Comparison of the sealing of different dentin bonding adhesives in root-end cavities: a bacterial leakage study

Kürşat Er, DDS, PhD,[a] Tamer Taşdemir, DDS, PhD,[a] Gülçin Bayramoğlu, DDS, MD,[b] and Şeyda Hergüner Siso, DDS, PhD,[c] Trabzon and Sivas, Turkey
KARADENIZ TECHNICAL UNIVERSITY AND CUMHURIYET UNIVERSITY

Objective: To compare the root-end sealing of the different dentin-bonding adhesives (DBAs) in root-end cavities using a bacterial leakage system.
Study design: One hundred extracted single-rooted teeth were instrumented, and root-ends were resected and ultrasonically prepared. All teeth were then randomly assigned to 6 experimental groups (n = 15) and 2 control groups (positive and negative control; n = 5) for root-end filling with iBond (Heraeus Kulzer, Hanau, Germany), G-bond (GC Co., Tokyo, Japan), Xeno III Bond (Dentsply/Caulk, Milford, DE), AdheSe Bond (Ivoclar Vivadent, Schaa, Liechenstein), Clearfil Protect Bond (Kuraray, Osaka, Japan), or Clearfil Tri-S Bond (Kuraray). All preparations were further restored with a hybrid resin composite (Renew; Bisco, Schaumburg, IL). Nail varnish was applied to all external root surfaces to the level of the resected root-ends to prevent lateral microleakage. Samples were sterilized in an ethylene oxide sterilizer for 12 h. The apical 3-4 mm of the roots were immersed in brain-heart infusion culture medium with phenol red indicator within culture chambers. The coronal access of each specimen was inoculated every 48 h with a suspension of *Enterococcus faecalis*. Bacterial leakage was monitored every 24 h for 4 weeks. The data obtained were analyzed using a chi-squared test, with $\alpha = .05$ as the level for statistical significance.
Results. All positive controls leaked within 24 h; none of the negative controls leaked. Under the conditions of the study, despite some variations, there were no statistically significant differences in rate of bacterial leakage among the experimental test materials at 1-4 weeks ($P > .05$).
Conclusions. The type of DBA had no influence in the bacterial leakage of the root-end fillings. **(Oral Surg Oral Med Oral Pathol Oral Radiol Endod 2008;106:976-82)**

Studies have demonstrated that bacteria play an important role in the development of apical pathosis.[1,2] In addition, microorganisms and their by-products or toxins have the ability to penetrate obturated root canal systems and adversely affect the periradicular tissues.[3] The goal of nonsurgical endodontic therapy (initial and retreatment) is to remove these irritants from the root canal system and 3-dimensionally fill the canal space. In those instances where nonsurgical root canal treatment is unsuccessful, periradicular surgery may be necessary to facilitate healing.[4] The surgical procedures involve exposure of the tooth apex, periradicular curettage, resection and preparation of root-end, and placement of a root-end filling. The quality of the apical seal is an important determinant for successful periradicular surgery.[5] Many materials have been proposed to seal the root-end preparation, including amalgam, gutta-percha, zinc oxide–eugenol cements, composite resin with and without dentin-bonding adhesives (DBAs), polycarboxylate cement, glass ionomer cement, compomers, resin-modified glass ionomers, 4-META-TBB resin cements, and mineral trioxide aggregate (MTA).[5-7] Ideal characteristics of a root-end filling material include sealing the apical portion of the root canal system in 3 dimensions, periradicular tissue tolerance, not promoting and preferably inhibiting the growth of pathogenic organisms, not staining tooth structure or periradicular tissues, and adhering or bonding to the tooth.[8] These characteristics, if attainable, may provide a fluid- and bacteria-tight seal and allow for regeneration or repair of periradicular tissues.[4]

Composite resins are in use as a root-end filling material, but they may be subject to microleakage and cause irritation of vital tissues. To prevent microleakage, the use of DBAs has been proposed. Conventional DBAs require the sequential application of conditioner, primer, and bonding agents in several clinical steps. A number of new DBAs have been developed recently. To reduce technique-sensitive and materials-related factors that affect bond strength a self-etch approach involving either one (conditioner–primer–bonding

[a]Department of Endodontics, School of Dentistry, Karadeniz Technical University.
[b]Department of Microbiology, School of Medicine, Karadeniz Technical University.
[c]Department of Restorative Dentistry, School of Dentistry, Cumhuriyet University.
Received for publication Aug 25, 2007; returned for revision Feb 18, 2008; accepted for publication Feb 19, 2008.
1079-2104/$ - see front matter
© 2008 Mosby, Inc. All rights reserved.
doi:10.1016/j.tripleo.2008.02.023

Table I. The materials used and their composition

Material	*Batch no.*	*Manufacturer*	*Composition*
iBond (1-step self-etch)	010075	Heraeus Kulzer, Hanau, Germany	4-META, UDMA, gluturaldehyde, acetone, water, camphorquinone
G-Bond (1-Step self-etch)	0503281	GC Co, Tokyo, Japan	4-MET, phosphate ester monomer, UDMA, water, acetone, silica filler, photo initiator
Xeno III Bond (1-step self-etch)	04411001721	Dentsply/Caulk, Milford, DE	Liquid A: HEMA, water, ethanol, BHT, nanofiller; liquid B: pyro-EMA-SK, Pem-F, UDMA, BHT, EPD
AdheSe Bond (2-step self- etch)	G13221	Ivoclar Vivadent, Schaan, Liechenstein	Primer: phosphoric acid acrylate, bis-acrylic acid amine, water,initiators, stabilizers; bonding component: HEMA, highly dispersed silicone dioxide, initiators, stabilizers
Clearfil Protect Bond (2-step self- etch)	41114	Kuraray, Osaka, Japan	Primer: HEMA, MDP, MDPB, water; bond: MDP, bis-GMA, HEMA, hydrophobic dimethacrylate, di-camphoroquinone, N,N-diethanol-p-toluidine, silanated colloidal silica, surface treated sodium fluoride
Clearfil tri-S Bond (1-step self- etch)	41116	Kuraray, Osaka, Japan	MDP, bis-GMA, HEMA, camphorquinone, ethly alcohol, water, silanated colloidal silica
Renew (hybrid composite resin)	0400003469	Bisco, Schaumburg, IL	Monomer bis-EMA, dimethacrylate, filler (59%wt, 73%vol: bariumglass, silica, titanium dioxide

4-META, 4-methacryloyloxy ethyl trimellitic anhydride; *4-MET*, 4-methacryloylox ethyl trimellitic acid; *HEMA*, hydroxyethyl methacrylate; *PEM-F*, pentamethacryloxyethyl-cyclophosphazen mono fluoride; *BHT*, 2,6-di-tert-butyl-p hydroxy toluene; *EPD*, p-dimethylamino ethyl benzoate; *pyro-EMA*, tetramethacryoxyethyl-pyrophosphate; UDMA, urethane dimethacrylate; *MDP*, 10-methacryoloyloxydecyl dihydrogen phosphate; *5-NMSA*, *N*-methacryloxyl-5-aminosalicylic acid; *MDPB*, 12-methacryloyloxydodecylpyridinium bromide; *bis-GMA*, bis-phenol a diglycidylmethacrylate; *bis-EMA*, ethoxylated BisGMA.

agent) or 2-step self-etch systems (conditioner-primer, bonding agent) application has been developed.[9,10] These DBAs have decreased the application time, increased consistency of bond strength of composite resins to dentin, and produced leak-free restorations. It is assumed that these bondings improve the adhesive capability and bonding strength of resins to tooth structure by promoting penetration, impregnation, and entanglement of the coupling agents into dentinal substrates where they polymerize in situ and create zones of resin-reinforced dentin layers.[11]

Many in vitro methods have been used to evaluate the sealing ability of root canal filling materials by using dyes, scanning electron microscopy, fluid filtration techniques, electrochemical methods, radioisotopes, and bacteria.[12-17] Among these techniques, the bacterial leakage model is considered to be the most clinically relevant.

The purpose of the present study was to compare the root-end sealing of the different DBAs using an in vitro bacterial leakage method.

MATERIALS AND METHODS

The root-end filling materials used in this study and their manufacturers are shown in Table I.

Selection and preparation of teeth

One hundred freshly extracted single-rooted human mature maxillary premolars that were approximately of the same dimensions were selected. Preoperative radiographs and an operating microscope (Zeiss, Oberkochen, Germany) at ×20 magnification were used to ensure that the teeth did not have root caries, fractures, multiple canals, lateral radicular canals, calcifications, periradicular resorptive changes, or excessive curvatures. All teeth were cleaned free of attached soft tissue, autoclaved, and stored in saline solution until required.

The teeth were sectioned at the cementoenamel junction with a multipurpose bur (Dentsply Maillefer, Tulsa, OK) in a high-speed handpiece with continuous water spray. The length of roots was adjusted to approximately 16 mm. Patency of the apical foramen was determined with a #15 K-file (Dentsply Maillefer). When the file tip appeared flush with the apical foramen, the length of the file was recorded; the working length was determined to be 1 mm short of the measured length. All teeth were instrumented using a set of ProTaper nickel-titanium instruments (Dentsply Maillefer) including S1, S2, F1, F2, and F3 with a Tri Auto ZX (Morita, Kyoto, Japan) endodontic handpiece. Irrigation with 5.25% NaOCl was accomplished between instruments. A final flush of 17% EDTA followed by 5.25% NaOCl was used to remove the smear layer. With continuous water spray, the apical 3 mm of the root was removed with a multipurpose bur perpendicular to the long axis of the root. Afterward, root-end cavities (3 mm depth) were created using diamond ultrasonic retrotips (ENAC, Osada, Japan).

To provide an intracanal matrix to pack the root-end filling material against, a gutta-percha cone was firmly inserted into the canal leaving a root-end void of 3 mm that was confirmed by radiographs. The root canals were dried with sterile paper points before the root-end fillings were placed.

Root-end obturation

The roots were then randomly divided into 6 experimental groups (n = 15) and 2 control groups (n = 5) and were filled as follows.

Group 1 (iBond). The root-end cavities were cleaned and dried. The self-etching, self-priming, bonding agent iBond (Heraeus Kulzer, Hanau, Germany) was applied onto the cavities according to the manufacturer's instructions. Three additional layers of iBond were applied followed by a 30-s waiting time. The surfaces were gently air dried until no movement of the adhesive film was detected (visibly glossy surface) followed by light polymerization for 20 s.

Group 2 (G-Bond). G-Bond (GC Co., Tokyo, Japan) was applied on the cavity surface in accordance with the manufacturer's instructions. The bonding agent was gently dried with an air syringe, followed by light curing for 10 s.

Group 3 (Xeno III). An equal amount of Xeno III (Dentsply/Caulk, Milford, DE) liquid B was dispensed into liquid A and thoroughly mixed for 5 s. The adhesive mixture was applied to the dentin surfaces and left undisturbed for 20 s. A gentle stream of air was applied to the adhesive for at least 2 s to remove the solvent. The adhesive was then light polymerized for 10 s.

Group 4 (AdheSe). The AdheSe (Ivoclar Vivadent, Schaa, Liechenstein) primer was applied to dentin with continuous rubbing for 30 s, and the excess primer was dispersed and air dried with oil-free compressed air from an air syringe until the mobile liquid film disappeared. The bonding resin AdheSe Bond was applied, gently air blown, and light cured for 10 s.

Group 5 (Clearfil Protect Bond). The Clearfil Protect (Kuraray, Osaka, Japan) self-etching primer was applied to the dentin surface with a brush and left in place for 20 s. After drying the etched surface with mild air flow, the bonding was applied on the etched-primed dentin, gently air blown, and light-cured for 10 s.

Group 6 (Clearfil Tri-S Bond). Clearfil Tri-S Bond (Kuraray) was applied to the entire cavity wall for 20 s. After conditioning the tooth surface for 20 s, the entire adherent surface was dried by blowing high-pressure air for more than 5 s while spreading the bond layer thinly. The adhesive was then light polymerized for 10 s.

The cavities were then incrementally restored with a hybrid resin composite (Renew; Bisco, Schaumburg, IL). Each increment was cured for 40 s using a curing unit (Hilux; Benlioglu Dental, Ankara, Turkey). The intensity of light was at least 500 mW/cm. Verification of the unit light intensity output was checked every 10 samples with the digital-readout light meter available with the unit.

For the 5 negative controls, the entire root surface was coated with 2 layers of nail varnish after filling the root-end preparation with red rope wax. Positive controls consisted of 5 teeth with unfilled root-end preparation.

After placement of the root-end fillings, all samples were examined under ×20 magnification to ensure proper adaptation and that there were no voids or marginal discrepancies of the material. The intracanal barrier was removed and radiographs were taken to verify the density and depth of the filling material. The restored teeth were stored in an incubator at 37°C in 100% humidity for 48 h.

Bacterial leakage test

The model used in this study was modified from a technique developed by Maltezos et al.[15] The tapered ends of 1.5-mL polypropylene centrifuge tubse (Greiner, Mannheim, Germany) were cut, and the obturated roots were inserted into the tubes until the roots protruded through the end. The junction between each tube and root was sealed with cyanoacrylate to prevent leakage of the connection. Two coats of nail varnish were applied to all external root surfaces to the level of the resected root-ends. The entire apparatus was then gas sterilized using ethylene oxide for a 12 h cycle

Table II. Samples that leaked in each group at various time intervals

Root-end filling	*Week 1*	*Week 2*	*Week 3*	*Week 4*	*Total*
iBond	2/15	0/15	1/15	1/15	4/15
G-Bond	1/15	0/15	1/15	2/15	4/15
Xeno III Bond	2/15	1/15	0/15	1/15	4/15
AdheSe	1/15	2/15	1/15	1/15	5/15
Clearfil Protect Bond	0/15	0/15	1/15	1/15	2/15
Clearfil Tri-S Bond	2/15	0/15	1/15	1/15	4/15
Positive control	5/5	—	—	—	—
Negative control	0/5	0/5	0/5	0/5	0/5

using the Anprolene AN 74C Gas Sterilizer (Andersen Products, Haw River, NC). To ensure sterilization, the whole system was incubated at 37°C for 3 days. Any test apparatus that showed signs of turbidity in the brain-heart infusion (BHI) broth was discarded.

A volume of 7-8 mL of BHI broth (Difco, Detroit, MI) with 0.01% phenol red indicator was introduced into sterile 13 × 100 mm disposable culture tubes. The previously assembled portions of tooth and its container were then inserted in the culture tubes such a minimum of 3-4 mm of the apical part of each root was immersed in BHI broth. Each assembled apparatus was then placed in a 16 × 125 mm sterile test tube and covered with a polypropylene cap. The bacterial suspension of the *E. faecalis* organism (American Type Culture Collection 29212, RSHSE, Ankara, Turkey) was used to contaminate root canals. Each access cavity was aseptically inoculated with 15 μL of freshly prepared *Enterococcus faecalis* culture (10^6 cells/mL) twice per week. All samples were incubated in a humid environment at 37°C for 4 weeks and examined daily for color change from red to orange-yellow each day. When the color of the broth had changed to orange-yellow, 10 μL of the culture broth was subcultured onto sheep blood agar to confirm purity. To assure sterility, all testing was performed in a class II laminar airflow cabinet.

Statistical analysis

Statistical analysis of the data was accomplished using a chi-squared test. The level of significance was 5% ($P < .05$).

RESULTS

The numbers of samples with bacterial leakage at the end of each week are listed in Table II. No growth was observed when checking the sterilization of the whole apparatus. None of the negative controls showed any color change indicating no leakage and all of the positive controls demonstrated a color change indicating leakage within the first 24 h. The broth cultures producing growth were then subcultured onto sheep blood agar to confirm that there was no contamination of the samples by bacteria other than *E. faecalis*. After 4 wk of comparing the bacterial penetration values, total penetration was observed in 26% of groups 1-3, 6 and 33% of the group 4, and 13% of the group 5. There were no statistically significant differences in rate of bacterial leakage among the experimental test materials at 1-4 weeks ($P > .05$).

DISCUSSION

Restoration of root-end cavities is a critical final step of endodontic surgical procedures. Besides, proper adhesive placement is critical for the success of bonded restorations.[18] Self-etch adhesives facilitate application in practice by removing the acid-etching step. There are a number of other advantages that can be listed in comparison with total etching, such as the moisture content when applied in dentin and that the resin monomers diffuse to the same depth to which the surface was demineralized, thus providing better interlocking, and there is a reduced risk of postoperative sensitivity.[19] The bonding mechanism of self-etch adhesives is based on changing the chemical composition of the substrate, commonly referred to as hybridization; the surface layer of dentin/enamel is practically dissolved and the resultant porosity filled by resin.[20] Two types of self-etch adhesives were used in this study: one was a one-step system that combined the conditioner, primer, and adhesive resin for a single application (iBond, G-Bond, Xeno III Bond, Clearfil Tri-S Bond); the other was a two-step system that combined conditioning and priming into 1 bottle but had a separate adhesive resin that was applied to complete the bonding procedure (Clearfil Protect Bond, AdheSe Bond).

Apical leakage studies show that the ideal root-end preparation is highly dependent on the depth/shape of the cavity preparation and root-end bevel.[7,21] Leakage increases as the angle of the bevel increases and the depth of the preparation decreases. Some minimal apical leakage has been observed with 1 mm root-end filling when the angle of the bevel was 0 degrees.[22] However, from a practical perspective, a root-end preparation depth of 3 mm or more is desirable.[7] This

increases the contact length of the material in the cavity and decreases the probability of leakage. Besides, the depth of less than 3 mm most likely does not remove all of the lateral canals and apical ramifications, therefore posing a risk of reinfection and eventual failure. Various authors[22-25] have suggested a slightly concave root-end cavity for the resin composites with DBAs, allowing the adhesive to cover denuded dentin tubules and the exposed root canals. In contrast, traditional cavity design (3 mm depth, class I cavity) was used in this study as in earlier investigations.[13,15,26] According to some authors,[22-25] this cavity design for root-end preparation is most likely not ideal for a root-end filling with composite, owing to an unfavorable C-factor (bonded/unbonded area ratio). But recent studies[27,28] showed that the C-factor can be reduced using appropriate techniques. Nikolaenko et al.[27] found that using an incremental technique high bond strengths to deep cavity floors can be achieved. Besides, He et al.[28] found that cavity size also was an influential factor in class I cavities, as well as the filling technique. They reported that, in large-sized cavities (5 mm depth), a high C-factor was risky for bonding, in which the incremental technique increased the microtensile bond strength to the cavity floor. However, in the small cavities (3 mm depth), the bond strength at the cavity floor was not affected as much by the filling technique. Additionally, in the small cavities the resin composite at the bottom of the deep cavity can be cured completely. In the present study, to reduce C-factor the root-end cavity was prepared to a 3 mm depth and filled with a hybrid resin composite with different DBAs using incremental technique.

Occlusal loads acting on the tooth have been reported to increase periapical hydrostatic pressure, and this in turn affected the seal between the filling material and the root canal wall.[29] A direct relationship between the magnitude of occlusal pressure and the volume of periapical leakage has also been described.[30] Although many earlier studies evaluated the effects of orthograde fluid leakage and apical seal in root-filled teeth, only a few investigations have been conducted to examine the response in the periapical region to biting forces and the role of cyclic biting forces on periapical fluid dynamics.[31-33] Blum et al.[31] demonstrated that in vitro leakage due to masticatory forces increased over time and was influenced by cavity depth. At all stages of the masticatory cycles, the 1.5 mm cavities showed more leakage than the 3 mm cavities. Peters and Peters,[32] using a computer-controlled masticator to assess the marginal adaptation of 2 root-end filling materials (Super EBA and MTA) in vitro, concluded that both materials displayed excellent marginal adaptation before masticatory loading. After loading, the amount of continuous margin for both root end filling materials decreased slightly but was still high. Kishen[33] investigated the stress distribution pattern in the periapical region caused by biting forces and the role of cyclic biting loads on periapical fluid movement. According to the results, biting forces caused bending of the periapical bone and cyclic biting forces contributed to retrograde fluid movement into the root canal space and extraradicular region. These dynamics may provide an ideal environment for any surviving bacterial species to grow and cause persistent endodontic infections. The effect of masticator forces in the use of DBAs as a root-end filling material must be investigated in a future study.

Adamo et al.[34] compared MTA, Super-EBA, amalgam (with and without ProBond), and TPH composite resin (with ProBond DBA) as root-end filling materials, using a bacterial microleakage model. There were no statistically significant differences between the 5 groups. Additionally, Economides et al.,[26] Pichardo et al.,[35] and Yazdi et al.[36] found that the use of DBAs seemed to be useful in preventing leakage, because they seem to improve sealing ability. In contrast, Murray et al.[37] did not recommend the use of DBAs over the blood-contaminated resected root apices without root-end preparation. In the present study, we compared the root-end sealing of different DBAs. Under the conditions of the study, despite some variations, there were no statistically significant differences in rate of bacterial leakage among the experimental test materials at 1-4 weeks. But Clearfil Protect Bond group showed the least bacterial leakage (2 of 15) among the experimental groups. This result may be due to its special content (12-methacryloyloxydodecylpyridinium bromide [MDPB]), which is an antibacterial monomer synthesized by combining an antibacterial agent and a methacryloyl group and shows antibacterial activity against oral bacteria.[35,38] According to us, the presence of an antibacterial material in DBA might be an advantage because of its disinfecting capability.

Many leakage models have been developed to study the sealing of root-end filling materials. The bacterial model system used in the present study was chosen because it is more clinically relevant.[15] The bacterial species used was *E. faecalis* that is a gram-positive facultative anaerobic bacterium frequently found in asymptomatic failing root canal–treated teeth and is present in large quantities in the oral cavity.[39] Recently, Gomes et al.[40] showed that *E. faecalis* is frequently found in primary necrotic cases. Evans et al.[41] showed that it is resistant to calcium hydroxide because of its proton pump. *Enterococcus faecalis* can also survive by genetic polymorphisms and its ability to bind to dentin, invade dentinal tubules, and survive starvation.[39] It is a

nonfastidious easy-to-grow facultative anaerobic bacterium of significant clinical importance. Other bacteria commonly associated with endodontic infections may require symbiotic support from other bacteria, but *E. faecalis* has been reported to survive and successfully thrive alone.[40]

The use of the in vitro bacterial leakage model used in the present study, although more clinically relevant than dyes or isotopes, has some inherent drawbacks. With this model, the inherent antibacterial activity of the material itself may have affected the outcome in addition to the sealing ability.[15] In addition, only 1 microorganism was evaluated, whereas in vivo the oral microflora are variable and mixed. Polymicrobial cultures should be used in future studies to better simulate clinical conditions, and the long-term sealing of DBAs must be evaluated. The presence of saliva, blood, and purulence in the surgical field was not taken into consideration, and they could ultimately affect the sealing of the materials tested. The use of extracted teeth may have altered the physical characteristics of dentin, especially when autoclaving and ethylene oxides are used.[42] In the present study, only a qualitative measure of bacterial leakage was determined: by the color change in the culture medium. Therefore, the sensitivity of this method is limited. The critical concentration of bacteria required to cause color change was not determined; samples without a demonstrable color change may have also contained bacteria, but they were not plated or assessed spectrophotometrically. A future investigation in the use of DBAs as a root-end filling material may be of how well the marginal adaptation and seal are maintained in the presence of periradicular moisture.

CONCLUSION

Based on the results, the type of DBA had no influence in the bacterial leakage of the root-end fillings. More research is needed to evaluate the potential with this approach.

The authors thank Prof. Murat Ertürk from the Department of Microbiology, School of Medicine, Karadeniz Technical University, for his technical support.

REFERENCES

1. Kakehashi S, Stanley HR, Fitzgerald RJ. The effects of surgical exposures of the dental pulps in germ-free and conventional laboratory rats. Oral Surg Oral Med Oral Pathol Oral Radiol Endod 1965;20:340-9.
2. Möller AJ, Fabricius I, Dahlen G, Ohman AE, Heyden G. Influence on periapical tissues of indigenous oral bacteria and necrotic pulp tissue in monkeys. Scan J Dent Res 1981;89:475-84.
3. Torabinejad M, Eby WC, Naidorf IJ. Inflammatory and immunological aspects of the pathogenesis of human periapical lesions. J Endod 1985;11:479-88.
4. Montellano AM, Schwartz SA, Beeson TJ. Contamination of tooth-colored mineral trioxide aggregate used as a root-end filling material: a bacterial leakage study. J Endod 2006;32:452-5.
5. Gutmann JL, Harrison JW. Surgical endodontics. 1st ed. Boston: Blackwell Scientific; 1991.
6. Chong BS, Pitt Ford TR. Root-end filling materials: rationale and tissue response. Endod Top 2005;11:114-30.
7. Kim S, Kratchman J. Modern endodontic surgery concepts and practice: a review. J Endod 2006;32:601-23.
8. Gartner AH, Dorn SO. Advances in endodontic surgery. Dent Clin North Am 1992;36:357-79.
9. Kitasako Y, Nakajima M, Pereira PN, Okuda M, Sonoda H, Otsuki M, Tagami J. Monkey pulpal response and micro tensile bond strength beneath a one-application resin bonding system in vivo. J Dent 2000;28:193-8.
10. Van Meerbeek B, De Munck J, Yoshida Y, Inoue S, Vargas M, Vijay P, et al. Buonocore memorial lecture. Adhesion to enamel and dentin: current status and future challenges. Oper Dent 2003;28:215-35.
11. Goerig AC, Mueninghoff LA. Management of the endodontically treated tooth. Part II: Technique. J Prost Dent 1983;49:491-7.
12. Spanberg LSW. Experimental endodontics. 1st ed. Boca Raton, FL: CRC Press; 1990.
13. Fogel HM, Peikoff MD. Microleakage of root-end filling materials. J Endod 2001;27:456-8.
14. Xavier CB, Weismann R, de Oliveira MG, Demarco FF, Pozza DH. Root-end filling materials: apical microleakage and marginal adaptation. J Endod 2005;31:539-42.
15. Maltezos C, Glickman GN, Ezzo P, He J. Comparison of the sealing of Resilon, Pro Root MTA, and Super-EBA as root-end filling materials: a bacterial leakage study. J Endod 2006;32:324-7.
16. Eldeniz AU, Hadimli HH, Ataoglu H, Ørstavik D. Antibacterial effect of selected root-end filling materials. J Endod 2006;32:345-9.
17. Bortoluzzi EA, Broon J, Bramante CM, Garcia RB, de Moraes IG, Bernardineli N. Sealing of MTA and radiopaque portland cement with and without calcium chloride for root-end filling. J Endod 2006;32:897-900.
18. Velasquez LM, Sergent RS, Burgess JO, Mercante DE. Effect of placement agitation and placement time on the shear bond strength of 3 self-etching adhesives. Oper Dent 2006;31:426-30.
19. Lopes GC, Baratieri LN, de Andarama MA, Vierira LCC. Dental adhesion: present state of the art and future perspectives. Quintessence Int 2002;33:213-24.
20. Inoue S, Van Meerbeek B, Vargas M, Yoshida Y, Lambrechts P, Vanherle G. Adhesion mechanism of self etching adhesives. In: Tagami J, Toledano M, Prati C, editors. Proceedings of Third International Kuraray Symposium on Advanced Adhesive Dentistry. Cirimido, Italy; Grafiche Erredue: 2000. p. 131–48.
21. Johnson BR. Considerations in the selection of a root-end filling material. Oral Surg Oral Med Oral Pathol Oral Radiol Endod 1999;87:398-404.
22. Andreasen JO, Munksgaard EC, Fredebo L, Rud J. Periodontal tissue regeneration including cementogenesis adjacent to dentin-bonded retrograde composite fillings in humans. J Endod 1993;19:151-3.
23. Gilheany PA, Figdor D, Tyas MJ. Apical dentin permeability and microleakage associated with root end resection and retrograde filling. J Endod 1994;20:22-6.
24. Rud J, Rud V, Munksgaard EC. Retrograde root filling with dentin-bonded modified resin composite. J Endod 1996;22:477-80.
25. Vignaroli PA, Anderson RW, Pashley DH. Longitudinal evalu-

ation of the microleakage of dentin bonding agents used to seal resected root apices. J Endod 1995;21:509-12.
26. Economides N, Kokorikos I, Gogos C, Kolokouris I, Staurianos C. Comparative study of sealing ability of two root-end-filling materials with and without the use of dentin-bonding agents. J Endod 2004;30:35-7.
27. Nikolaenko SA, Lohbauer U, Roggendorf M, Petschelt A, Dasch W, Frankenberger R. Influence of C-factor and layering technique on microtensile bond strength to dentin. Dent Mater 2004;20:579-85.
28. He Z, Shimada Y, Tagami J. The effects of cavity size and incremental technique on microtensile bond strength of resin composite in class I cavities. Dent Mater 2007;23:533-8.
29. Dobo-Nagy C, Fejerdy P, Angyal J, Harasztosi L, Daroczi L, Beke D, Wesselink PR. Measurement of periapical pressure created by occlusal loading. Int Endod J 2003;36:700-4.
30. Esber S, Blum JY, Chazel JC, Parahy E. Effect of masticatory cycles on apical leakage of obturated teeth. J Endod 1998;24:322-5.
31. Blum JY, Esber S, Parahy E, Franquin J-C. Effect of masticatory cycles on tooth compression and resultant leakage of amalgam retrofills. J Endod 1997;23:605-9.
32. Peters CI, Peters OA. Occlusal loading of EBA and MTA root-end fillings in a computer-controlled masticator: a scanning electron microscopic study. Int Endod J 2002;35:22-9.
33. Kishen A. Periapical biomechanics and the role of cyclic biting force in apical retrograde fluid movement. Int Endod J 2005;38:597-603.
34. Adamo HL, Buruiana R, Schertzer L, Boylan RJ. A comparison of MTA, Super-EBA, composite and amalgam as root-end filling materials using a bacterial microleakage model. Int Endod J 1999;32:197-203.
35. Pichardo MR, George SW, Bergeron BE, Jeansonne BG, Rutledge R. Apical leakage of root-end placed Super EBA, MTA, and Geritore restorations in human teeth previously stored in 10% formalin. J Endod 2006;32:956-9.
36. Yazdi PM, Schou S, Jensen SS, Stoltze K, Kenrad B, Sewerin I. Dentine-bonded resin composite (retroplast) for root-end filling: a prospective clinical and radiographic study with a mean follow-up period of 8 years. Int Endod J 2007;40:493-503.
37. Murray MJ, Loushine RJ, Weller RN, Kimbrough F, Pashley DH. Use of self-etching adhesives to seal resected apices. J Endod 2004;30:538-40.
38. Imazato S, Ehara A, Torii M, Ebisu S. Antibacterial activity of dentine primer containing MDPB after curing. J Dent 1998;26:267-71.
39. Stuart CH, Schwartz SA, Beeson TJ, Owats CB. Enterococcus faecalis: its role in root canal treatment failure and current concepts in retreatment. J Endod 2006;32:93-8.
40. Gomes BP, Pinheiro ET, Sousa EL, Jacinto RC, Zaia AA, Ferraz CC, et al. *Enterococcus faecalis* in dental root canals detected by culture and by polymerase chain reaction analysis. Oral Surg Oral Med Oral Pathol Oral Radiol Endod 2006;102:247-53.
41. Evans M, Davies JK, Sundqvist G, Figdor D. Mechanisms involved in the resistance of *Enterococcus faecalis* to calcium hydroxide. Int Endod J 2002;35:221-8.
42. White JM, Goodis HE, Marshall SJ, Marshall GW. Sterilization of teeth by gamma radiation. J Dent Res 1994;73:1560-7.

Reprint requests:

Kürşat Er, DDS, PhD
Karadeniz Technical University
School of Dentistry
Department of Endodontics
61080 Trabzon, Turkey
kursater@ktu.edu.trp