

ORIGINAL ARTICLE

Comparison of bond strength of self-etch adhesive to pulp chamber dentin after placement of calcium hydroxide and various antibiotic pastes

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Abstract

Objective. The aim of this study was to evaluate the effect of calcium hydroxide, double antibiotic paste (DAP) and triple antibiotic paste (TAP) with minocycline, cefaclor and amoxicillin on the micro tensile bond strength (μ TBS) of self-etch adhesive to pulp chamber dentin. **Materials and methods.** Sixty mandibular first molars were cut horizontally and randomly divided into a control group and five experimental groups, which received an intra-canal dressing, as follows: calcium hydroxide, DAP, TAP with minocycline, TAP with cefaclor and TAP with amoxicillin. After storing the specimens for 4 weeks, the medicaments were removed by irrigation with 10 mL each of the following solution: 2.5% NaOCl, 17% EDTA and distilled water. A self-etch adhesive (Clearfil S3 Bond, Okayama, Japan) was applied and composite resin (Clearfil Majesty Posterior, Kuraray Medical Inc., Japan) was placed into the cavity. A μ TBS test was performed on each specimen using a universal test machine. **Results.** The DAP reduced the μ TBS of self-etch adhesive compared to the control group, calcium hydroxide and TAP with minocycline and with cefaclor ($p < 0.05$). However, the other medicaments did not result in a decreased μ TBS of self-etch adhesive to pulp chamber dentin as compared to the control group ($p > 0.05$). **Conclusions.** The use of DAP resulted in a reduced μ TBS of self-etch adhesive to pulp chamber dentin.

Key Words: Adhesion, double antibiotic paste, micro tensile bond strength, minocycline, triple antibiotic paste

Introduction

The re-vascularization of necrotic pulps has become an alternative conservative treatment option to the apexification procedure for necrotic immature permanent teeth. The revascularization procedure allows for the thickening of the root walls by mineralized tissue and continued physiological root development [1,2]. One of the most important stages of the revascularization procedure is the disinfection of the root-canal system. In order to ensure the effective elimination of infection from the root-canal system, several investigators have used a triple antibiotic paste (TAP) [3–5].

Consisting of ciprofloxacin, metronidazole and minocycline, TAP was developed by Hoshino et al. [1]. Several reports have indicated that it has good antimicrobial properties [1,4,5]. Moreover,

Gomes-Filho et al. [6] showed it to be biocompatible over the various experimental periods examined. Despite these positive features, however, several case reports have shown that minocycline causes visible crown discoloration [7,8]. Recently, antibiotic alternatives to minocycline have been proposed for use in combination with ciprofloxacin and metronidazole, including amoxicillin, cefaclor and doxycycline [2,9–12]. Double antibiotic paste (DAP), a combination of metronidazole and ciprofloxacin, has been used as an alternative to TAP because of the risk of discoloration caused by minocycline [2,13,14]. Recently, the cytotoxic effects of TAP, DAP, minocycline and their influence on cytokine mRNA expression levels on human periodontal ligament fibroblasts were investigated. TAP and minocycline were found highly cytotoxic to human periodontal ligament fibroblasts, leading to a decrease of the cell

viability value by comparison with DAP [15]. Additionally, residual TAP remained on the root dentin regardless of removal efforts with different irrigation methods because of its penetration and binding into dentin [16]. Furthermore, DAP was found to be as effective as TAP against endodontic bacteria at high dilutions [17]. Various chemical agents used in root canal treatment can alter the chemical structure of dentin and the mineral content of dentin surfaces [18–20]. Alterations in the original mineral ratio between organic and inorganic components may affect the adhesion of dental materials to hard tissues [21,22]. During re-vascularization procedures, the root and coronal dentine is exposed to antibiotic pastes. Yassen et al. [23] reported that antibiotic pastes and calcium hydroxide may alter the chemical structure of the dentin.

There is limited data on the effect of the antibiotic pastes on the bond strength of self-etch adhesive to pulp chamber dentin. Therefore, the aim of this study was to evaluate the effect of calcium hydroxide, DAP and TAP with minocycline, cefaclor and amoxicillin on the micro-tensile bond strength (μ TBS) of self-etch adhesive to pulp chamber dentin. The null hypothesis was that there would be no differences between the medicaments in terms of the μ TBS of self-etch adhesive to pulp chamber dentin.

Materials and methods

Sixty mandibular first molars fitting the inclusion criteria (below) were selected from a collection of teeth that had been extracted for reasons unrelated to this study. The specimens were stored in 4°C distilled water and used within 3 month after extraction. Before the experimental procedures, the specimens were immersed for disinfection in 0.5% Chloramine-T solution (Merck, Germany) for 48 h. Soft tissues and calculus were removed mechanically from the root surfaces with a periodontal scaler. The teeth were verified radiographically as having a pulp chamber without calcification. Excluded from the study were any teeth having caries or restorative material near the pulp chamber, immature root apices, root canal treatment, internal/external resorption or cracks and fractures on the crown or cervical part of the root.

The specimens were cut horizontally 3 mm above and 2 mm below the cemento-enamel junction with a precision saw (Isomet 1000; Buehler, Lake Bluff, IL) at low speed under water cooling. The pulp tissue was removed with a spoon excavator to avoid contact with the pulpal floor. The apical opening of the root canal was sealed with boxing wax to prevent irrigant extrusion. Then, a protocol was performed according to the American Association of Endodontists (AAE) Regenerative Endodontics Committee [24]. First, copious and gentle irrigation with 20 mL 1.25 % NaOCl was performed for 5 min through the pulp

chamber via a size 27 gauge needle (Ultradent, South Jordan, UT, USA), followed by 20 mL saline for 5 min. The excessive fluid was aspirated and the root canals were dried with paper points (Dentsply Maillefer, Ballaigues, Switzerland). Because the effect of intra-canal medicaments on the bond strength of self-etch adhesive to pulp chamber dentin was to be evaluated, the medicaments were applied into the root canals and the pulpal floor was contaminated with the medicament. The specimens were randomly divided into a control group (without intra-canal dressing) and five experimental groups, which received an intra-canal dressing ($n = 10$), as follows:

- *Control group*: The specimens in this group were left empty;
- *Calcium hydroxide*: The specimens in this group received calcium hydroxide (Spotdent, Izmir, Turkey);
- *DAP*: The specimens in this group received equal portions of metronidazole (Eczacıbası, Istanbul, Turkey) and ciprofloxacin (Biofarma, Istanbul, Turkey);
- *TAP with minocycline*: The specimens in this group received equal portions of metronidazole, ciprofloxacin and minocycline (Ratiopharm, Ulm, Germany);
- *TAP with cefaclor*: The specimens in this group received cefaclor (Sanovel, Istanbul, Turkey), metronidazole and ciprofloxacin; and
- *TAP with amoxicillin*: The specimens in this group received amoxicillin (Bilim, Istanbul, Turkey), metronidazole and ciprofloxacin.

All medicaments were mixed with distilled water (at a powder/liquid ratio of 3:1). The medicaments were placed in the pulp chamber with a 21 gauge needle and the volumes of medicaments used were ~0.2 mL. The cavity was temporarily sealed with a cotton pellet and hydraulic temporary restorative material (MD-Temp; Meta Biomed Co. Ltd, Cheongju, Korea) and the specimens were then kept at 37°C with 100% humidity for 4 weeks. After 4 weeks, the intra-canal dressing was removed by rinsing with 10 mL of 2.5% NaOCl [25]. Then, irrigation was continued, using 10 mL of 17% EDTA and, finally, 10 mL of distilled water. The excessive fluid was aspirated and the root canals were dried with paper points.

A self-etch adhesive (Clearfil S3 Bond, Kuraray Medical Inc., Okayama, Japan) was applied to the whole cavity with an applicator brush. The cavity was dried with a mild air flow. The bond was light cured for 10 s using an LED light-curing unit (Valo Cordless, Ultradent, South Jordan, UT) with an output of 1000 mW/cm². The (shade A3) composite resin, Clearfil Majesty Posterior (Kuraray Medical Inc.), was inserted into the cavity using an incremental technique. Each increment of composite materials was cured for 20 s using an LED light-curing unit.

All specimens were further stored in distilled water at 37°C for 24 h. Then, they were serially sectioned perpendicular to the pulpal floor with the diamond saw to obtain several slabs with approximate dimensions of 2 mm × 1 mm (a size selected after a pilot study revealed that premature failure occurred when attempting to obtain 1 mm × 1 mm slabs). The slabs were carefully evaluated under a stereomicroscope (Zeiss Stemi 2000C; Carl Zeiss, Jena, Germany) at 32× magnification in order to ensure proper orientation of the pulp chamber with regard to the force orientation for bond strength test. For each specimen, two suitable slabs were selected for the μ TBS test ($n = 20$).

The definitive thicknesses of the slices were recorded after measurement using a digital caliper. The specimens were then attached to an apparatus suitable for the μ TBS test with a cyanoacrylate adhesive (Pattex Super Glue; Türk Henkel, Inc., Istanbul, Turkey). Then, a μ TBS test was performed on each specimen using a universal test machine (AGS-X; Shimadzu Corporation, Tokyo, Japan) at a crosshead speed of 1 mm/min. The force at the time of the failure was recorded in MPa by dividing the force into the bonding area size.

After the test procedure, each specimen was visually examined under a stereomicroscope at 32× magnification to evaluate failure mode. Four types of failure were classified: adhesive failure (between the dentin and composite), cohesive failure in composite resin, cohesive failure in dentin and mixed (a combination of cohesive and adhesive failures).

Statistical analysis

The Kolmogorov-Smirnov statistical test for normality revealed a normal data distribution ($p > 0.05$). Tests of homogeneity of variance revealed that the variances were homogeneous ($p > 0.05$). Analysis of variance (ANOVA) and Fisher's Least Significant Difference (LSD) *post hoc* tests were performed to evaluate the effect of medicament on μ TBS. To compare the failure mode among the groups, a chi-square test was performed ($p = 0.05$). All statistical analyses were performed using IBM® SPSS® Statistics 20 software (IBM SPSS Inc., Chicago, IL).

Results

μ TBS values for the groups are shown in Figure 1. Statistical analysis revealed that none of the tested medicaments except DAP decreased the μ TBS of self-etch adhesive to pulp chamber dentin as compared to the control group ($p > 0.05$). DAP reduced the μ TBS of self-etch adhesive compared to the control group, calcium hydroxide and TAP with minocycline and with cefaclor ($p < 0.05$). Representative image of the junction between the composite

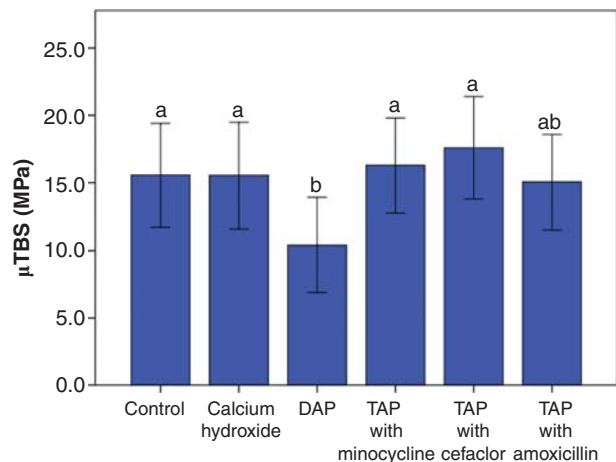


Figure 1. Mean micro tensile bond strength (μ TBS) of self-etch adhesive to pulp chamber dentin values after the application of calcium hydroxide, double antibiotic paste (DAP), triple antibiotic paste (TAP) with minocycline, cefaclor and amoxicillin. The different letters indicate statistically significant differences. Error bars represent confidence intervals.

resin and the dentin treated with different medicaments are shown in Figure 2.

Adhesive failure between cement and dentin was the most frequent type of failure mode in all groups

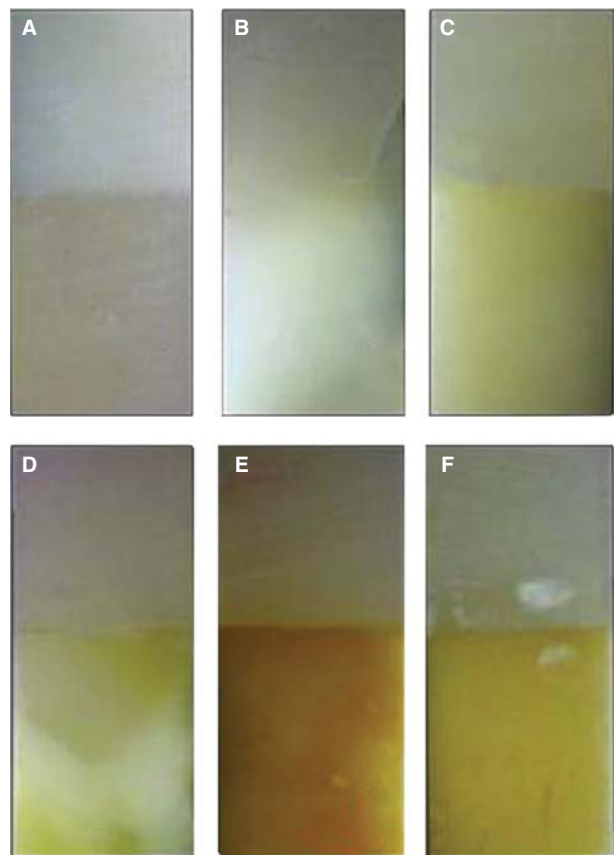


Figure 2. Representative image of the junction between the composite resin and the dentin treated with different medicaments. (A) Control group, (B) calcium hydroxide, (C) DAP, (D) TAP with amoxicillin, (E) TAP with minocycline and (F) TAP with cefaclor.

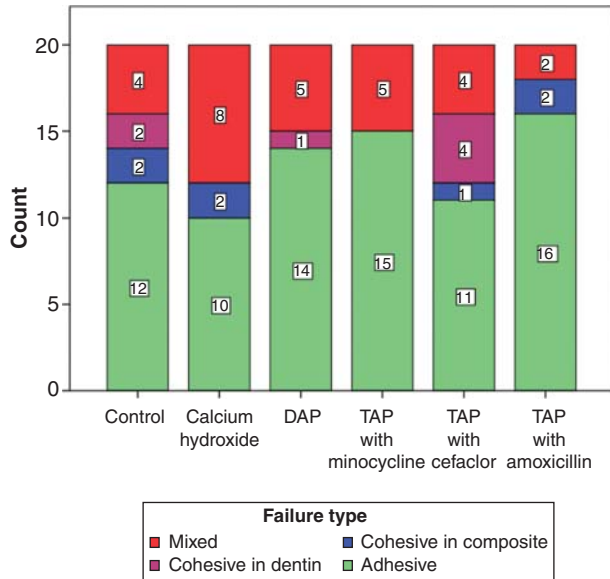


Figure 3. Failure type by group.

(Figure 3). A chi-square test revealed that there were no significant differences among the groups according to failure type ($p > 0.05$).

Discussion

Regenerative endodontic procedures depend on chemical debridement of the root canal system with minimal to no mechanical instrumentation. After the disinfection procedure, the treatment strategy includes intra-canal medicament removal followed by the placement of mineral trioxide aggregate and restorative material [12,26]. Several intra-canal medicaments such as calcium hydroxide and various antibiotic pastes have been used for disinfection of the root canal system [2,9–12,27]. However, it has been shown that it is difficult to completely remove these intra-canal medicaments from the root canals [25]. Moreover, a recent study showed antibiotic pastes to cause significant decrease in the microhardness of root dentin [28] and alter the chemical structure of the dentin [23]. The inability of complete removal of antibiotic pastes and the effects of the intra-canal medicaments on the dentin may influence the bond strength of self-etch adhesive to pulp chamber dentin of molar teeth. Therefore, this study compared the application of calcium hydroxide, DAP and TAP with minocycline, cefaclor and amoxicillin to a control group on the μ TBS of self-etch adhesive to pulp chamber dentin in the molar teeth.

According to the findings, DAP placement resulted in decreased μ TBS of self-etch adhesive to pulp chamber dentin. Therefore, the null hypothesis that there would be no differences between the medicaments in terms of the μ TBS of self-etch adhesive to pulp chamber dentin can be rejected. The lowest

bond strength values after DAP application could depend on a number of factors. Tanase et al. [29] indicated that minocycline in the TAP binds to calcium ions and demineralizes dental hard tissue via chelation to form an insoluble complex in the tooth matrix. The lower pH value of TAP than DAP may affect the dentinal–resin interface, which, in turn, could enhance the μ TBS of self-etch adhesive. The significant reduction in μ TBS of self-etch adhesive in DAP compared with TAP with minocycline and with cefaclor, that is, may be explained by the higher pH of DAP than TAP [28]. Following the demonstration of successful clinical and radiographic outcome for the regenerative endodontic treatment approach in single rooted teeth [2,7,11], researchers have also employed this treatment approach on molar teeth [30–32]. Thus, in the present study mandibular first molars were used.

Clearfil S3 Bond was preferred as a self-etch adhesive in the present study. Self-etch adhesives have weak acids in their primer composition, which results in less change in the dentinal wall structure than with the strong acids of total etch systems. Residual calcium is an important factor for dentin adhesion. It has been claimed that removal of calcium may be more detrimental for adhesives that rely on dentin acid etching than for adhesives based on self-etching primers [33]. This could be an explanation for the non-affected μ TBS values shown in the present study for TAP with minocycline. There are no data in the available literature on the μ TBS of self-etch adhesive concerning the comparison of intra-canal medicaments. Further investigations with a similar experimental set-up should be performed to re-assess the results.

Only limited data are available in the literature regarding the effect of antibiotic pastes on the μ TBS of self-etch adhesive to pulp chamber dentin, so the full range of findings of this study could not be directly compared to other studies. Only the values for the control group in the present study could be compared with those of previous studies in which the effect of irrigating solutions on the μ TBS of adhesives was evaluated. In one such study, the μ TBS of Clearfil S3 Bond to pulp chamber dentin was found to be 36.36 Mpa for saline irrigation [34]. In the present study, however, the μ TBS of Clearfil S3 Bond to pulp chamber dentin was 15.57 MPa for the control group (using NaOCl followed by saline irrigation solutions). In a study by Yurdagüven et al. [35], the μ TBS of a total etch (XP-Bond) and a self-etch (Clearfil SE Bond) adhesive system to dentin surfaces treated using endodontic irrigants were evaluated. They used distilled water as the control and found the μ TBS of the self-etch adhesive system to be 55.53 MPa much higher than the values obtained in the present study. The difference between the results of the studies may explained by the use of NaOCl in

the present study and the different adhesive systems used.

A recent study has demonstrated that it was difficult to remove TAP from root canals using irrigating solutions [25]. This also found that the use of 2.5% NaOCl improved the removal of TAP. Based on these findings in the present study, the intra-canal dressing was removed by rinsing with 10 mL of 2.5% NaOCl; then, irrigation was continued using 10 mL of 17% EDTA and finally 10 mL of distilled water.

As mentioned, because of the premature failure when attempting to obtain 1 mm × 1 mm dimension slabs, ~2 mm × 1 mm dimension slabs were obtained in this study. However, the definitive thicknesses of the slices were recorded using a digital caliper and the bond strength expressed in MPa at failure was calculated by dividing the load in Newtons by the area of the bonded interface. Thereby, the bonded interface difference was eliminated.

Conclusions

DAP reduced the μ TBS of self-etch adhesive to pulp chamber dentin as compared to a control group. However, calcium hydroxide and TAP with minocycline, cefaclor and amoxicillin did not, decrease the μ TBS of self-etch adhesive to pulp chamber dentin.

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References

- [1] Hoshino E, Kurihara-Ando N, Sato I, Uematsu H, Sato M, Kota K, et al. In-vitro antibacterial susceptibility of bacteria taken from infected root dentine to a mixture of ciprofloxacin, metronidazole and minocycline. *Int Endod J* 1996;29:125–30.
- [2] Iwaya SI, Ikawa M, Kubota M. Revascularization of an immature permanent tooth with apical periodontitis and sinus tract. *Dent Traumatol* 2001;17:185–7.
- [3] Reynolds K, Johnson JD, Cohenca N. Pulp revascularization of necrotic bilateral bicuspids using a modified novel technique to eliminate potential coronal discoloration: a case report. *Int Endod J* 2009;42:84–92.
- [4] Thibodeau B, Teixeira F, Yamauchi M, Caplan DJ, Trope M. Pulp revascularization of immature dog teeth with apical periodontitis. *J Endod* 2007;33:680–9.
- [5] Sato I, Ando-Kurihara N, Kota K, Iwaku M, Hoshino E. Sterilization of infected root-canal dentine by topical application of a mixture of ciprofloxacin, metronidazole and minocycline in situ. *Int Endod J* 1996;29:118–24.
- [6] Gomes-Filho JE, Duarte PC, de Oliveira CB, Watanabe S, Lodi CS, Cintra LT, et al. Tissue reaction to a triantibiotic paste used for endodontic tissue self-regeneration of nonvital immature permanent teeth. *J Endod* 2012;38:91–4.
- [7] Kim JH, Kim Y, Shin SJ, Park JW, Jung IY. Tooth discoloration of immature permanent incisor associated with triple antibiotic therapy: a case report. *J Endod* 2010;36:1086–91.
- [8] Miller EK, Lee JY, Tawil PZ, Teixeira FB, Vann WF Jr. Emerging therapies for the management of traumatized immature permanent incisors. *Pediatr Dent* 2012;34:66–9.
- [9] Thomson A, Kahler B. Regenerative endodontics—biologically-based treatment for immature permanent teeth: a case report and review of the literature. *Aust Dent J* 2010;55:446–52.
- [10] Sato T, Hoshino E, Uematsu H, Noda T. In vitro antimicrobial susceptibility to combinations of drugs on bacteria from carious and endodontic lesions of human deciduous teeth. *Oral Microbiol Immunol* 1993;8:172–6.
- [11] Bezgin T, Yilmaz AD, Celik BN, Sonmez H. Concentrated platelet-rich plasma used in root canal revascularization: 2 case reports. *Int Endod J* 2014;47:41–9.
- [12] Tawfik H, Abu-Seida AM, Hashem AA, Nagy MM. Regenerative potential following revascularization of immature permanent teeth with necrotic pulps. *Int Endod J* 2013;46:910–22.
- [13] Hargreaves KM, Diogenes A, Teixeira FB. Treatment options: biological basis of regenerative endodontic procedures. *J Endod* 2013;39:S30–43.
- [14] Akcay M, Arslan H, Yasa B, Kavrik F, Yasa E. Spectrophotometric analysis of crown discoloration induced by various antibiotic pastes used in revascularization. *J Endod* 2014;40:845–8.
- [15] Yadlapati M, Souza LC, Dorn S, Garlet GP, Letra A, Silva RM. Deleterious effect of triple antibiotic paste on human periodontal ligament fibroblasts. *Int Endod J* 2014;47:769–75.
- [16] Berkhoff JA, Chen PB, Teixeira FB, Diogenes A. Evaluation of triple antibiotic paste removal by different irrigation procedures. *J Endod* 2014;40:1172–7.
- [17] Sabrah AHA, Yassen GH, Gregory RL. Effectiveness of antibiotic medicaments against biofilm formation of enterococcus faecalis and porphyromonas gingivalis. *J Endod* 2013;39:1385–9.
- [18] Ari H, Erdemir A. Effects of endodontic irrigation solutions on mineral content of root canal dentin using ICP-AES technique. *J Endod* 2005;31:187–9.
- [19] Cobankara FK, Erdogan H, Hamurcu M. Effects of chelating agents on the mineral content of root canal dentin. *Oral Surg Oral Med Oral Pathol Oral Radiol Endod* 2011;112:e149–54.
- [20] Zhang K, Kim YK, Cadenaro M, Bryan TE, Sidow SJ, Loushine RJ, et al. Effects of different exposure times and concentrations of sodium hypochlorite/ethylenediaminetetraacetic acid on the structural integrity of mineralized dentin. *J Endod* 2010;36:105–9.
- [21] Hennequin M, Pajot J, Avignant D. Effects of different pH values of citric acid solutions on the calcium and phosphorus contents of human root dentin. *J Endod* 1994;20:551–4.
- [22] Rotstein I, Dankner E, Goldman A, Heling I, Stabholz A, Zalkind M. Histochemical analysis of dental hard tissues following bleaching. *J Endod* 1996;22:23–5.
- [23] Yassen GH, Chu T-MG, Eckert G, Platt JA. Effect of medicaments used in endodontic regeneration technique on the chemical structure of human immature radicular dentin: an in vitro study. *J Endod* 2013;39:269–73.
- [24] American Association of Endodontists. Considerations for regenerative procedures. American Association of Endodontists, 2012. Available online at www.aae.org/Dental_Professionals/Considerations_for_Regenerative_Procedures.aspx. accessed 5 November 2012.
- [25] Arslan H, Capar ID, Saygili G, Uysal B, Gok T, Ertas H, et al. Efficacy of various irrigation protocols on the removal of triple antibiotic paste. *Int Endod J* 2014;47:594–9.
- [26] Zhu W, Zhu X, Huang GTJ, Cheung GSP, Dissanayaka WL, Zhang C. Regeneration of dental pulp tissue in immature teeth

- with apical periodontitis using platelet-rich plasma and dental pulp cells. *Int Endod J* 2013;46:962–70.
- [27] Soares Ade J, Lins FF, Nagata JY, Gomes BP, Zaia AA, Ferraz CC, et al. Pulp revascularization after root canal decontamination with calcium hydroxide and 2% chlorhexidine gel. *J Endod* 2013;39:417–20.
- [28] Yassen GH, Chu TM, Eckert G, Platt JA. Effect of medications used in endodontic regeneration technique on the chemical structure of human immature radicular dentin: an in vitro study. *J Endod* 2013;39:269–73.
- [29] Tanase S, Tsuchiya H, Yao J, Ohmoto S, Takagi N, Yoshida S. Reversed-phase ion-pair chromatographic analysis of tetracycline antibiotics. Application to discolored teeth. *J Chromatogr B Biomed Sci Appl* 1998;706:279–85.
- [30] Nosrat A, Seifi A, Asgary S. Regenerative endodontic treatment (revascularization) for necrotic immature permanent molars: a review and report of two cases with a new biomaterial. *J Endod* 2011;37:562–7.
- [31] Cehreli ZC, Isbitiren B, Sara S, Erbas G. Regenerative endodontic treatment (revascularization) of immature necrotic molars medicated with calcium hydroxide: a case series. *J Endod* 2011;37:1327–30.
- [32] Sonmez IS, Akbay Oba A, Erkmen Almaz M. Revascularization/Regeneration performed in immature molars: case reports. *J Clin Pediatr Dent* 2013;37:231–4.
- [33] Perdigao J, Eiriksson S, Rosa BT, Lopes M, Gomes G. Effect of calcium removal on dentin bond strengths. *Quintessence Int* 2001;32:142–6.
- [34] Barutçigil C, Arslan H, Ozcan E, Harorli O. Micro-tensile bond strength of adhesives to pulp chamber dentin after irrigation with Ethylenediaminetetraacetic acid. *J Conserv Dent* 2012;15:242–5.
- [35] Yurdagüven H, Tanalp J, Toydemir B, Mohseni K, Soyman M, Bayirli G. The effect of endodontic irrigants on the microtensile bond strength of dentin adhesives. *J Endod* 2009;35:1259–63.