/mL for *Candida*) was added to all TSB wells of the third microtiter plate. Growth was detected in each well, confirming that negative growth when observed was not a consequence of carryover effect by NaOCl.

The detection limit of the qualitative test was calculated and tested by serial 10-fold dilutions, showing that negative growth indicated killing of 99.99% or more of the microbial cells. All the tests were performed in triplicate.

Data Analysis

The growth was defined as 0 = no growth in the three parallel tests; 1/3 and 2/3 = partial growth, when one or two parallel tests showed positive growth; and 1 = full growth, when all three parallel test showed positive growth. The reliability of the "growth" measurement was studied evaluating growth in three repeated plates. For each sample, the reliability (consistency of growth) was assessed by Cohen's kappa and was found to be highly reliable ($\kappa = 0.97$).

The results for each microorganism were analyzed separately. First, the presence of growth was compared with the time of incubation and concentrations of BSA and NaOCl applying bivariate Spearman correlation (threshold for significance chosen as $p < 0.05$). An overall effect of three factors for each microbe was assessed by means of linear multiple regression analysis. All statistical analyses were performed with the statistical package SPSS v 15.0 (SPSS for Windows; SPSS Inc, Chicago, IL).

Results

All four microbes tested were completely killed within 30 seconds by 0.03% NaOCl when no BSA was present. The inhibition of NaOCl by BSA was directly dependent on their quantitative relationships in all experiments (Fig. 1).

Spearman correlation showed significant associations at the 0.01 level for all the microorganisms and times tested. *S. epidermidis* was the most sensitive microbe tested and was completely killed in 30 seconds by 0.12% NaOCl even in the presence of 6.66% BSA. When incubated for 6 minutes or 30 minutes, BSA interfered with the antimicrobial activity of NaOCl only when the concentrations of NaOCl were below 0.06%. Because of the high sensitivity to NaOCl, *S. epidermidis* was also exposed to concentrations of NaOCl as low as 0.0002%. Independent of time, *S. epidermidis* was completely killed by 0.0017% NaOCl when no BSA was present.

The three strains of *E. faecalis* (VP3-181, GEL 31, and GEL 32) showed little variation in susceptibility to NaOCl. Full-concentration (6.66%) BSA was able to prevent the complete killing of two *E. faecalis* strains (GEL 31 and GEL 32) by 2% NaOCl at 30 seconds. After longer incubation times, all three *E. faecalis* strains were killed.

The joint effect of NaOCl and BSA concentrations and the time of incubation were studied in linear multiple regression models for each microorganism separately. The possible presence of multicollinearity was tested in all models and was not a problem because tolerance values were always equal to unity (ie, the effects of all studied factors [concentrations of NaOCl and BSA and time of exposure]); all presented independent effects with growth in all linear multiple regression models. The three factors were always influencing growth in different directions but in a consistent manner (ie, NaOCl concentration and increased time of exposure always had an inhibiting effect on growth, whereas the increasing concentration of BSA always had a growth facilitating effect). This pattern was consistently observed in models for all microorganisms. An exposure time presented the smallest influence among three studied factors, and its effect did not reach statistical significance in LMR models for *E. faecalis* (VP3-181, GEL 31, and GEL 32).

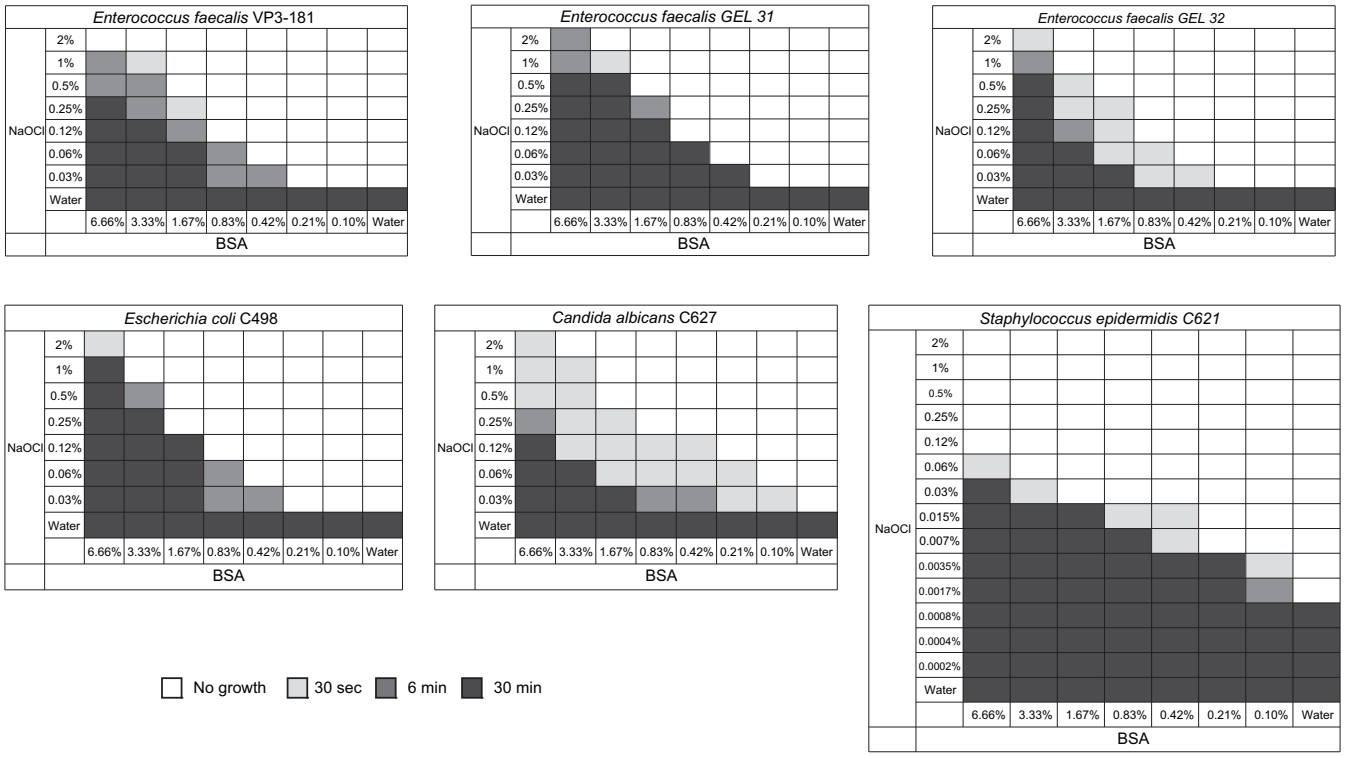

Figure 1. The survival of the four microbes after incubation with NaOCl and BSA at various concentrations for 30 seconds, 6 minutes, and 30 minutes. The darkest color shows survival at 30 minutes, the two darkest colors indicate the area of survival after 6 minutes of incubation, and all three colors (gray shades) show the survival after 30 seconds of incubation. In those very few experiments ($\kappa = 0.97$) in which both positive and negative growth was detected in the three parallel tests, the result is shown as growth (survival).

Discussion

The results of this study verified the effectiveness of NaOCl against bacteria and yeasts in planktonic culture. All microbes were killed rapidly even by low concentrations (0.03%) of NaOCl, and, after 30 seconds of incubation, no viable cells were detected. Although the results are in line with several previous reports, contradictory results have also been published recently. Vianna et al (12) reported that 0.5% and 1.0% NaOCl required 30 and 20 minutes, respectively, to completely kill *E. faecalis, S. aureus*, and *C. albicans*, and Radcliffe et al (13) showed that 0.5% NaOCl killed *E. faecalis* only after 30 minutes of incubation. The detection level in the present study was high, and, when no growth was detected, 99.99% or more of the microbial cells had been killed. Therefore, the fast killing in the present study cannot be explained by the poor detection level. A closer analysis of the two studies in which 20 to 30 minutes was required for complete eradication of the bacteria by even relatively high NaOCl concentrations shows that the microbes were not suspended in water before being challenged with the antimicrobial irrigant. Instead, NaOCl was added directly to the liquid culture, and sampling was done directly from this culture at predetermined time intervals. Therefore, it is possible that the organic load by the culture medium contributed to the inhibition of the antimicrobial activity of NaOCl in these studies. Moreover, the pH of the culture medium is reduced by the production of organic acids by the microbes, although the magnitude of the reduction is not known in individual cases. Therefore, the lower pH is also likely to play a role by neutralizing the alkalinity of NaOCl. In fact, the studies with a long killing time may to some extent reflect the reality *in vivo* in which the presence of organic matter is likely to occur. However, it is important that the various confounding factors are kept under control and calculated whenever possible.

The antibacterial effect of sodium hypochlorite is well established. However, *in vivo* studies have shown that NaOCl cannot predictably eliminate the root canal infection (30–32). In apical periodontitis, bacteria penetrate dentinal tubules and lateral ramifications from the main canal, colonizing the entire root canal system (33, 34). Although the concentration of NaOCl is supposed to be on a high level in the coronal and middle parts of the main canal, it is likely that in the peripheral parts of the root canal system the concentration and activity are lower. In addition to the well-documented difficulty for the irrigant to reach the apical canal, fins, and various canal ramifications, the irrigant will be diluted by inflammatory exudate and other fluids present in these locations. The studies by Haapasalo et al (9) and Portenier et al (15, 35) have shown that different organic and inorganic compounds present in the necrotic root canal environment have an inhibitory effect on local medicaments and disinfectants commonly used in endodontics. The inhibitory effect of dentin powder on the killing of *E. faecalis* by 1% NaOCl has been shown (9). However, the effect of other inorganic and organic components present in the root canal environment on the NaOCl antimicrobial activity has not been established. Sassone et al (18) investigated the inhibitory effect of 0.5% BSA on the antimicrobial activity of 1% and 5% NaOCl and reported no inhibition in killing of the microbes. This result is in fact in accordance with the present study because no inhibition of killing was shown at these concentrations with any of the microbes in our experiments. Higher BSA concentrations were not studied by Sassone et al (21).

Albumin is the main protein in human serum. It is present in inflammatory exudates (19, 20), gingival crevicular fluid (24, 25), and dentinal fluid (26). Its concentration in plasma is between 3.5% and 5% and up to 4% in inflammatory exudate (36). The total protein concentration in human serum is between 5.5% and 7.5%, which

corresponds well to the highest BSA concentration (6.66%) used in the present study (37). The results showed that BSA can have an inhibitory effect on the killing by NaOCl of all the four species tested. However, the inhibition is dependent on the relative concentrations of both NaOCl and BSA. The control experiments in which sterilized water was used instead of NaOCl or BSA showed that BSA had no antimicrobial effect against the tested microorganisms.

Baker (18) stated that the reaction between egg albumin and NaOCl results in the degradation of protein and the reduction of hypochlorite, whereas approximately two to nine molecules of NaOCl are reduced for each amino acid residue attacked. Wasil et al (20) reported that components from serum, mainly albumin, have antioxidant activity by scavenging HOCl and protect against the inactivation of α_1-antiproteinase by HOCl, whereas the BSA structure can be modified or even degradated upon hypochlorite oxidation (19, 38). It is likely that, in a corresponding manner, BSA has a concentration-dependent inhibitory effect on the killing of microbes by NaOCl. The presence of high concentrations of BSA consumes some of the NaOCl, which results in the delayed killing of the microflora and in some cases even survival of some microbes. Clegg et al (39) studied the killing of mixed plaque bacteria in experimental biofilm formed on dentin surface and showed that even 15 minutes of exposure to 3% NaOCl was not enough to eradicate all bacteria as revealed by culturing. The results show the strong difference between studies in which organic matter is present and studies with planktonic bacteria, which are killed in seconds by very low NaOCl concentrations. It is possible that organic matter in general has the ability to inhibit the microbicidal activity of NaOCl by mechanisms similar to that described by Wasil et al (20). Besides that, biofilms can provide a reduced contact area between bacteria and NaOCl and can also present barriers to the penetration of NaOCl and consuming available chlorine.

In conclusion, BSA has a concentration-dependent inhibitory effect on the killing of bacteria and yeasts by NaOCl. This effect can partly help us to better understand the reasons for the poorer performance of NaOCl *in vivo* as compared with *in vitro* conditions.

References

1. Friedman S, Abitbol S, Lawrence HP. Treatment outcome in endodontics: the Toronto Study. Phase 1: initial treatment. J Endod 2003;29:787–93.
2. Farzaneh M, Abitbol S, Lawrence HP, et al. Toronto Study. Treatment outcome in endodontics—the Toronto Study. Phase II: initial treatment. J Endod 2004;30:302–9.
3. Marquis VL, Dao T, Farzaneh M, et al. Treatment outcome in endodontics: the Toronto Study. Phase III: initial treatment. J Endod 2006;32:299–306.
4. Waltimo T, Sirén E, Torkko H, et al. Fungi in therapy-resistant apical periodontitis. Int Endod J 1997;30:96–101.
5. Molander A, Reit C, Dahlen G, et al. Microbiological status of root-filled teeth with apical periodontitis. Int Endod J 1998;31:1–7.
6. Peciuliene V, Balciuniene I, Eriksen H, et al. Isolation of Enterococcus faecalis in previously root-filled canals in a Lithuanian population. J Endod 2000;26:593–5.
7. Haapasalo M, Ørstavik D. In vitro infection and disinfection of dentinal tubules. J Dent Res 1987;66:1375–9.
8. Waltimo TM, Orstavik D, Sirén EK, et al. In vitro susceptibility of Candida albicans to four disinfectants and their combination. Int Endod J 1999;32:421–9.
9. Haapasalo H, Sirén E, Waltimo T, et al. Inactivation of local root canal medicaments by dentine: in vitro study. Int Endod J 2000;33:126–31.
10. Gomes BPFA, Ferraz CCR, Vianna ME, et al. In vitro antimicrobial activity of several concentrations of sodium hypochlorite and chlorhexidine gluconate in the elimination of Enterococcus faecalis. Int Endod J 2001;34:424–8.
11. Zehnder M, Kosicki D, Luder H, et al. Tissue-dissolving capacity and antibacterial effect of buffered and unfuffered hypochlorite solutions. Oral Surg Oral Med Oral Pathol Oral Radiol Endod 2002;94:756–62.
12. Vianna ME, Gomes BP, Berber VB, et al. In vitro evaluation of the antimicrobial activity of chlorhexidine and sodium hypochlorite. Oral Surg Oral Med Oral Pathol Oral Radiol Endod 2004;97:79–84.
13. Radcliffe CE, Podtouridou L, Qureshi R, et al. Antimicrobial activity of varying concentrations of sodium hypochlorite on the endodontic microorganisms Actinomyces israelii, A. naeslundii, Candida albicans and Enterococcus faecalis. Int Endod J 2004;37:438–46.
14. Portenier I, Waltimo T, Ørstavik D, et al. The susceptibility of starved, stationary phase, and growing cells of Enterococcus faecalis to endodontic medicaments. J Endod 2005;31:380–6.
15. Portenier I, Haapasalo H, Rye A, et al. Inactivation of root canal medicaments by dentine, hydroxylapatite and bovine serum albumin. Int Endod J 2001;34:184–8.
16. Wright NC. The action of hypochlorites on amino-acids and proteins. Biochem J 1926;20:524–32.
17. Wright NC. The action of hypochlorites on amino-acids and proteins. The effect of acidity and alkalinity. Biochem J 1936;30:1661–7.
18. Baker WRW. Studies on the reaction between sodium hypochlorite and proteins. Biochem J 1947;41:337–42.
19. Yan LJ, Traber MG, Kobuchi H, et al. Efficacy of hypochlorous acid scavengers in the prevention of protein carbonyl formation. Arch Biochem Biophys 1996;327:330–4.
20. Wasil M, Halliwell B, Hutchison DC, et al. The antioxidant action of human extracellular fluids. Effect of human serum and its protein components on the inactivation of alpha 1-antiproteinase by hypochlorous acid and by hydrogen peroxide. Biochem J 1987;243:219–23.
21. Sassone LM, Fidel R, Fidel S, et al. The influence of organic load on the antimicrobial activity of different concentrations of NaOCl and chlorhexidine in vitro. Int Endod J 2003;36:848–52.
22. Tollefsen T, Saltvedt E. Comparative analysis of gingival fluid and plasma by crossed immunoelectrophoresis. J Periodontal Res 1980;15:96–106.
23. Tögl-Leimüller A, Egger G, Porta S. Albumin as one-way transport vehicle into sites of inflammation. Exp Pathol 1986;30:91–6.
24. Bickel M, Cimasoni G, Andersen E. Flow and albumin content of early (pre-inflammatory) gingival crevicular fluid from human subjects. Arch Oral Biol 1985;30:599–602.
25. Mäkelä M, Söderling E, Paunio K, et al. Protein composition of crevicular fluid before and after treatment. Scand J Dent Res 1991;99:413–23.
26. Knutsson G, Jontell M, Bergenholtz G. Determination of plasma proteins in dentinal fluid from cavities prepared in healthy young human teeth. Arch Oral Biol 1994;39:185–90.
27. Peciuliene V, Reynaud AH, Balciuniene I, et al. Isolation of yeasts and enteric bacteria in root-filled teeth with chronic apical periodontitis. Int Endod J 2001;34:429–34.
28. Reynaud af Geijersstam AH, Ellington MJ, Warner M, et al. Antimicrobial susceptibility and molecular analysis of Enterococcus faecalis originating from endodontic infections in Finland and Lithuania. Oral Microbiol Immunol 2006;21:164–8.
29. Machado E, Coque TM, Canto R, et al. Emergence of CTX-M b-lactamaseproducing Enterobacteriaceae in Portugal: report of an Escherichia coli isolate harbouring blaCTX-M-14. Clin Microbiol Infect 2004;10:755–7.
30. Byström A, Sundqvist G. Bacteriologic evaluation of the effect of 0.5 percent sodium hypochlorite in endodontic therapy. Oral Surg Oral Med Oral Pathol 1983;55:307–12.
31. Bystrom A, Sundqvist G. The antibacterial action of sodium hypochlorite and EDTA in 60 cases of endodontic therapy. Int Endod J 1985;18:35–40.
32. Card SJ, Sigurdsson A, Orstavik D, et al. The effectiveness of increased apical enlargement in reducing intracanal bacteria. J Endod 2002;28:779–83.
33. Nair PN. Light and electron microscopic studies of root canal flora and periapical lesions. J Endod 1987;13:29–39.
34. Peters LB, Wesselink PR, Buijs JF, et al. Viable bacteria in root dentinal tubules of teeth with apical periodontitis. J Endod 2001;27:76–81.
35. Portenier I, Haapasalo H, Orstavik D, et al. Inactivation of the antibacterial activity of iodine potassium iodide and chlorhexidine digluconate against Enterococcus faecalis by dentin, dentin matrix, type-I collagen, and heat-killed microbial whole cells. J Endod 2002;28:634–7.
36. Bergenholtz G, Jontell M, Tuttle A, et al. Inhibition of serum albumin flux across exposed dentine following conditioning with GLUMA primer, glutaraldehyde or potassium oxalates. J Dent 1993;21:220–7.
37. Hoefs JC. Serum protein concentration and portal pressure determine the ascitic fluid protein concentration in patients with chronic liver disease. J Lab Clin Med 1983;102:260–73.
38. Sharonov BP, Govorova NJ, Lyzlova SN. Serum protein degradation by hypochlorite. Biochem Int 1989;19:27–35.
39. Clegg MS, Vertucci FJ, Walker C, et al. The effect of exposure to irrigant solutions on apical dentin biofilms in vitro. J Endod 2006;32:434–7.