

An Analysis of the Antimicrobial Activity of Ten Root Canal Sealers - A Duration Based *in vitro* Evaluation

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Aim and Design: Sealers with prolonged antimicrobial activity are needed by the fact that microorganisms are commonly recovered from teeth with failed endodontic therapy. The aim of this study was to evaluate the duration of antimicrobial activity of ten root canal sealers (Apexit plus, Tubli Seal Xpress, Endoflas FS, Endomethasone, Endomethasone N, AH Plus, Epiphany, EndoRez, Ketac Endo, Roeko Seal) against *Enterococcus faecalis* and *Candida albicans* by the agar diffusion test. The zones of inhibition were examined immediately and after 24, 48, 72 hours, 5 and 7 days. Statistical analysis was done using Kruskal - Wallis test and Mann Whitney U test.

Results and conclusions: Against *Enterococcus faecalis*, Endoflas FS showed the largest inhibitory zones immediately and 24 hours after manipulation, whereas, there was no significant difference between Endoflas FS and Endomethasone after 48 hours. Against *Candida albicans*, Endoflas FS performed better than the other sealers. All the sealers (except AH Plus, Epiphany and Roeko Seal) demonstrated higher antimicrobial action in the first 24 hours after manipulation. The antimicrobial action of all the sealers (except AH Plus and Roeko Seal which showed no antimicrobial activity in any studied time and Epiphany which ceased to show any antimicrobial action after 24 hours) decreased significantly with time.

Keywords: Root canal sealers; agar diffusion; microbiology; antimicrobial activity; duration; Zinc oxide – eugenol; resin sealer; calcium hydroxide; glass ionomer; silicone sealer.

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INTRODUCTION

The main objective of endodontic treatment is the elimination of microorganisms from the root canal system and prevention of subsequent reinfection. Biomechanical cleaning and shaping, followed by three dimensional obturation of the root canal space are the common procedures to achieve this goal.

However, the presence of microorganisms has been reported even after thorough chemo mechanical preparation of the root canal system.^{1,2} Conventional canal debridement procedures may not be able to completely eliminate the bacteria from the root canal system. This becomes more important taking into consideration, the complex anatomy of the

root canal system i.e., apical deltas, ramifications, lateral and accessory canals. Microbial persistence and growth in dentinal tubules, lateral canals and apical ramifications has been proved.³ The residual organisms, together with that re-entering from the oral cavity, if the access cavity is not sealed adequately, rapidly repopulate the empty canals between appointments and can induce or sustain apical periodontitis secondary to pulpal pathosis.^{4,5}

Bacteria that colonize the root canal system interact with the host tissue and cause periradicular disease.^{5,6} Disinfection of the root canal system and filling the root canal space with a material which is compatible with the host tissues, allows periradicular healing. The root canal sealers basically hold the cones of the filling material together and fill the space that the obturating material is unable to fill.^{7,8} The use of sealers with antibacterial activity is important in decreasing or eliminating micro organisms from the root canal space and increasing the overall success rate of endodontic therapy.^{2,7} This is more important in young permanent molars, taking into consideration the fact that these teeth have to be retained for proper growth and development.

The duration of antibacterial activity of the sealers is also important in this regard.⁷ A wide variety of sealers are available today, ranging from zinc oxide – eugenol to resin based sealers, glass ionomer based sealers and calcium hydroxide sealers. Though there are studies that have investigated the antimicrobial action of root canal sealers, there are very few

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studies that have made such evaluations on duration based protocol.^{9, 10}

To the knowledge of the authors, there is no study till date which has evaluated and compared the duration of antimicrobial activity of zinc oxide eugenol based sealers (with and without antimicrobial additives), resin sealers, calcium hydroxide sealers, glass ionomer sealers and silicone based sealers.

The objective of this study was to investigate the antimicrobial activity of ten endodontic sealers (Apexit Plus, Tubli Seal Xpress, Endoflas FS, Endomethasone, Endomethasone N, AH Plus, Epiphany, EndoRez, Ketac Endo and Roeko Seal) against two microorganisms most commonly isolated from pulpal and periapical pathologies as well as failed endodontic therapy -*Enterococcus faecalis* and *Candida albicans*^{3, 6, 9, 10, 11, 12} at different times after manipulation.

MATERIALS AND METHODS

The sealers used in this study were:

- One calcium hydroxide based sealer: Apexit Plus (Ivoclar Vivadent, Liechtenstein)

- Four zinc oxide–eugenol based sealers: Tubli Seal Xpress (Kerr Corporation, Orange, CA, USA), Endomethasone and Endomethasone N (Septodont, Saint–Maur–des–Fosses Cedex, France), Endoflas FS (Sanlor Laboratories, Florida, USA)
- Three resin based sealers: AH Plus (Dentsply De Trey GmbH, Konstanz, Germany), Epiphany (Jeneric Pentron Clinical Technologies, CT, USA), EndoRez (Ultradent Products Inc, UT, USA)
- One glass ionomer based sealer: Ketac Endo Applicap (ESPE Dental AG, Seefeld, Germany)
- One silicone based sealer: Roeko Seal Automix (Coltène Whaledent, Switzerland). The composition of these sealers is given in Table 1.

The species of microorganisms used in this study were an aerobe and one facultative anaerobe *Candida albicans* (ATCC 10556) and *Enterococcus faecalis* (ATCC 29212), which were grown on Brain Heart Infusion Agar (BHI agar with 5% defibrinated sheep blood) plates (Hi Media Labs, Bangalore, India).

The microorganisms were subcultured in appropriate cul-

Table 1. Composition of the Root Canal Sealers used in the study

SEALER	MANUFACTURER	COMPOSITION
APEXIT PLUS	Ivoclar Vivadent, Liechtenstein	BASE: Calcium hydroxide / Calcium oxide (36.9%), Hydrated colophony, highly dispersed silicon oxide filler and phosphoric acid alkyl ester ACTIVATOR: Disalicylates, Bismuth hydroxide/ bismuth carbonate, fillers
ENDOMETHASONE	Septodont, Saint–Maur–des–Fosses Cedex, France	POWDER: Hydrocortisone acetate, polyoxymethylene (paraformaldehyde), thymol iodide, barium sulfate, zinc oxide, magnesium stearate LIQUID: Eugenol
ENDOMETHASONE N	Septodont, Saint–Maur–des–Fosses Cedex, France	POWDER: Hydrocortisone acetate, thymol iodide, barium sulfate, zinc oxide, magnesium stearate LIQUID: Eugenol
TUBLI SEAL XPRESS	Kerr Corporation, Orange, CA, USA	BASE: Mineral oil, barium sulfate, zinc oxide, lecithin, cornstarch. ACCELERATOR: Eugenol (4-allyl-2-methoxy phenol), dimeric acid resin, thymol iodide.
ENDOFLAS FS	Sanlor Laboratories, Florida, USA	POWDER: Zinc oxide, Iodoform, calcium hydroxide, barium sulfate, LIQUID: Eugenol
AH PLUS	Dentsply De Trey GmbH, Konstanz, Germany	PASTE A: Diepoxide, calcium tungstate, zirconium oxide, silica, iron oxide pigments PASTE B: 1- adamantine amine, N, N' – Dibenzyl – 5 – oxa- nonandi-amine-1,9- TCD diamine, calcium tungstate, zirconium oxide, silica, silicone oil
EPIPHANY	Jeneric Pentron Clinical Technologies, CT, USA	Urethane dimethacrylate, Bisphenol Glycidyl methacrylate, PEGDMA, EBPADMA, silane treated barium borosilicate glass, aluminium oxide, barium sulfate, silica, calcium hydroxide, bismuth oxychloride, amines, peroxide, photo initiator, stabilizers and pigments.
ENDOREZ	Ultradent Products Inc, UT, USA	BASE: Diurethane Dimethacrylate, benzoyl peroxide. CATALYST: 2,2' – (p-tolylimino) ethanol, Triethylene Glycol dimethacrylate,
KETAC ENDO APPLICAP	ESPE Dental AG, Seefeld, Germany	Polymaleinate glass ionomer
ROEKO SEAL AUTOMIX	Coltène Whaledent, Switzerland	Polydimethyl siloxane, silicone oil, paraffin base oil, platinum catalyst, zirconium dioxide

ture media and under gaseous conditions to confirm their purity. The methodology used was adopted from Gomes et al.^{11,12} The microbes were individually inoculated into tubes containing 5 mL of sterile 0.9 % saline solution. The suspension was adjusted spectrophotometrically at 800 nm (Optical Density 800), which was used to match the turbidity of 1.5×10^8 CFU mL⁻¹ (equivalent to 0.5 McFarland standard). Five hundred μ L of each test microorganism suspension was used to inoculate glass bottles containing 50 mL of BHI agar (Hi Media Labs, Bangalore, India) at 46° C, mixed and poured onto 130 mm plates containing a previously set layer of Mueller Hinton agar (Hi Media Labs).

Wells of 3mm diameter and 4mm depth were punched in the agar plates with a sterile punch. Sealers were manipulated according to the manufacturers' instructions and placed in these wells. The plates were maintained at room temperature for 2 hours, to allow diffusion of the agents through the agar, and then incubated for 24–48 hours in a carbon dioxide incubator (Jouan, Saint Herblain, France), in an atmosphere of 10 % carbon dioxide. This was considered as the "immediate group". Stainless steel tubes of 3mm diameter and 4mm depth were made. The sealers were manipulated and placed in the sterile stainless steel tubes, which were placed in a humid atmosphere at 37 °C. This resulted in the formation of sealer tablets, which were used after 24, 48, 72 hours,

5 days and 7 days. These were designated as "24 hours," "48 hours," "72 hours," "5 days" and "7 days" samples respectively. These samples, after removal from the stainless steel tubes, were placed on agar plates and maintained at room temperature for 2 hours, and then incubated for 24–48 hours in a carbon dioxide incubator, in an atmosphere of 10% carbon dioxide.

Zones of inhibition of microbial growth around the sealers were measured and recorded. The inhibitory zone was considered to be the shortest distance (mm) between the outer margin of the sealer tablet and the initial point of microbial growth. Five replicates of each sealer were prepared for each of the microorganisms. So, for every studied time, each sealer had five samples against each of the microorganisms. The results were tabulated and statistically analyzed. The comparison of the action of the sealers against each microorganism was statistically analyzed using Kruskal - Wallis one way ANOVA test and the action of the sealer against each microorganism at the studied periods of time was analyzed using Mann Whitney U test.

RESULTS

The mean values of growth inhibition produced by the sealers against the microorganisms are given in Tables 2 and 3.

Table 2. Mean values of growth inhibition, in mm, produced by the sealers against *Enterococcus faecalis*

SEALER	Immediate	24 Hours	48 Hours	72 Hours	5 Days	7 Days
AH Plus	0.0	0.0	0.0	0.0	0.0	0.0
Epiphany	2.0 (0.55) ^a	0.0	0.0	0.0	0.0	0.0
EndoRez	2.0 (0.00) ^a	1.0 (0.00) ^a	0.0	0.0	0.0	0.0
Tubli Seal Xpress	10.0 (0.00) ^b	14.0 (0.55) ^b	10.5 (0.57) ^a	7.0 (0.35) ^a	5.0 (0.35) ^a	3.5 (0.55) ^a
Endomethasone	10.8 (0.50) ^b	14.4 (0.89) ^b	12.5 (0.25) ^b	8.4 (0.76) ^b	5.5 (0.25) ^a	3.0 (0.45) ^a
Endomethasone N	8.50 (0.20) ^c	11.0 (0.45) ^c	7.0 (0.0) ^c	5.0 (0.30) ^c	3.0 (0.0) ^b	2.0 (0.35) ^a
Endoflas FS	12.0 (0.25) ^c	16.5 (0.45) ^d	14.0 (0.91) ^b	10.0 (0.25) ^b	8.0 (0.45) ^c	5.5 (0.35) ^b
Apexit Plus	2.8 (0.0) ^a	3.0 (0.55) ^e	2.0 (0.0) ^d	0.0	0.0	0.0
Ketac Endo	7.0 (0.55) ^c	9.0 (0.0) ^f	5.5 (0.55) ^e	3.0 (0.65) ^c	0.0	0.0
Roeko Seal	0.0	0.0	0.0	0.0	0.0	0.0

Table 3. Mean values of growth inhibition, in mm, produced by the sealers against *Candida albicans*

SEALER	Immediate	24 Hours	48 Hours	72 Hours	5 Days	7 Days
AH Plus	0.0	0.0	0.0	0.0	0.0	0.0
Epiphany	3.70 (0.20) ^a	0.0	0.0	0.0	0.0	0.0
EndoRez	5.00 (0.45) ^b	3.00 (0.00) ^a	0.0	0.0	0.0	0.0
Tubli Seal Xpress	10.00 (0.87) ^c	15.50 (0.32) ^b	8.00 (0.56) ^a	5.00 (0.45) ^a	2.00 (0.25) ^a	2.00 (0.00) ^a
Endomethasone	10.00 (0.89) ^c	14.00 (0.20) ^b	9.00 (0.71) ^a	5.00 (0.20) ^a	3.00 (0.90) ^a	2.00 (0.00) ^a
Endomethasone N	9.50 (0.25) ^c	12.45 (0.00) ^c	6.50 (0.00) ^b	3.50 (0.25) ^b	1.50 (0.35) ^a	1.50 (0.31) ^a
Endoflas FS	13.20 (0.89) ^d	17.20 (0.64) ^d	10.20 (0.45) ^c	8.20 (0.68) ^c	6.20 (0.32) ^b	5.80 (0.24) ^b
Apexit Plus	5.80 (0.55) ^b	9.00 (0.20) ^e	3.80 (0.76) ^d	1.80 (0.52) ^b	0.0	0.0
Ketac Endo	8.00 (0.30) ^c	10.00 (0.32) ^e	5.00 (0.55) ^d	2.00 (0.77) ^b	0.0	0.0
Roeko Seal	0.00 (0.00)	0.0	0.0	0.0	0.0	0.0

n = 5 for each sealer – time duration combination

Numbers in parentheses indicate standard deviation

Bold numbers indicate statistically significant groups (for each sealer – time duration comparison)

Different alphabets indicate statistically difference amongst the sealers; Same alphabets indicate no statistical difference (Comparison amongst sealers for each duration of time studied).

No multiple comparisons amongst the sealers at different time durations were done.

The results show a statistically significant difference in the antimicrobial action of the sealers with time ($p < 0.005$). The 24 hour group showed significantly better antimicrobial activity for all the sealers tested in this study, except AH Plus, Epiphany and Roeko Seal, which showed no antimicrobial activity 24 hours after manipulation.

For both *Enterococcus faecalis* and *Candida albicans*, Endoflas FS showed the highest zones of inhibition at all durations studied, except against *C.albicans* in the 48 hours group where the difference between Endoflas FS and Endomethasone was not significantly different ($p > 0.05$). Endomethasone was significantly better than Endomethasone N against both the microbes at all durations studied, except against *C.albicans* in the immediate and 7 days groups. The resin sealer AH Plus and silicone based sealer Roeko Seal did not exhibit any antibacterial activity in any studied duration of time.

DISCUSSION

The most important step for successful endodontic therapy is the chemo mechanical preparation of the root canal system. However, the filling quality is also of equal importance, in which the sealer has an important role to play. It is important that the sealer be bactericidal or at least not encourage bacterial growth.^{7, 10, 12, 13} Microorganisms may persist in the root canal system even after chemomechanical preparation and use of intra canal medicaments.^{3, 4, 12} A sealer with antimicrobial activity can thus be considered advantageous in order to eliminate the remaining microbes present in the root canal system and to prevent re-infection.^{7, 11, 13, 14} Many of these cements may also be used as the sole filling material in the case of primary teeth. So it is mandatory that these materials have antimicrobial activity in ensuring success of therapy.

Most of the studies only evaluate the immediate antimicrobial activity of the materials. It would be more relevant if the duration of antimicrobial action is determined. To achieve this, we placed the material in stainless steel tubes in an incubator and used the sealer tablets after 24, 48, 72 hours, 5 days and 7 days.^{11, 12}

The agar diffusion method has been widely used to test the antimicrobial activity of dental materials and medicaments.^{7, 9, 10, 11, 12, 13, 14, 15} The advantage of this method is that it allows direct comparison of the materials against the organisms, indicating which material has the potential to eliminate bacteria in the local micro environment of the root canal system. However, the disadvantage of this method is that, the result does not depend only on the toxicity of the material for the particular organism, but is also highly influenced by the ability of the material to diffuse across the medium. So, care was taken to keep the plates at room temperature for 2 hours, to allow diffusion of the materials through the agar and the plates were then incubated at 37°C, under appropriate conditions, as proposed by Gomes *et al.*^{11, 12}

The results of our study shows that the zinc oxide eugenol based sealers had a higher antimicrobial activity than the other sealers. Zinc oxide eugenol based sealers have served

as the benchmark against which other sealers are compared, as it meets most of Grossman's requirements.^{7, 8} Bactericidal agents have been added to improve the antimicrobial efficacy of zinc oxide eugenol sealers. To evaluate whether the addition of these agents significantly improves the antimicrobial activity of these sealers, it is essential to compare it with zinc oxide eugenol sealers with different bactericidal agents and sealers without these additives. So, four zinc oxide eugenol based sealers were chosen for this study. Endoflas FS contains iodoform as the bactericidal agent, while Endomethasone contains paraformaldehyde. However, the most important disadvantage of Endomethasone is that paraformaldehyde is cytotoxic and can impair tissue repair.¹¹ This led to the introduction of Endomethasone N (similar to Endomethasone, without paraformaldehyde in its composition). TubliSeal Xpress is a zinc oxide eugenol sealer without bactericidal additives.

Our study showed that Endoflas FS showed the largest inhibitory zones against both the microorganisms at all times after manipulation. The high antimicrobial activity of Endoflas FS was probably due to the presence of iodoform and eugenol, both of which have antibacterial action. Eugenol acts by protein denaturation, while iodoform is an oxidizing agent.¹⁵ Also, even after the material sets, surface hydrolysis of the chelate (zinc eugenolate), results in release of eugenol, thus explaining the effective antibacterial activity of this substance even after 72 hours. This is in accordance with previous studies which have shown that zinc oxide eugenol based sealers possess a strong and persistent antibacterial activity.^{7, 10, 11, 16}

Endomethasone performed better than Endomethasone N against *E.faecalis* at all times after manipulation, whereas against *C.albicans*, in the immediate group, there was no significant difference. This result is in contrast to that of Gomes *et al.*¹¹ But, Endomethasone is expected to have a better antimicrobial action because of the paraformaldehyde in its composition. This component being absent in Endomethasone N could probably explain the reduced antibacterial activity. However, it was surprising to note that Tubli Seal Xpress had antimicrobial activity similar to Endomethasone, except against *E.faecalis* in the 48 and 72 hours groups. Tubli Seal is a zinc oxide eugenol sealer without any antibacterial additives. The antimicrobial activity is attributed to the eugenol in this sealer.¹⁵ This calls into question, the need for addition of antimicrobial agents, especially toxic agents like paraformaldehyde, in zinc oxide eugenol sealers.

The calcium hydroxide based sealer (Apexit Plus) showed smaller zones of inhibition when compared to Endoflas FS and Endomethasone. The material showed no antibacterial activity after 48 hours against *E.faecalis*, whereas against *C.albicans*, it was significantly better than the resin sealers, but there was no significant difference when compared to the glass ionomer sealer (Ketac Endo). Apexit Plus was ineffective against *C.albicans* in the 5 days and 7 days group. The antimicrobial action of calcium hydroxide is based on

its dissociation into calcium ions and hydroxyl ions, which creates a high pH environment and inhibits enzymes essential for microbial metabolism. The pH necessary for this action should be greater than 12.5. The pH of Apexit Plus is 8.5, which makes the material ineffective against *E. faecalis*.^{8,12,16} However, disintegration of the material is disadvantageous in that, it will result in loss of apical seal.

The action of calcium hydroxide decreased significantly after 48 hours. The poor performance of calcium hydroxide may be explained by the fact that, owing to its high pH, the material may precipitate on the agar and reduce its diffusion. We speculate that the reduction in antimicrobial activity of calcium hydroxide may also be attributed to the buffering effect of agar, the experimental condition of incubation in 10% carbon dioxide and the presence of polymers in the composition, which may limit the release and diffusion of hydroxyl ions.^{11,12,17}

The glass ionomer sealer (Ketac Endo) showed antibacterial activity comparable to that of Endomethasone N in the immediate and 24 hour groups, against both the microbes evaluated in this study. But the action reduced drastically after 48 hours. The antibacterial activity of this material is attributed to fluoride, the release of which decreases with time.¹⁸

Resin based sealers (AH plus, Epiphany and EndoRez) and silicone based sealers (Roeko Seal) were introduced because of the advantages like high radiopacity, low solubility, slight shrinkage and antimicrobial efficacy.^{8,11,13,14} This is the first study which has compared the antimicrobial activity of Epiphany, Roeko Seal and EndoRez. In our study, the epoxide – amine resin based sealer AH plus showed no zones of inhibition against any micro organism. Though some studies¹¹ claim that the polymerization reaction in AH Plus could result in antibacterial reaction, this explanation is only speculative and such an antimicrobial action would only be a contact inhibition for a short duration.¹⁷ The elimination of formaldehyde release from AH plus, makes it an ineffective bactericidal agent. The results of this study are in accordance with previous studies that have evaluated the antimicrobial action of AH Plus.^{11,13,14,17} The other resin based sealer Epiphany, showed minimal zones of inhibition against both the microorganisms studied, immediately after manipulation, but showed no action thereafter. This is contrasting to the results of Bodrumlu *et al*,²⁰ who found Epiphany to have no antibacterial activity. We speculate that the reason for the antimicrobial activity of Epiphany is the minimal amount of calcium hydroxide in its composition.

Roeko Seal (an addition silicone) showed absolutely no antimicrobial activity at all durations studied. In contrast, it is interesting to note that EndoRez had significant antimicrobial activity (compared to AH Plus and Epiphany) in the immediate group against *E. faecalis*, and in the immediate, 24 and 48 hours group against *C. albicans*. This is in contrast to the results of Sipert *et al*¹⁹ and Eldeniz *et al*.²¹ The antimicrobial activity of EndoRez can be explained by the presence of benzoyl peroxide in its composition. Benzoyl peroxide is

a known antibacterial and effective antifungal agent.²² Though a part of the chemical cure process, the free peroxide present in the sealer could have attributed to the antibacterial activity.

CONCLUSION

In our study, all the sealers tested, except AH Plus, Epiphany and Roeko Seal, demonstrated a higher antimicrobial action in the first 24 hours after manipulation. This is interesting, knowing that microbes can remain in the ramifications of the root canal system after chemo mechanical preparation. Even within the limitations of the *in vitro* evaluation in this study, it was possible to compare the antimicrobial activity of the sealers over time. None of the sealers showed significant antimicrobial action after 72 hours. This can be attributed to the fact that, as the material sets, the diffusion of the active antimicrobial components decreases. So, all efforts should be made to reduce the endodontic microbes to a minimum, using powerful irrigant solutions, intra canal medicaments whenever necessary and sealers with antimicrobial activity and an effective coronal seal to ensure success of endodontic therapy.

REFERENCES

1. Molander A, Reit C, Dahlen G. The antimicrobial effect of calcium hydroxide in root canals pre treated with 5% potassium iodide. *Endod Dent Traumatol*, 15: 205–209, 1999.
2. Sjogren U, Hagglund B, Sundqvist G, Wing K. Factors affecting the long-term results of endodontic treatment. *J Endod*, 16: 498–504, 1990.
3. Love RM and Jenkinson HF. Invasion of dentinal tubules by oral bacteria. *Crit Rev Oral Biol Med*, 13: 171–183, 2002.
4. Grossman LI. *Endodontic practice – 10th edition*, Lea and Febrieger, pp 297, 1982.
5. Moller AJ, Fabricius L, Dahlen G. Apical periodontitis development and bacterial response in endodontic treatment – experimental root canal infections in monkeys with selected bacterial strains. *Eur J Oral Sci*, 112: 207–215, 2004.
6. Siqueira JF, Rôças IN, Santos RN. Detection of putative oral pathogens in acute periradicular abscesses by 16S rDNA directed polymerase chain reaction. *J Endod*, 27: 164–167, 2001.
7. Grossman LI. Antimicrobial effect of root canal cements. *J Endod*, 6: 594–597, 1980.
8. Spangberg L. Instruments, materials and devices. In: Cohen and Burns Pathways of the pulp - 8th edition, Mosby, pp 550–552, 2000.
9. Abdul Kader A, Deguid R, Saunders EM. The antimicrobial activity of endodontic sealers to anaerobic bacteria. *Int Endod J*, 29: 280–283, 1996.
10. Kaplan AE, Picca M, Macchi RI, Molgatini SL. Antimicrobial effect of six endodontic sealers – an *in vitro* evaluation. *Endod Dent Traumatol*, 15: 42–45, 1999.
11. Gomes BPFA, Vianna ME, Sena NT, Zaia AA, Ferraz CCR, Souza Filho FJ. *In vitro* evaluation of antimicrobial activity of five root canal sealers. *Braz Dent J*, 15: 30–35, 2004.
12. Gomes BPFA, Vianna ME, Sena NT, Zaia AA, Ferraz CCR, Souza Filho FJ. *In vitro* evaluation of antimicrobial activity of calcium hydroxide combined with chlorhexidine gel used as intracanal medication. *Oral Surg Oral Med Oral Pathol Oral Radiol Endod*, 102: 544–550, 2006.
13. Pizzo G, Giammanco GM, Nicolosi G, Cumbo E. *In vitro* antibacterial activity of endodontic sealers. *J Dent*, 34: 35–40, 2006.

14. Pumarola J, Berastegui E, Brau E, Canalda C, Jimenez de Anta MT. Antimicrobial activity of seven root canal sealers. *Oral Surg*, 74: 216–220, 1992.
15. Michael Pelczar. Control of microorganisms by chemical agents. In: *Microbiology*. Tata Mc Graw Hill - 5th edition, pp 494, 1998.
16. Fuss Z, Weiss EI, Shalhav M. Antibacterial activity of calcium hydroxide containing endodontic sealers on *Enterococcus faecalis in vitro*. *Int Endod J*, 30: 397–402.
17. Siqueira JF and Goncalves RB. Antibacterial activities of root canal sealers against selected anaerobic bacteria. *J Endod*, 22: 79–80, 1996.
18. Shalhav M, Fuss Z, Weiss EI. *In vitro* antibacterial activity of a glass ionomer endodontic sealer. *J Endod*, 23: 616–619, 1997.
19. Sipert CR, Hussne RP, Nishiyama CK, Torres SA. *In vitro* antimicrobial activity of Fill Canal, Sealapex, Mineral Trioxide Aggregate, Portland cement and EndoRez. *Int Endod J*, 38: 539–543, 2005.
20. Bodrumlu E, Semiz M. Antibacterial Activity of a New Endodontic Sealer against *Enterococcus faecalis*. *J Can Dent Assoc*, 72: 637, 2006.
21. Eldeniz AU, Erdemir A, Hadimli HH et al. Assessment of antibacterial activity of EndoREZ. *Oral Surg Oral Med Oral Pathol Oral Radiol Endod*, 102: 119–126, 2006.
22. Burkhart CG, Burkhart CN, Isham N. Synergistic antimicrobial activity by combining an allylamine with benzoyl peroxide with expanded coverage against yeast and bacterial species. *Br J Dermatol*, 154: 341–344, 2006.