Clin Oral Invest (2015) 19:1437–1442
DOI 10.1007/s00784-014-1364-5

ORIGINAL ARTICLE

Comparison of bleaching efficacy of two bleaching agents on teeth discoloured by different antibiotic combinations used in revascularization

Bilal Yasa · Hakan Arslan · Merve Akcay · Fevzi Kavrik · Huseyin Hatirli · Bulent Ozkan

Received: 30 June 2014 / Accepted: 13 November 2014 / Published online: 22 November 2014

Abstract
Objectives To investigate the whitening effects of different bleaching agents on teeth discoloured by different antibiotic combinations of ciprofloxacin and metronidazole with minocycline, doxycycline, amoxicillin or cefaclor.
Materials and methods Forty extracted bovine incisors were collected and discoloured with triple antibiotic pastes (TAP) with minocycline, doxycycline, amoxicillin and cefaclor throughout 30 days. The specimens were then randomly divided into two subgroups and each group received different bleaching materials: 35 % hydrogen peroxide and sodium perborate. Spectrophotometric measurements were obtained on the buccal surfaces of the crown, firstly in the beginning, then on the 4th, 8th and 12th days after the placement of the bleaching materials. The acceptability threshold was set to 3.5. The ΔE values were calculated and the data was analysed using the repeated measures analysis of variance (P=.05).
Results All the test groups induced colour changes exceeding the acceptability threshold 30 days after the antibiotic pastes were placed. The 35 % hydrogen peroxide was more effective than sodium perborate in the whitening of discoloured teeth by antibiotic pastes (P=.001). The whitening effect after the 8th and 12th days was significantly more than after 4 days of treatment (P <.001). The discolouration caused by the TAP with minocycline and cefaclor showed greater whitening compared to the TAP with doxycycline and amoxicillin groups (P <.05).
Conclusions The whitening treatment effect of 35 % hydrogen peroxide on teeth discoloured by antibiotic pastes seems to have significantly outperformed the sodium perborate treatment. Both bleaching agents were allowed to bleach the teeth gradually each day and the effects on the 8th and 12th days were superior to the one on the 4th day.
Clinical relevance The use of 35 % hydrogen peroxide could be advantageous to bleach the teeth discoloured with antibiotic pastes compared to sodium perborate.

Keywords Amoxicillin · Bleaching · Cefaclor · Doxycycline · Hydrogen peroxide · Minocycline · Sodium perborate

Introduction

Revascularisation enables the thickening of the root walls by mineralized tissue and the continuation of physiological root development. It has become an alternative conservative treatment option to the apexification procedure for immature permanent teeth [1]. One of the most important stages of the revascularisation procedure is the disinfection of the root canal system. In order to eliminate infection from the root canal system effectively, several investigators have used antibiotic pastes [1–4]. However, several studies have demonstrated that the antibiotic pastes induced crown discolouration [5–7].

The internal bleaching technique is a minimally invasive, simple, and cost-effective intervention and causes only slight risks, if performed correctly [8]. Among the internal bleaching agents, hydrogen peroxide was used at different

B. Yasa · H. Hatirli
Department of Restorative Dentistry, Faculty of Dentistry, Izmir Katip Celebi University, Izmir, Turkey

H. Arslan
Department of Endodontics, Faculty of Dentistry, Atatürk University, Erzurum, Turkey

M. Akcay (✉) · F. Kavrik
Department of Pediatric Dentistry, Faculty of Dentistry, Izmir Katip Celebi University, Izmir, Turkey
e-mail: merve.akcay@ikc.edu.tr

B. Ozkan
Department of Biostatistics and Medical Informatics, Izmir Katip Celebi University, Izmir, Turkey

concentrations ranging from 5–35 %. Because of its low molecular weight, it can penetrate dentin and can release oxygen. The oxygen breaks the double bonds of the organic and inorganic compounds inside the dentinal tubules. Sodium perborate, another internal bleaching agent, is available as powder. It is easier to control and is safer than concentrated hydrogen peroxide solutions [9].

There are no studies available that compare the whitening effect of different bleaching agents on teeth discoloured by different antibiotic combinations used for regenerative procedures. Therefore, this study was conducted using different bleaching agents (35 % hydrogen peroxide as well as sodium perborate) to assess their whitening effects on the teeth discoloured by different antibiotic pastes of ciprofloxacin and metronidazole combined with minocycline, doxycycline, amoxicillin or cefaclor. The null hypotheses of this study were; (1) teeth would not be differentially discoloured by the tested antibiotic pastes and (2) there would be no significant differences between the tested bleaching agents on the teeth discoloured with different antibiotic pastes.

Materials and methods

Power analysis

The necessary sample size was calculated as 40 to give 84 % power, 0.48 effect size and 0.05 significance level accompanied by a repeated measures design with 2 between factors, antibiotic paste (4 levels) and bleaching material (2 levels), and 1 within factor, time with 3 levels.

Sample selection and preparation

Forty bovine maxillary incisors were collected and disinfected by immersion in 0.5 % Chloramine-T solution (Merck, Germany) for 48 h. The specimens were stored in distilled water until they were used. Each specimen was sectioned using a cylindrical bur under water-cooling to obtain a standardised root length of 10 mm apical to the facial cementoenamel junction (CEJ).

The bucco-lingual and mesial-distal dimensions at the CEJ and the thickness between the root canal wall and external root surface were measured using electronic digital callipers and were recorded for each specimen. On the basis of the sum of these three measurements, teeth with similar measurements were distributed equally across the eight groups. Statistical analysis by one-way ANOVA confirmed no significant differences among the groups in terms of their bucco-lingual dimensions (P=.694), mesial-distal dimensions (P=.757) and root canal wall thickness (P=.517).

The pulp tissue was apically removed using a spoon excavator and the root canal was irrigated with 10 mL of 5.25 % sodium hypochlorite for 2 min to remove any remaining pulp tissue, and then with 10 mL of 17 % EDTA for 2 min, followed by 10 mL of distilled water in order to simulate clinical irrigation. After the root canal space had been dried using cotton, the pulp chamber was filled with a hydraulic temporary filling material (MD-Temp; META Biomed Co Ltd, Cheongju, Korea) 2 mm coronal to the CEJ. A cotton pellet was loosely placed on the temporary filling material up to the facial CEJ. The specimens were then apically filled using syringe according to the following 4 groups:

TAP with minocycline: The specimens in this group received equal portions of metronidazole, (Eczacıbası, Istanbul, Turkey), ciprofloxacin (Biofarma, Istanbul, Turkey) and minocycline (Ratiopharm, Ulm, Germany) mixed with distilled water (at a powder/liquid ratio of 3:1).

TAP with doxycycline [10]: The specimens in this group received equal portions of doxycycline (Deva, Istanbul, Turkey), metronidazole and ciprofloxacin mixed with distilled water as mentioned above.

TAP with amoxicillin [11]: The specimens in this group received amoxicillin (Bilim, Istanbul, Turkey), metronidazole and ciprofloxacin.

TAP with cefaclor [3]: The specimens in these groups received cefaclor (Sanovel, Istanbul, Turkey), metronidazole and ciprofloxacin.

The volumes of the antibiotics used were approximately 0.5 mL. The apical openings were sealed with sticky wax, and all the specimens were stored at 100 % humidity in an incubator at 37 °C for 30 days. A coronal endodontic access cavity (approximately 7×9 mm) was performed with a size #9 round bur. The temporary filling material and cotton pellet were removed, and the antibiotic pastes were removed by using 10 mL of 5.25 % NaOCl agitated using an ultrasonic file. The root was then filled with the temporary filling material (META Biomed Co., Ltd.) from the apical foramen to the CEJ. A 2.0-mm thick glass-ionomer cement base (3M ESPE, Ketac Molar, Seefeld, Germany) was placed at the level of the CEJ. Each group was divided into 2 subgroups to determine the whitening effects of the bleaching agents as follows (n=5):

35 % hydrogen peroxide: The pulp chamber was filled with 35 % hydrogen peroxide (Opalescence®Endo; Ultradent Products Inc., South Jordan, UT, USA) by using its own syringe.

Sodium perborate: The pulp chamber was filled with the mixture of tetrahydrate sodium perborate (Sultan Healthcare, Hackensack, NJ, USA) and distilled water by using a plastic amalgam carrier.

After the access cavity of the tooth was sealed with provisional adhesive restoration (Filtek Ultimate, 3M ESPE,

Seefeld, Germany), the bleaching agents were left for 4 days in distilled water at 37° C. Then, the composite resin was removed, the bleaching agent was washed out with water, and a fresh bleaching agent was placed into the access cavity. This procedure was repeated three times (at days 4, 8 and 12) with a 4-day interval.

Tooth colour assessment

The colour measurements were recorded 30 days after the placement of the antibiotic pastes (baseline), as well as on the days 4, 8 and 12 after the placement of the bleach. The colour of each specimen was assessed by the CIE-Lab system, which was defined according to the International Commission on Illumination in 1967 and referred to as CIE-Lab (Commission Internationale de L'Eclairage, 1978), in L*a*b* mode by using a noncontact-type spectrophotometre (Spectro Shade™ Micro, MHT, Milan, Italy) on the buccal surfaces of the crown. A standardised circular strip with a diameter of 6 mm was bonded to the buccal surface of the crown 2 mm coronal to the CEJ to ensure that the colour measurement was performed on the same region in each turn with a vertical angle [12]. The colour measurements were performed thrice on a white background at each time point, and then the mean of the three measurements was calculated. The acceptability threshold was set to 3.5.

According to the manufacturer's recommendation, the spectrophotometer was calibrated on the calibration tile for each group at each time point. L^* represents lightness, from 0=black to 100=white; a^* represents the measurement along the green-red coordinate and b^* represents the measurement along the blue-yellow coordinate. The total colour differences or the distances between the two colours (ΔE) were calculated using the following formula:

$$\Delta E* = \sqrt{\Delta L*^2 + \Delta a*^2 + \Delta b*^2}$$

Statistical analysis

All the statistical analyses were carried out at a significance level of 0.05 and confidence interval of 95 %. A one-way analysis of variance (ANOVA) was used to determine if there were statistically significant differences in regards to the baseline colours. The repeated measures ANOVA with 2 between factors and 1 within factor were used for the evaluation of the results. The within factor was "time" with three levels. All the statistical analyses were performed using the IBM SPSS Statistics software version 20 (SPSS; IBM, Chicago, IL, USA).

Results

All of the test groups induced colour changes exceeding the acceptability threshold 30 days after the antibiotic pastes had been placed (Fig. 1). The samples discoloured by various antibiotic pastes had a significantly different baseline ΔEs (P <.001). The TAP with minocycline induced the most discolouration among the groups, and the TAP with amoxicillin did the least. The differences between the TAP with minocycline and the other antibiotic pastes were statistically significant (P <.05). However, there were no statistically significant differences between the TAP with doxycycline, the TAP with amoxicillin and the TAP with cefaclor (P >.05).

The repeated measures analysis of variance indicated that the whitening effect of the 35 % hydrogen peroxide was superior to that of the sodium perborate (P=.001) (Fig. 2). The whitening in the discoloured teeth with the antibiotic pastes on the 8th and 12th days after the placement of bleach material was also superior to the one on the 4th day (P <.001). However, there was no statistically significant difference between the 8th and 12th-day measurements (P=.175) (Table 1). The discolouration caused by the TAP with minocycline and cefaclor was more whitening compared to the TAP with doxycycline and amoxicillin groups (P <.05) (Fig. 3).

Discussion

Various antibiotic pastes are currently used in endodontics to disinfect the root canal system [1–3, 5, 11]. According to the results of this study, all the tested antibiotics induced crown discolouration. However, the TAP with minocycline induced significantly more discolouration than the other tested antibiotic pastes. The first null hypothesis which states that the teeth would not be differentially discoloured by the tested antibiotic pastes was rejected. Previous studies have found that the TAP with minocycline resulted in discolouration [5, 13, 14]. The results of these studies partly confirm our findings. The mechanisms by which the TAP with minocycline impacts on coronal tooth discolouration may be related to the binding of minocycline to the calcium ions via chelation [15]. However, the mechanisms of the others should be studied in the future. Another interesting finding in the present study was that, the discolouration caused by the TAP with minocycline and cefaclor has also shown more whitening effect compared to the TAP with doxycycline and amoxicillin groups.

The main aim of this study was to assess the whitening effects of 35 % hydrogen peroxide and sodium perborate on the teeth discoloured by different antibiotic combinations. The highest level of whitening effect of the bleaching agents occurred on the 12th day. The whitening effect also increased day by day and exceeded the acceptability threshold from the 4th day of the evaluation in all the antibiotic paste subgroups

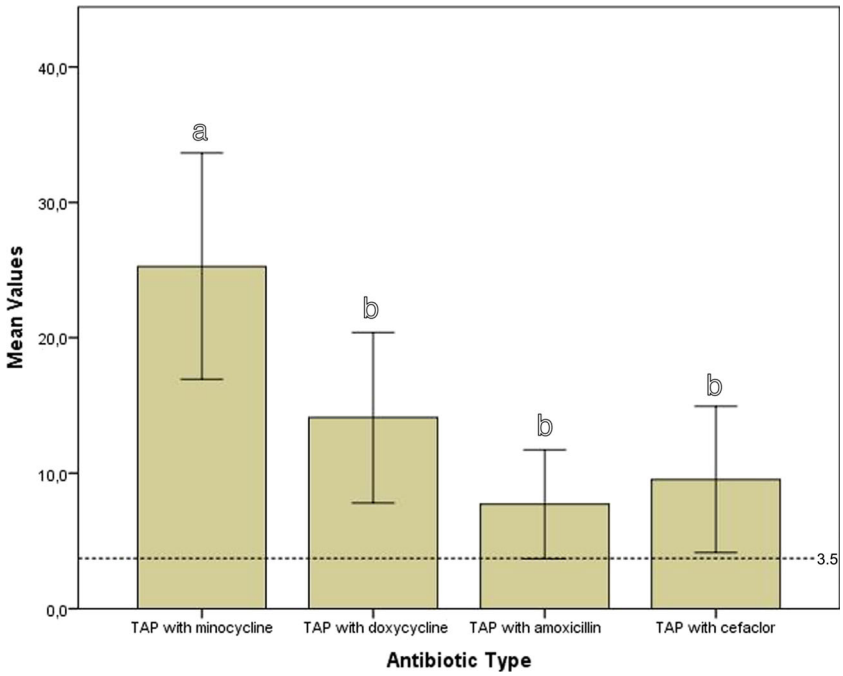

Fig. 1 Colour changes of the groups at 30 days after the placement of the antibiotic pastes. The *different letters* show the statistically significant differences between the groups (P <.05). Note: the discolouration exceeded the acceptability threshold

except for the doxycycline and amoxicillin with sodium perborate groups. Within the limitations of this experimental study, the whitening effect of the 35 % hydrogen peroxide was superior to that of the sodium perborate. Therefore, the second null hypothesis could also be partly rejected. However, there are no previous studies to use as a reference to compare the results of bleaching agents on teeth discoloured with different antibiotic pastes. Lim et al. [16] artificially stained the teeth using whole blood, and whitened them using different bleaching agents. They found that 35 % hydrogen

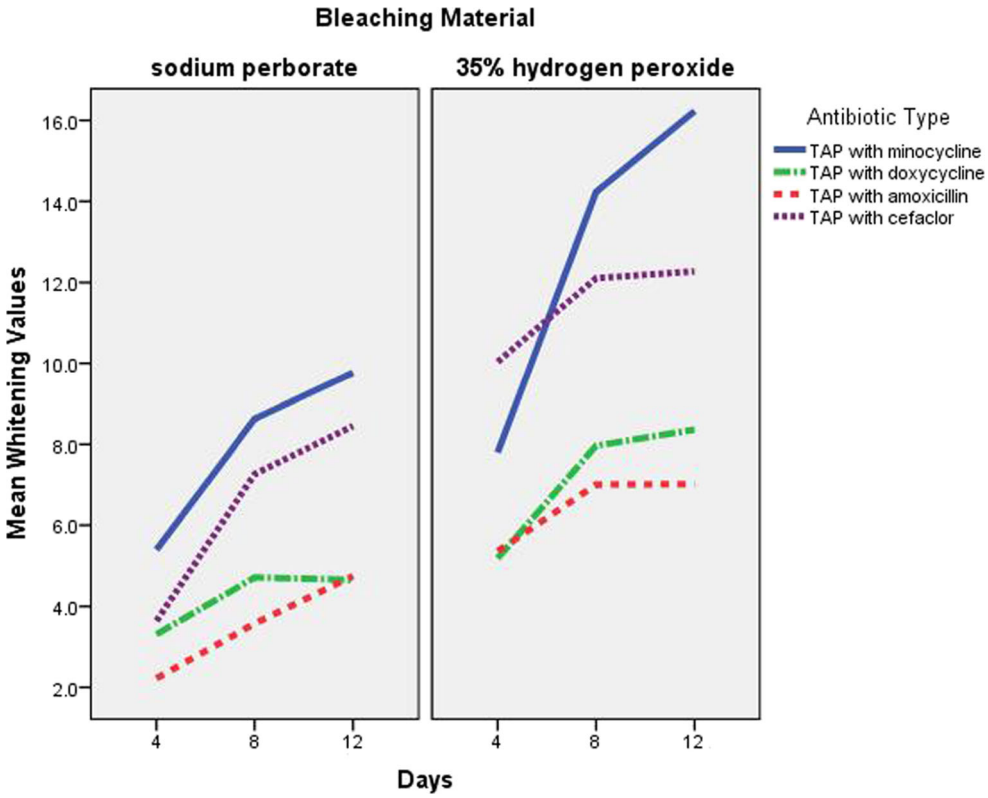

Fig. 2 Whitening effects of the bleaching materials according to various antibiotic pastes at different time periods. The whitening on the 8th and 12th days after the placement of the bleach material was superior to the one on the 4th day (P <.001). There was no statistically significant difference between the 8th and 12th day measurements (P=.175). The discolouration caused by the TAP with minocycline and cefaclor were more whitening, compared to the TAP with doxycycline and amoxicillin groups (P <.05)

Table 1 Mean whitening values and standard deviation of the bleaching materials according to various antibiotic pastes at 4th, 8th and 12th days

Antibiotic Type	Bleaching material	4th day	8th day	12th day
TAP with minocycline	Sodium perborate	5.39±1.82	8.63±1.62	9.77±1.67
	35 % Hydrogen peroxide	7.80±3.15	14.23±8.74	16.23±11.77
TAP with doxycycline	Sodium perborate	3.31±1.18	4.72±2.07	4.66±2.89
	35 % Hydrogen peroxide	5.18±1.85	7.97±3.54	8.36±1.70
TAP with amoxicillin	Sodium perborate	2.22±0.72	3.58±1.92	4.75±2.74
	35 % Hydrogen peroxide	5.36±1.60	7.01±1.48	7.02±1.87
TAP with cefaclor	Sodium perborate	3.64±1.61	7.26±2.35	8.45±3.12
	35 % Hydrogen peroxide	10.04±2.09	12.11±2.91	12.28±3.42

The acceptability threshold was set to 3.5. The whitening effect of the 35 % hydrogen peroxide was superior to that of the sodium perborate (P=.001). The whitening on the 8th and 12th days after the placement of the bleach material was superior to the one on the 4th day (P <.001). There was no statistically significant difference between the 8th day and 12th day measurements (P=.175). The discolouration caused by the TAP with minocycline and cefaclor were more whitening compared to the TAP with doxycycline and amoxicillin groups (P <.05)

peroxide was more effective than sodium perborate for intracoronal bleaching. This result is in agreement with the results obtained in the present study.

Sodium perborate occurs in the form of mono-, tri- or tetrahydrate. Although tetrahydrate sodium perborate presents small amount of active oxygen (10.4 %) compared with the other forms, it remains active until the changing time [17]. The high concentration of the bleaching agents promotes faster and effective tooth bleaching [16]. However, considering the biological aspects, it is recommended to use the low concentration agent to avoid the damage of dental and periodontal tissues. But, possible damage can be prevented by using a correct cervical sealing [18]. When the severe discolouration caused by TAPs is considered, the use of high concentration of bleaching agents is more predictable. Previous studies have conceded that, hydrogen peroxide, especially in combination with sodium perborate, is very effective for bleaching discoloured teeth [17, 19]. However, high concentration hydrogen peroxide liquid can be very caustic when used as a bleaching agent [9]. This caustic effect can be more dramatic in immature teeth, having more widened dentinal tubules in comparison to mature teeth.

In this study, bovine incisors were used to assess the discolouration by various antibiotic pastes. Schilke et al. [20] reported that, the number of dentinal tubules in bovine coronal dentine did not significantly differ from the dentine of human teeth. In the present study, we assessed the coronal discolouration in bovine incisors; therefore, the results of the aforementioned study suggest that, bovine coronal dentine may be suitable for the assessment of discolouration [14]. Another reason for the use of bovine teeth was that the pulp was removed from the apical aspect; therefore, that was more predictable in the bovine model.

Previous studies on root canal treatment and revascularisation treatment have looked into the effects of antibiotic pastes left in the root canal over various periods of time [3, 21]. In the previous studies, the pastes were left in the canal for up to 6 weeks [5, 6, 22, 23]. In this study, the antibiotic pastes were left in the root canals up to 30 days. After this period, all of the tested antibiotics induced crown discolouration exceeding the acceptability threshold.

In a clinical setting, the combination of blood with residual antibiotic pastes remaining in the canal may have contributed to staining. In the present study, only the staining after placement of the antibiotic pastes was evaluated. This was the limitation for this study.

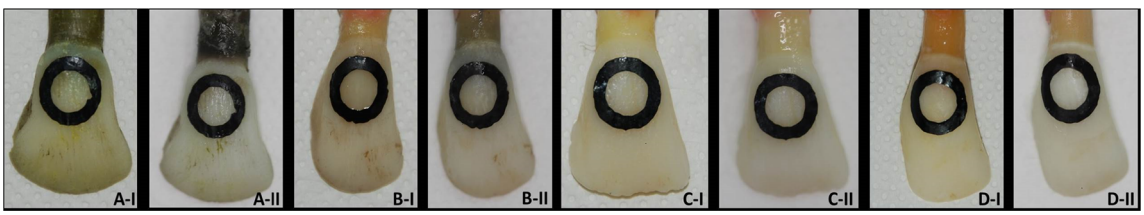

Fig. 3 Representative images of the specimens before (30 days after the placement of antibiotic pastes) and after the bleaching procedures (on the 12th day) by group. The whitening effect of 35 % hydrogen peroxide on teeth discoloured by TAP with minocycline (*A-I* and *A-II*). The whitening effect of sodium perborate on teeth discoloured by TAP with doxycycline (*B-I* and *B-II*). The whitening effect of 35 % hydrogen peroxide on teeth discoloured by TAP with amoxicillin (*C-I* and *C-II*). The whitening effect of sodium perborate on teeth discoloured by TAP with cefaclor (*D-I* and *D-II*)

Conclusion

For the intracoronal bleaching in the teeth discoloured with antibiotic pastes, 35 % hydrogen peroxide was more effective than sodium perborate. The whitening effects of both bleaching agents gradually increased day after day, and the effects on the 8th and 12th days were superior to the one on the 4th day. However, there was no statistically significant difference between the 8th and 12th day measurements.

Conflict of interest The authors declare that there are no conflicts of interests in writing this article.

References

1. Iwaya SI, Ikawa M, Kubota M (2001) Revascularization of an immature permanent tooth with apical periodontitis and sinus tract. Dent Traumatol 17:185–187
2. Reynolds K, Johnson JD, Cohenca N (2009) Pulp revascularization of necrotic bilateral bicuspids using a modified novel technique to eliminate potential coronal discolouration: a case report. Int Endod J 42:84–92
3. Bezgin T, Yilmaz AD, Celik BN, Sonmez H (2013) Concentrated platelet-rich plasma used in root canal revascularization: 2 case reports. Int Endod J. doi:10.1111/iej.12144
4. Tawfik H, Abu-Seida AM, Hashem AA, Nagy MM (2013) Regenerative potential following revascularization of immature permanent teeth with necrotic pulps. Int Endod J. doi:10.1111/iej.12079
5. Kim JH, Kim Y, Shin SJ, Park JW, Jung IY (2010) Tooth discoloration of immature permanent incisor associated with triple antibiotic therapy: a case report. J Endod 36:1086–1091
6. Miller EK, Lee JY, Tawil PZ, Teixeira FB, Vann WF Jr (2012) Emerging therapies for the management of traumatized immature permanent incisors. Pediatr Dent 34:66–69
7. Akcay M, Arslan H, Yasa B, Kavrik F, Yasa E (2013) Spectrophotometric analysis of crown discoloration induced by various antibiotic pastes used in revascularization. J Endod. doi:10.1016/j.joen.2013.09.019
8. Zimmerli B, Jeger F, Lussi A (2010) Bleaching of nonvital teeth. A clinically relevant literature review. Schweiz Monatsschr Zahnmed 120:306–320
9. Plotino G, Buono L, Grande NM, Pameijer CH, Somma F (2008) Nonvital tooth bleaching: a review of the literature and clinical procedures. J Endod 34:394–407
10. Ritter AL, Ritter AV, Murrah V, Sigurdsson A, Trope M (2004) Pulp revascularization of replanted immature dog teeth after treatment with minocycline and doxycycline assessed by laser Doppler flowmetry, radiography, and histology. Dent Traumatol 20:75–84
11. Thomson A, Kahler B (2010) Regenerative endodontics—biologically based treatment for immature permanent teeth: a case report and review of the literature. Aust Dent J 55:446–452
12. Arslan H, Akcay M, Yasa B, Hatirli H, Saygili G (2014) Bleaching effect of activation of hydrogen peroxide using photon-initiated photoacoustic streaming technique. Clin Oral Investig. doi:10.1007/s00784-014-1255-9
13. Nosrat A, Homayounfar N, Oloomi K (2012) Drawbacks and unfavorable outcomes of regenerative endodontic treatments of necrotic immature teeth: a literature review and report of a case. J Endod 38:1428–1434
14. Lenherr P, Allgayer N, Weiger R, Filippi A, Attin T, Krastl G (2012) Tooth discoloration induced by endodontic materials: a laboratory study. Int Endod J 45:942–949
15. Tanase S, Tsuchiya H, Yao J, Ohmoto S, Takagi N, Yoshida S (1998) Reversed-phase ion-pair chromatographic analysis of tetracycline antibiotics. Application to discolored teeth. J Chromatogr B Biomed Sci Appl 706:279–285
16. Lim MY, Lum SO, Poh RS, Lee GP, Lim KC (2004) An in vitro comparison of the bleaching efficacy of 35% carbamide peroxide with established intracoronal bleaching agents. Int Endod J 37:483–488
17. Weiger R, Kuhn A, Lost C (1994) In vitro comparison of various types of sodium perborate used for intracoronal bleaching of discolored teeth. J Endod 20:338–341
18. Dietschi D (2006) Nonvital bleaching: general considerations and report of two failure cases. Eur J Esthet Dent 1:52–61
19. Rotstein I, Zalkind M, Mor C, Tarabeah A, Friedman S (1991) In vitro efficacy of sodium perborate preparations used for intracoronal bleaching of discolored non-vital teeth. Endod Dent Traumatol 7:177–180
20. Schilke R, Lisson JA, Bauss O, Geurtsen W (2000) Comparison of the number and diameter of dentinal tubules in human and bovine dentine by scanning electron microscopic investigation. Arch Oral Biol 45:355–361
21. Er K, Kustarci A, Ozan U, Tasdemir T (2007) Nonsurgical endodontic treatment of dens invaginatus in a mandibular premolar with large periradicular lesion: a case report. J Endod 33:322–324
22. Sato I, Ando-Kurihara N, Kota K, Iwaku M, Hoshino E (1996) Sterilization of infected root-canal dentine by topical application of a mixture of ciprofloxacin, metronidazole and minocycline in situ. Int Endod J 29:118–124
23. Hoshino E, Kurihara-Ando N, Sato I, Uematsu H, Sato M, Kota K, Iwaku M (1996) In-vitro antibacterial susceptibility of bacteria taken from infected root dentine to a mixture of ciprofloxacin, metronidazole and minocycline. Int Endod J 29:125–130