

THE IN VITRO EFFECT OF 2% CHLORHEXIDINE ON DENTINE HARDNESS

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ABSTRACT

The objective of the study was to investigate the effect of 2% Chlorhexidine on the hardness of different levels of root dentine when used as root canal irrigation in a clinically relevant time interval.

Twenty extracted teeth were endo-dentically cleaned and shaped and each root was sectioned into three sections; cervical, middle and apical and then the three sections were mounted on an acrylic resin disc shape mould. After polishing the coronal surface of each section, microhardness was measured using a Wallace microhardness appliance at five locations at 1 mm distance from the root canal lumen. Specimens were then irrigated with 2% Chlorhexidine for one hour and microhardness was measured again.

Result showed no statistically significant ($p > 0.05$) difference in the decrease of dentine hardness before or after application of 2% Chlorhexidine in any root section.

In conclusion Chlorhexidine found to have no significant effect on root dentine microhardness.

Key words: Chlorhexidine, irrigation, microhardness, root dentine, Wallace microhardness appliance

INTRODUCTION

The most common cause of pulpal disease are bacteria or their toxins. When the pulp is invaded, damage is often irreversible.¹ The main objectives of using an irrigating solution during root canal treatment are removal of pulpal and dentinal debris from the root canal system, disinfecting the root canal from invading microorganisms² and smear layer removal.³ Ideally they should clean and flush out debris from the root canal. Lubricate files, and offer antimicrobial and tissue dissolution effects.⁴

However, apart from the beneficial effects, irrigants may exhibit detrimental effects on dentine or on root canal filling materials. Irrigants affect apical seal or root filled teeth⁵ and resin dentine bond stability.⁶ Some irrigants could affect flexural strength⁷ ultimate tensile strength⁸ punch shear strength⁹, microhardness of root dentine¹⁰ or tooth surface strain.^{11,12}

Evaluating the effect of irrigants on mechanical properties of dentine is of utmost importance as the understanding of the mechanical properties of dentine is the first step towards predicting the behavior of the dentine/restoration interface¹³ and for evaluation of dental materials when considering the design or life-time duration¹⁴.

Hardness expresses the resistance a material offers to scratching, indentation, elastic impact, cutting and permanent deformation.¹⁵ It is measured by applying a predetermined load onto a surface using an indenter and measuring the dimensions of the residual impression left on the material after withdrawal of the indenter.¹⁶ Hardness can be related to other dentine mechanical properties such as Young's modulus and yield stress.^{10,17,18,19} If dentine hardness is reduced to a critical limit, this may result in permanent deformation that could affect the final restored tooth.

Chlorhexidine was introduced to dentistry in the 1950's and has been used in a range of forms (gluconate, acetate, hydrochlorate).²⁰ It is a wide spectrum antimicrobial agent that has medical and dental uses.²¹ It is miscible in water²² and because of its high solubility in water; the digluconate salt is the most common form of Chlorhexidine.²³ It acts by disruption of bacterial membrane.²⁴

Chlorhexidine has efficient antimicrobial that encouraged its use in endodontics alone or as an adjunct to sodium hypochlorite.²⁸ Its antimicrobial effectiveness has been confirmed by different studies in vivo.^{26,27,28} and in vitro^{4,10,29,30,31} In addition, it has been used as an intracanal medicament.²⁸ Chlorhexidine has the ad-

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vantage of adsorbing to dentinal wall and this provides an important residual antimicrobial activity in the root canal²⁰ but without significant effect on the shear bond strength of composite to dentine.³²

The effect of irrigants on microhardness has been investigated¹⁷ however, these studies did not produce clear conclusions related to the time over which dentine specimens were exposed to the irrigating solution. The aim of this study was to investigate the effect of 2.0% Chlorhexidine digluconate on the microhardness of apical root dentine in a clinically relevant time.

METHODOLOGY

Twenty anterior and posterior teeth, which were previously extracted were randomly selected for this experiment. In the posterior teeth the palatal root of upper molars and the distal root of lower molars were selected. The roots of the selected teeth were examined by magnifying loupes (Keeler Ltd., Berks, UK) for the presence of cracks or carious defects. Teeth with root caries, fillings, cracks, roots with more than one canal or roots of less than 10 mm in length were excluded.

External surfaces of the selected teeth were cleaned using a sharp scalpel, disinfected by rinsing with approximately 0.5% sodium hypochlorite (Milton, Proctor and Gamble Professional, Surrey, UK) following which the teeth were kept in sterile water at room temperature.

Crowns were removed at the cemento-enamel junction using a water cooled low speed diamond wheel saw (Model 650, South Bay technology Inc., CA, USA). The pulpal tissues were removed using size 210 k files (Sterifile, K type file, Quality Endodontic Distributors Ltd., UK) accompanied by copious irrigation with sterile water. Using a size 10 k file, the length between canal orifice and apical foramen was measured. The working length was considered to be 1 mm shorter than the measured length. To facilitate root canal preparation after measuring the working length, roots were mounted in a silicon lab operating putty (SHERA SIL GmbH and Co. KG., Lemforde, Germany) and canals were prepared using k type (Sterifile) and Hedstroem type files (Hedstroem type file, Quality Endodontic Distributors Ltd., UK) size Apical preparation was enlarged to size 45 and the canal was shaped by stepping back at a 1 mm increment to size 70. The canal was irrigated with at least one ml of sterile water after each file used.

Each root was fixed into the sectioning machine clamps and the cervical, middle and the apical

third were sectioned horizontally using diamond sheel saw. The three sections from each root were positioned in a disc shaped hollow acrylic mould of 6 mm thickness. The apical surface of each section was covered by a small disc of adhesive paper to prevent entry of arcyll during the pouring procedure. The sections were inverted in the mould, so that the coronal surface of each section faced the smooth surface of the working table. The Acrylic resin (De Trey RR, Dentsply, Weybridge, UK) was mixed according to manufacturer's instructions and poured into the moulds with the apical part of each section (covered with adhesive paper) facing upward. The acryl was poured to cover each section and to the same level of the upper surface of the mould. Following setting, the acrylic disc was released from the mould, inverted, and checked that it was parallel to upper and lower surfaces.

Each section was examined with a sharp probe to inspect the presence of any arcyll either on the coronal surfaces or in the root canal. The coronal surface of each embedded section was ground to a smooth finishing using a series of increasingly fine wet Silicon Carbide papers (Bukfast Tools Ltd. Manchester, UK). The root canal of each section was dried using paper points (Dentsply Ltd, Weybridge, UK) The microhardness was measured using a Wallace hardness instrument (H.W. Wallace Co.Ltd., Croydon, UK) under good illumination and magnification at five locations 1 mm from the lumn. (Fig 1)

- Marks on acryl taken as a guide before the irrigation phase
- Marks on acryl taken as a guide after the irrigation phase

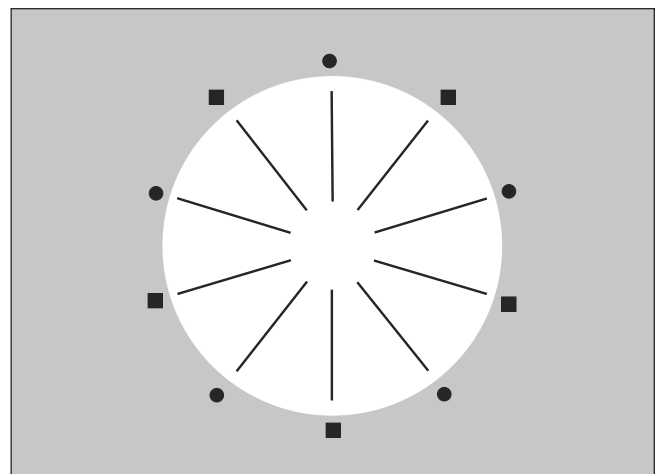


Fig 1: Marks on the acryl that were taken as guides for the indentations made into dentine 1 mm from root canal lumen before and after the irrigation phase.

TABLE 1: MEDIAN VALUES FOR THE CERVICAL, THE MIDDLE, AND THE APICAL SECTION OF DENTINE BEFORE AND AFTER IRRIGATION WITH CHLORHEXIDINE (n=20)

Root Section	Cervical	Middle	Apical
Before	53.95 (19.9)	56.05 (16.3)	54.15 (21.1)
After	53.20 (22.6)	57.25 (14.0)	55.60 (19.8)
P-values*	0.131	0.126	0.350

*Comparing hardness medians before and after values Wilcoxon Signed Ranks Test

A small piece of non-porous electric tape was cut to size that cover dentine surface around the root canal of each section using a rubber dam punch (Claudius Ash, Hertfordshire, UK). A fresh solution prepared at room temperature was used (SIGMA ALDRICH, Inc., Steinheim, Germany). The canal portion of the root segments was irrigated with 2.0% Chlorhexidine digluconate using an irrigating syringe with a 27 gauge needle. The lumen was completely filled with the irrigant, which was kept for 1h. Although the coronal surface of the root sections was masked with the non-porous adhesive tape, caution was exercised to avoid over flooding the canal. If the level of irrigant decreased more was added to keep the lumen filled. After 1h, the irrigant was washed away with sterile samples dried. Hardness was measured again in the same manner as discussed above at five different sites than those measured before irrigation (Fig.1). Throughout the experiment and to avoid excessive drying of the dentine, care was taken to keep the specimens hydrated; they were placed into a plastic container in plastic bags containing sterile water.

RESULTS

The Hardness data obtained were analyzed using SPSS software (11.5). For each root section five hardness measurements were taken before the introduction of the irrigant, and five hardness measurements after the introduction of the irrigant, leading to 600 hardness readings overall from the dial gauge. The mean value of the five readings was calculated. The microhardness value before irrigation was considered as the control measurement for the microhardness after the irrigation. Analysis of the microhardness data using this test indicated that there was no statistically significant difference. The median and range values and the p-values for the Wilcoxon Signed Ranks Test are shown in Table1.

DISCUSSION

Evaluating the effect of irrigation dentine properties is essential as detrimental effect may jeopardize

overall treatment success. Three section of each root were examined to assure that Chlorhexidine could affect certain level than other levels.

In this study, the specimens were prepared in a similar manner to the root canal preparation used in clinical situation and the experimental approach was sequential. That is, each specimen was examined before and after irrigation. In this way, each specimen was used as its own reference. This had the advantage of minimizing the structural variation of different teeth and established a reasonable base line for later evaluation.¹⁷

Because microhardness calculations are based on the induced permanent surface deformation, which remains after removal of the load¹⁰, extra care was taken that the indentations after the irrigation phase, to extra care was taken that the indentations after the irrigation phase were made in different sites from the sites indented before irrigation phase, to exclude the possibility of re-indentation (F1).

Before the irrigation phase, the coronal surface of each specimen was covered with a non-porous adhesive tape. The tape was perforated with an endodontic punch to allow for irrigation of the root canal. The punch was made to a diameter equal to or smaller if possible, than the diameter of each specimen's root canal. The tape was useful in preventing direct contact of the irrigant to the coronal surface of the specimen and prevented scratching by the irrigating needle on the occlusal surface that might have occurred during root canal irrigation. Saleh and Ettman⁽¹⁷⁾ found that the adhesive discs proved satisfactory sealing and leakage control when using Indian ink dye tracer applied into canal spaces and incubated at 37 C for 5h.

In this study, microhardness was measured only at 1000 um (1mm) from the root canal lumen because it was thought that a statistically significant difference in root dentine microhardness at this distance might be of clinical significance especially after the reduction in root dentine thickness associated with root canal prepa-

ration. The Wallace tester indentations were created at a distance of 1 mm from the root canal lumen by using good illumination (spot light) and magnifying loupes. Because of the likely difference in dentine microhardness at different locations around the root canal lumen, five indentations at five different locations were taken before and after the irrigation phase at a distance of 1 mm from the lumen of the root canal and the mean of the five readings was calculated.

The concentrations of Chlorhexidine that have been investigated either *in vivo*^{26,28} or *in vitro*^{4,10,29,30} were between 0.12% and 2%. The concentration of Chlorhexidine used in this study was the highest concentration of Chlorhexidine used clinically in order to establish the maximum possible effect of Chlorhexidine on root dentine microhardness.

The antimicrobial activity of the irrigant was dependent on the microorganism in the biofilm and on exposure time.³³ In this study, the irrigating solution was left for one hour in the root canal to compensate for the factors usually associated with endodontic preparation, such as instrumentation, using a heated solution or exchange of the irrigating solutions.³⁴ Considering all the previous factors and in addition to the fact that retreatment or complicated cases might consume more time than straightforward cases, it was decided to expose root canal sections to the irrigating solution for 1 hour.

The values of root dentine microhardness found in this study before irrigating were similar to previous studies using Vickers indenter instrument. The median microhardness value for control specimens described in previous studies. Chng et al. (2002)³⁵ found VHN of the dentine at the CEJ to be approximately 58, Cruz-Filho et al. (2000)³⁶ found the VHN to vary between 37.72 and 51.64 and Slutzky-Goldberg et al. (2000)³⁷ found the VHN value for root dentine to be 50 at 500µm and 55 at 1 mm distance from the root canal lumen.

Although Chlorhexidine was shown to cause a statistically significant increase in the bond strength of adhesive materials to root dentine³⁸, the mild increase or decrease of VHN in this study found to be significant and could be attributed to the localized in dentine microhardness¹³.

Chlorhexidine was found to be similar to sodium hypochlorite in its ability to penetrate the whole length of dentinal tubules³⁷ but was unable to dissolve tissues^{40,41,42} or to remove smear layer⁴³ and this is one of

the disadvantages of Chlorhexidine⁴⁴ which was found to be similar to normal saline.⁴⁵

The result of this study was in contrary to the result from the work by Oliveira et al. 2007⁴⁶ who found a statistically significant decrease in the microhardness of root dentine when 2.0% Chlorhexidine gluconate was used on 10 apical specimens only for 15 minutes which could not be explained by the authors and could not be confirmed by any other study. This might be due to difference methods used in the study such as difference in exposure time.

Depending on these facts, the inability of Chlorhexidine to effect root dentine microhardness found in this study might be explained by its inability to dissolve the organic tissue of root dentine.

CONCLUSION

It was found that Chlorhexidine digluconate did not affect the root dentine microhardness.

REFERENCES

- 1 Levin LG, Law AS, Holland GR, Abbott PV, Roda RS identify and Define All Diagnostic Terms for Pulpal Health and Disease States. *Journal of Endodontics* 2009;35: 1645-57.
- 2 Haapasalo M, Endal U, Zandi H, Coil J. Eradication of endodontic infection by instrumentation and irrigation solutions. *Endodontics Topis* 2005;10:77-102.
- 3 Torabinejad M, Khademi AA, Bavagoli J, Cho Y, Johnson WB, Bozhilov K, Kim J, Shabahang S.A new solution for the removal of the smear layer. *Journal of Endodontics* 2003; 29:170-75.
- 4 Estrela CR, Estrela C, Reis C, Bammann LL, Pecora JD, Control of microorganisms *in vitro* by endodontic irrigants. *Brazilian Dental Journal* 2003; 14:187-92.
- 5 Engel GT, Goodell GG, McClanahan SB. Sealer Penetration and Apical microleakage in smear-free Dentin After a Final Rinse with Either 70% Isopropyl Alcohol or peridex. *Journal of Endodontics* 2005; 31:620-23.
- 6 Carrilho MR, Carvalho RM, Di Hipolito V, Geraldini S, FR Tay Pashley DH, Tjderhane L. Chlorhexidine Preserves Dentine Bond *in Vitro* *Jurnal of Dental Research* 2007; 86; 90-95.
- 7 Sim TP, Knowles JC, Shelton J, Gulabivala K. Effect of sodium hypochlorite on mechanical properties of dentine and tooth surface strain. *International Endodontic Jurnal* 2001; 34: 120-32.
- 8 Fuentes V, Ceballos L, Osorio R, Toledano M, Carvalho RM, Pashley DH. Tensile strength and microhardness of treated human dentine. *Dental Materials* 2004; 20: 522-29.
- 9 Lee B-S, Hsieh T-T., Chi D.C-H, Lan W-H, Lin C-P. The role of organic tissue on the punch shear strength of human dentine. *Journal of Dentistry* 2004; 32: 1010-107.
- 10 White JD, Lacefield WR, Chavers LS, Eleazer PD. The effect of three commonly used endodontic material on the strength and hardness of root dentine. *Jurnal of Endodontics* 2002; 28: 828-30.
- 11 Sobhani OE, Gulabivala K, Knowles JC, Ng Y-L. The effect of irrigation time, root morphology and dentine thickness on

- tooth strain when using 5% sodium hypochlorite and 17% EDTA. *International Endodontic Journal* 2010; 43: 190-99.
- 12 Rajasinggham Y, Ng Y-L, Knowles K, Gulabivala K, The effect of sodium hypochlorite and ethylenediamineteraacetic acid irrigation, indivisually and in alteration on tooth surface strain. *International Endodontic Journal* 2010; 43: 31-40.
 - 13 Kinney JH, Balooch M, Marshall SJ, Marshall Jr GW, Weihs TP. Hardness and young's modulus of human peritubular and intertubular dentine. *Archeives of Oral Biology* 1996; 41: 9-13.
 - 14 Inuoue T, Saito M, Yamamoto M, Debari K, Kou K, Nishimura F Miyazaki T. Comparison of nanohardness between voronal and radicular intertubular dentin. *Dental Materials Journal* 2009; 28(3): 295-300.
 - 15 Hodge HC, McKay H. The microhardness of teeth. *Journal of the American Dental Association* 1933; 20: 227-33.
 - 16 Chicot D, Mercier D, Roudet F, Silva K, Staia MH, Lesage J. Comparison of instrumented knop and Vickers hardness measurements on various soft materials and hard ceramics. *Journal of the European Ceramic Society* 2007; 27: 1905-1911.
 - 17 Saleh AA, Ettman WM, Effect of endodontic irrigation solutions on microhardness or root canal dentine. *Journal of Dentistry* 1999; 27: 43-46.
 - 18 Sayin TC, Serper A, Chereli ZC, Otlu HG. The effect of EDTA, EGTA, EDTAC, and tetracycline-HCl with and without subsequent NaOCI treatment on the microhardness of root canal dentin. *Oral Surg Oral Med Oral Pathol Oral Radiol Endod* 2007; 104:418-24.
 - 19 Saghiri MA, Lotfi M, Saghiri AM, Vosoughhosseini S, Aeinehchi M, Ranjkesh B. Scanning Electron Micrograph and Surface Hardness of Mineral Trioxide Aggregate in the presence of Alkaline PH. *Journal of Endodontics* 2009; 35: 706-10.
 - 20 Leonardo MR, Filho MT, Silva LA, Bonifacio KC, Ito IY. In vivo antimicrobial activity of 2% Chlorhexidine used as root canal irrigation soulation. *Journal of Endodontics* 1999; 25: 167-71.
 - 21 Fardal O, Turnbull R. A review of the literature on use of Chlorhexidine in dentistry. *Journal of the American Denatl Association* 1986; 112: 863-67.
 - 22 Dollery C. Chlorhexidine In: Therapeutic drugs. Second edition, Churchill Livingstone, Edinburgh, UK. 1999;pp:175-77.
 - 23 Muzyka BC. Oral fungal infections. *Dental Clinics of North America* 2005; 49:49-65.
 - 24 Hiraishi N, You CK, King NM, Tay FR. Effect of Chlorhexidine incorporation into a self-etching primer on dentine bond strength of a luting cement. *Journal of Dentistry* 2010; 38:496-502.
 - 25 Janer LR, Hays GL, White RR. Comparison of the antimicrobial activity remaining after 2.0% Chlorhexidine endodontic irrigant regimens. *Journal of Dental research* 1996; 75:52.
 - 26 Ringel AM, Patterson SS, Newton CW, Miller CH, Mulhern JM. In vivo evaluation of Chlorhexidine gluconate solution and sodium hypochlorite solution as root canal irrigants *Journal of Endodontics* 1982; 8: 200-204.
 - 27 Zamany A, Safavi K, Spangberg LS The effect of Chlorhexidine as an endodontic disinfectant. *Oral Surgery, Oral Medicine, Oral Pathology, Oral Radiology and Endodontology* 2003; 96: 578-81.
 - 28 Ercan E., Ozekinci T., Atakul F., Gul K. Antibacterial activity of 2% Chlorhexidine gluconate and 5.25% sodium hypochlorite in infected root canal: in vivo study. *Journal of Joel Endodontics* 2004; 30: 84-87.
 - 29 Gomes BPFA, ferraz CCR, Vianna ME, Berber VB, Teixxeira FB, Souza-Filho FJ. In vitro antimicrobial activity of several concentration of sodium hypochlorite and Chlorhexidine gluconate in the elimination of *Enterococcus faecalis*. *International Endodontic Journal* 2001; 34: 424-28.
 - 30 Sassone LM, Fidel S, Vieira M, Hirata Jr R. The influence of organic load on the antimicrobial activity of different concentrations of NaOCI and Chlorhexidine in vitro. *International Endodontic Journal* 2003; 36: 848-52.
 - 31 Vianna ME, Gomes BP, Berber VB, Zaia AA, Ferraz CC, de Souza-Filho FJ. In vitro evaluation of the antimicrobial activity of Chlorhexidine and sodium hypochlorite. *Oral surgery, Oral Medicine, Oral Pathology, Oral Radiology and Endodontology* 2004; 97: 79-84.
 - 32 Perdigao J, Denehy GE, Swift EJ. Effect of v on dentine surfaces and shear bond strengths. *American Journal of Dentistry* 1994; 7: 81-84.
 - 33 Spratt DA, Pratten J, Wilson M, Gulabivala K. An in vitro evaluation of the antimicrobial efficacy of irrigants on biofilms of root canal isolates. *International Endodontic Journal* 2001; 34: 300-307.
 - 34 Spano JCE, Barbin EL, Santos TC, Gumaraes LF, Pecora JD. Solvent of sodium hypochlorite on bovine pulp and physicochemical properties of resulting liquid. *Brazilian Dental Journal* 2001; 12:154-57.
 - 35 Chng K.K Palamara JEA, Messes HH. Effect of hydrogen peroxide and sodium perborate on biomechanical properties of human dentine. *Journal of Endodontics* 2002; 28: 62-67.
 - 36 Cruz-Filho AM, Paula EA, Sousa-Neto MD. Effect of Different EGTA concentrations on dentine microhardness. *Brazilian Dental Journal* 2002; 13: 188-90.
 - 37 Slutzky-Goldberg I, Liberman R, Heling I. The effect of instrumentation with two different file types, each with 2.5% NaOCI irrigation on the microhardness of root dentine *Journal of Endodontics* 2002; 28: 311-12.
 - 38 Erdemir A, Ari H, Gungunes H, Belli S. Effect of medications for root canal treatment on bonding to root canal dentine. *Journal of Endodontics* 2004; 30: 113-16.
 - 39 Buck R, Eleazer PD, Staat RH. In vitro disinfection of dentinal tubules by various endodontic irrigants. *Journal of Endodontics* 1999; 25: 786-88.
 - 40 Evans MD, Baumgartner JC, Khemaleelakul S, Xia T. Efficacy of calcium hydroxide: Chlorhexidine paste as an intracanal medication in bovine dentine. *Journal of Endodontics* 2003; 29: 338-39.
 - 41 Okino LA, Siqueira EL, Santos M, Bombana AC, Figueiredo JAP. Dissolution of pulp tissue by aqueous solution of Chlorhexidine digluconate and Chlorhexidine digluconate gel. *International Endodontics Journal* 2004; 37: 38-41.
 - 42 Marley JT, Ferguson DB, Hartwell GR. Effect of Chlorhexidine digluconate as an endodontic irrigant on the spical seal: Short-trem result. *Journal of Endodontics* 2001; 27: 775-78.
 - 43 Ferraz CCR, Gomes BPFA, Zaia AA, Teixeira FB, Souza-Filho FJ. In vitro sassessment of the antimicrobial action and the mechanical ability of Chlorhexidine gel as an endodontic irrigant. *Journal of Endodontics* 2001; 27: 452-55.
 - 44 Mohammadi Z, Abbott PV. The properties and applications of Chlorhexidine in Endodnits. *International Endodontics Journal* 2009; 42: 288-302.
 - 45 Yamashita JC, Tanomaru Filho M, Leonardo MR, Rossi MA, Silva LAB. Scanning election microscopic study of the cleaning ability of Chlorhexidine as a root-canal irrigant. *International Endodontics Journal* 2003; 36: 391-94.
 - 46 Oliveira LD, Carvalho CA, Nunes C, Camargo R, Jorge AO. Et al. 2007 Effects of Chlorhexidine and sodium hypochlorite on the microhardness of root canal dentine. *Oral Surg Oral Med Oral Pathol Oral Radiol Endod* 2007; 104: 125-28.