Available online at www.sciencedirect.com

ScienceDirect

journal homepage: www.elsevier.com/locate/jpfa



Treatment outcome with a revascularization protocol using double and triple antibiotic pastes in immature necrotic teeth – A double blinded randomized control clinical trial

Saravanan Poorni[a,*], *Manali Ramakrishnan Srinivasan*[a], *Dasrathan Duraivel*[a], *Lodd Mahendra*[b], *S. Premkumari*[c]

[a] *Department of Conservative Dentistry and Endodontics, Sri Venkateswara Dental College and Hospital, Chennai, India*

[b] *Sri Venkateswara Dental College and Hospital, Chennai, India*

[c] *Department of Pharmacology, Sri Venkateswara Dental College and Hospital, Chennai, India*

ARTICLE INFO

Article history:

Available online 3 January 2017

Keywords:

Double antibiotic paste

Regenerative endodontics

Revasculasrisation protocol

Triple antibiotic paste

ABSTRACT

Aim: The aim of this clinical trial is to evaluate the regenerative potential of two antibiotic pastes in permanent teeth with necrotic pulp and open apex and compare it with a standard treatment using mineral trioxide aggregate.

Materials and methods: This interventional study was conducted to assess the revascularization potential. It was designed with two test arms and one control arm. LA was administered, access was established under rubber dam, irrigation was done, and each tooth received an antibiotic paste depending on the test arm to which it belonged. The control arm received a standard MTA apexification. Primary and secondary outcomes were assessed at baseline, 6 months and 12 months. Data were tabulated and analyzed.

Results: Though there was an increase in root length in the test arms, the difference was not statistically significant among the various groups at the different time intervals of assessment. Overall, the mean pain scores decreased over the study period in all the three groups. Though no root closure was found at baseline among the test groups, a statistically significant improvement in the root closure scores was observed in Group A, when compared to Group B, at the end of 6th and 12th month of intervention.

Conclusion: The results to this study show that the revascularization potential of triple antibiotic paste is superior to that of the double antibiotic group. The increase in the root length is statistically superior over the study period in the triple antibiotic arm when compared to that of the double antibiotic arm.

© 2016 Pierre Fauchard Academy (India Section). Published by Elsevier, a division of RELX India, Pvt. Ltd. All rights reserved.

* *Corresponding author at*: Department of Conservative Dentistry and Endodontics, Sri Venkateswara Dental College and Hospital, Off OMR Road, Thalambur, Kanchipuram District 603103, India. Tel.: +91 4427435059; fax: +91 4427435059; mobile: +91 9941123738.

E-mail address: poorniis@yahoo.com (S. Poorni).

http://dx.doi.org/10.1016/j.jpfa.2016.12.002

0970-2199/© 2016 Pierre Fauchard Academy (India Section). Published by Elsevier, a division of RELX India, Pvt. Ltd. All rights reserved.

1. Introduction

Pulpal necrosis of an immature tooth due to trauma poses many potential complications. It is difficult to insert an instrument into immature canal spaces using conventional endodontic techniques. The open apex is difficult or impossible to seal with conventional root filling methods because of the absence of an apical stop. Furthermore, the arrested development of the dentinal walls at the time of pulp necrosis leaves a weak tooth with thin dentinal walls that are susceptible to fracture.[1] For decades, such teeth have been treated by the apexification procedure, which involves placement of intracanal calcium hydroxide ($Ca(OH)_2$) to induce formation of a calcific barrier at the apex. The use of the $Ca(OH)_2$ based apexification technique has raised serious concern about the merit of this treatment approach due to the lengthy treatment period,[2,3] unpredictability of apical closure and susceptibility of cervical root fracture after prolonged exposure to $Ca(OH)_2$.[4]

More recently, the traditional apexification procedure has been modified by the introduction of artificial apical barrier methods with mineral trioxide aggregate (MTA). Although this approach might considerably shorten the treatment period, improve patient compliance, and result in favorable healing of the periapical tissues,[5,6] it still cannot stimulate the development of apical closure and thickening of radicular dentin.

Regenerative endodontic procedures can be defined as biologically based procedures designed to replace damaged structures, including dentin and root structures, as well as cells of the pulp-dentin complex.[7] One of the presently viable regenerative procedures is revascularization of the root canal. Revascularization depends mainly on disinfection of root canal, placement of matrix in canal for tissue growth, and impermeable seal of access opening. The primary parameter influencing success of this revascularization treatment is the disinfection of the root canal space.[8]

One combination that is effective against the bacteria commonly found in infected root canals is the use of Triple Antibiotic Paste (TAP), a combination of: (1) ciprofloxacin; (2) metronidazole; and (3) minocyclin. Hoshino et al.,[9] evaluated single and combined antibiotics against endodontic bacteria. They observed that the association of these three antibiotics eliminated bacterial colonization on the dentin surface.[10] Despite promising results, antibiotic paste may manifest some side effects such as crown discoloration due to the presence of minocycline.[11] In an attempt to minimize these undesirable effects, some authors have suggested decreasing the time of the antibacterial dressing to prevent discoloration.

Thus an attempt was made to eliminate the minocyline component, and a double antibiotic paste was formulated. The present randomized double blinded control trial was under taken to compare the clinical outcomes of Triple antibiotic paste versus a double antibiotic paste intracanal medicament with a standard treatment (immediate apexification with MTA) in single rooted maxillary permanent incisors with necrotic pulp and immature root development.

2. Aim

The aim of this clinical trial is to evaluate the regenerative potential of two antibiotic pastes in permanent teeth with necrotic pulp and open apex and compare it with a standard treatment using mineral trioxide aggregate.

3. Materials and methods

The present study was an interventional study conducted to assess the revascularization potential of a double anti-biotic paste when compared to that of a triple anti-biotic paste among a sampled population with necrotic immature pulp. Ethical clearance to conduct this study was obtained from the Institutions Review Board at Sri Venkeshwara Dental College and Hospitals. Individual informed consent was also obtained from the parents of the children who participated in this study, after explaining to them the treatment protocol. A total of 25 subjects who meet the inclusion and exclusion criteria were enrolled for the study.

3.1. *Inclusion criteria*

- Study subjects with a diagnosis of pulpal necrosis with apical periodontitis.
- Study subjects within the age group of 7–16 years.
- Study subjects having maxillary and mandibular restorable maxillary or mandibular anteriors, or lower premolar permanent tooth with open apices.
- Study subjects who gave consent to treatment plan with revascularization procedure.
- Study subjects belonging to ASA (American Society of Anesthetologists) Class I or II physical status and with no systemic health problems.

3.2. *Exclusion criteria*

- Study subjects with non-restorable teeth.
- Study subjects placed under ASA III and above physical status and having severe systemic disease that was considered constant threat to their life.
- Study subjects unable to give assent.

The study was designed with two test arms and one control arm.

- Test arm A consisted of study participants who received triple antibiotic paste as the intracanal medicament.
- Test arm B consisted of study participants who received double antibiotic paste as the intracanal medicament.
- Control arm consisted of study participants who received a standard treatment of mineral trioxide aggregate immediate apexification procedure.

Subjects were randomly divided into 2 test arms of 10 study participants each (Arm A and Arm B) and a control arm of 5 study participants. For allocation of the subjects, a

computer-generated list of random numbers was used with a randomization ratio of 2:2:1 using Random Allocation Software (Version 1.0 May 2004). Randomization was done by one of the operators who were not involved with the type of intervention.

Subjects enrolled in all three arms received a standard infiltration of 2% lignocaine with 1:80,000 epinephrine (Lignox, Indoco Remidies Ltd., India). LA was administered, teeth were isolated using a rubber dam (GDC Marketing, Punjab, India) and conventional access opening was initiated using Endo access bur (Maillefer, Dentsply, Ballaigues, Switzerland). Canal patency was checked with patency file (#10 K file) (Mani, Inc; Tochigi, Japan). The root canal system was copiously and slowly irrigated with 20 ml of 3% NaOCl (Prime Dental Products Pvt Ltd, Thane, India) followed by 20 ml of 2% chlorhexidine (Asep-RC, Anabond, Stedman, India). The canals were then dried with paper points (Maillefer, Dentsply, Ballaigues, Switzerland).

For subjects belonging to the test arm A, a mixture of ciprofloxacin, metronidazole, and minocycline paste as described by Hoshino et al.,[9] was placed with a lentulo spiral instrument to a depth of 8 mm into the canal. A double seal of Glass ionomer cement (GC Corporation, Tokyo, Japan) and composite (Denfil, Veronico, Gangwon, Korea) was then placed. While the subjects in the test arm B received a double antibiotic paste prepared similar to that of triple antibiotic paste excluding the Minocycline component. The procedure of double antibiotic paste placement was similar to that for test arm A. The patients were recalled after 3weeks. The access cavity was re-entered and the antibiotic paste was removed by gently irrigating with 20 ml of 3% NaOCl followed by 20 ml of 2% chlorhexidine. Canal was dried. No instrumentation was performed. A sterile file was used to stimulate apical tissues beyond the confines of the root canal to bleeding in the canal space, and a blood clot was induced and a double seal of glass ionomer cement and composite was placed. Access cavity was sealed using glass ionomer cement and Composite (Double Seal).

For subjects enrolled to the control arm, a standard instrumentation protocol using K files (Maillefer, Dentsply, Ballaigues, Switzerland) was followed and the chemomechanical instrumentation was performed with a copious irrigation of 3% sodium hypochlorite. Following this, mineral trioxide aggregate (MTA, Angelus, Brazil) was used to create a hard tissue barrier. The MTA was mixed and placed into the apical 3 to 4 mm of the canal using an amalgam carrier and hand plugger. A wet cotton pellet was placed against the MTA and left for at least 6 h and then the entire canal was filled with thermo-plasticised Gutta Percha (BeeFill, VDW, Munich, Germany). A double seal of Glass Ionomer Cement and composite was then placed.

The study participants were periodically reviewed after 6 months and 12 months to assess the primary and secondary outcome measures.

3.3. *Primary outcome measures*

- The primary outcome measure was a radiographic measurement of root length using a standard IOPA film, taken using a paralleling technique at the end of 6 months and 12 month among the test and control arm by a single examiner. The total length of the root was measured to the nearest millimeter using a ruler and a divider. This measurement was also done by a single examiner who was trained in this procedure before the start of the study.

3.4. *Secondary outcome measures*

- The secondary outcome measures were assessment of pain symptoms of the patients and subjective assessment of root closure using IOPA radiographs. Pain assessment was done using a Likert scale of 0–5, with 0 denoting no pain and 5 denoting extreme pain. The subjects were explained about the nature of the treatments done to them and were asked to show the picture of the VAS which best denotes their pain condition at baseline, 6 months and 12 months after the procedure was done.
- Assessment of the root closure was also done by grading the root closure on a Likert Scale of 0 to 5, with 0 denoting no closure and 5 denoting complete closure of the root apex. During all assessments, the examiner was informed about the tooth number to be assessed. Though efforts were taken to blind the group of the study subjects assessed, the time frame of the assessment was not blinded in all the groups.

The obtained data were entered into Microsoft EXCEL Sheet (MS Windows). The statistical analysis of the data was done using SPSS version 15 for Windows (SPSS Limited, Chicago, IL).

4. Results

Among the total participants, 16 were males and the rest were females. There was a male dominance in all the three groups. However, this difference in gender was not statistically significant between the groups. Table 1 shows the distribution of the study population in the test and control arms based on their gender. Table 2 shows the root length of the three arms at baseline, six months and twelve months. The increase in the root length was not statistically significant among the various groups at the different time intervals of assessment. However, in Group A subjects, the mean root length increased from 10.3 (1.78) mm to 11.08 (1.70) mm at sixth month and to 11.22 (1.56) mm at the end of 12th month. In Group 2, mean root length decreased from 11.50 (1.98) mm to 11.13 (2.16) mm at the end of 12th month, while it increased marginally from 10.1 (1.10) mm in the control group to 10.8 (1.7) mm at the end of the

Table 1 – Distribution of the study population in the test and control arms based on their gender.

	Gender		Total
	Male	Female	
Group A (TAP)	7	3	10
Group B (DAP)	6	4	10
Control (MTA)	3	2	5
Total	16	9	25

Table 2 – Distribution based on the mean root length of the study participants in the various groups at different time interval.

Time intervals		N	Mean	Std. deviation	F value	p value
Root length – baseline	Group A (TAP)	10	10.30	1.780	3.140	.052
	Group B (DAP)	10	11.50	1.987		
	Control (MTA)	5	10.10	1.101		
	Total	25	10.74	1.838		
Root Length – 6 months	Group A (TAP)	8	11.08	1.706	1.063	.358
	Group B (DAP)	7	11.00	1.859		
	Control (MTA)	5	10.11	1.167		
	Total	20	10.79	1.647		
Root length – 12 months	Group A (TAP)	7	11.22	1.563	1.052	.366
	Group B (DAP)	6	11.13	2.167		
	Control (MTA)	5	10.13	1.246		
	Total	18	10.84	1.700		

Table 3 – Distribution based on the mean distribution of the pain scores in the various groups at different time interval.

		N	Mean	Std. deviation	F value	p value
Pain – baseline	Group A (TAP)	10	1.95	1.317	2.352	.106
	Group B (DAP)	10	1.20	.894		
	Control (MTA)	5	1.50	.972		
	Total	25	1.56	1.128		
Pain – 6 months	Group A (TAP)	8	1.08	1.256	1.680	.203
	Group B (DAP)	7	.75	.622		
	Control (MTA)	5	.33	.707		
	Total	20	.76	.955		
Pain – 12 months	Group A (TAP)	7	.44	.726	2.000	.159
	Group B (DAP)	6	.75	.707		
	Control (MTA)	5	.13	.354		
	Total	18	.44	.651		

Table 4 – Distribution based on the mean subjective assessment toward root closure using a radiograph among the test groups.

	Group	N	Mean	Std. deviation	T test value	p value
Root closure – baseline	Group A (TAP)	10	.00	.000(a)		
	Group B (DAP)	10	.00	.000(a)		
Root closure – 6 months	Group A (TAP)	8	2.92	.494	.985	0.000**
	Group B (DAP)	7	2.08	.289		
Root closure – 12 months	Group A (TAP)	7	3.67	.500	.111	0.001**
	Group B (DAP)	6	2.63	.518		

intervention. Table 3 shows the mean distribution of the pain scores in the various groups at different time interval (baseline, 6 months and 12 months). Overall the mean pain scores decreased over the study period in all the three groups, though this difference was not statistically significant among the various groups at the different time intervals of intervention. Table 4 shows the mean subjective assessment regarding the perception toward root closure using a radiograph among the test groups. Though no root closure was found at baseline among the test groups, a statistically significant improvement in the root closure scores was observed in Group A, when compared to Group B, at the end of 6th and 12th month of intervention.

Figs. 1 and 2 shows the radiographs of a subject enrolled in the Test Arm A that is treated with Triple Antibiotic Paste, while Figs. 3 and 4 shows the radiographs of a subject enrolled in the Test Arm B who was treated with double antibiotic paste.

Figs. 5 & 6 shows the radiographs of subject enrolled in control arm treated with single step apexification using MTA.

5. Discussion

The present study has been designed as a randomized control trial (RCT). Randomized controlled trial is considered as the most rigorous method of determining whether a cause-effect



Fig. 1 – Preoperative radiograph of patient enrolled in Test arm A.



Fig. 2 – 12 months follow up radiograph of patient enrolled in Test arm A.

relationship exists between an intervention and outcome.[12] The present study was done to assess the revascularization potential of a triple versus a double antibiotic paste dressing in treating immature tooth. An immature tooth with early irreversible pulp involvement presents with thin divergent or parallel dentinal walls. This situation creates clinical challenges in disinfection, and as a result, affects the long-term outcome of the treatment.

Regenerative endodontics is the creation and delivery of tissues to replace diseased, missing and traumatized pulp. More than 50 years ago, this approach of endodontic treatment was presented by Nygaard-Ostby who introduced a revascularization method for re-establishing a pulp-dentin complex in permanent teeth with pulpal necrosis.[13,14] The first case of revascularization of a permanent immature tooth with necrotic pulp and apical periodontitis was published in 2001,[15] and since then several publications showcased the biological or regenerative approach for endodontic treatment.[16,8,17] Hence the present study was done to assess the potential of two antibiotic combinations in revascularization of necrotic immature pulp.

Irrigation protocols are another crucial component of regenerative endodontic therapy. NaOCl is included in the majority of clinical studies either as the only irrigator in combination with other irrigants, in a concentration ranging from 1 to 6%.[18] The combined protocol has been used in the present study as an effort to enhance the disinfection of the root canal. Concentrations of NaOCl higher than 3%, although having favorable antimicrobial action, are considered to be cytotoxic to periodontal ligament cells and stem cells of apical



Fig. 3 – Preoperative radiograph of patient enrolled in Test arm B.



Fig. 4 – 12 months follow up radiograph of patient enrolled in Test arm A.



Fig. 6 – Postoperative radiograph of patient enrolled in control arm.



Fig. 5 – Preoperative radiograph of patient enrolled in control arm.

papilla (SCAPs).[19,20] Hence a concentration of 3% NaOCl has been used in the current study.

Apical MTA plugs are more effective for accomplishing apexogenesis[21] and for strengthening the roots of traumatized immature permanent anterior teeth.[22] Thus apical barrier using MTA, an immediate apexification procedure was taken as the control for the current trial.

Because of the complexity of the root canal infection, it is unlikely that any single antibiotic could result in effective sterilization of the canal. More likely a combination would be needed to address the diverse flora encountered. The combination that appears to be most promising consists of metronidazole, ciprofloxacin, and minocycline.[9] Sato et al.,[23] investigated this drug combination in vitro and found it to be very effective in the sterilization of carious lesions, necrotic pulps, and infected root dentin of deciduous teeth. Hoshino et al.,[9] performed an in vitro study testing the antibacterial efficacy of these drugs alone and in combination against the bacteria of infected dentin, infected pulps, and periapical lesions. On the basis of these observations, triple antibiotic dressing was placed in the root canal to achieve disinfection, and encouraging results were obtained in this present study in both objective and subjective assessments of root closure.

However, minocycline was found to cause tooth discoloration because of photoactivation. minocycline binds to calcium ions via chelation to form an insoluble complex. Thus, it should be limited to the root canal because of the potential risk of tooth discoloration.[11] A biantibiotic paste, omitting minocycline from the formulation has also been

recommended but this mixture does not have the antibacterial potency of triple antibiotic paste. Cephalosporins (cefaclor) have also been suggested as an alternative to minocycline in the triple antibiotic paste.[24] Arestin[25] and amoxicillin[17] have also been recommended as alternatives to minocycline. However, no clinical trials exist on the efficacy of these alternative formulations on the success of revascularization treatment and hence a combination omitting the minocyclin was chosen to formulate the double antibiotic paste for the current trial.

The results of the present study showed that the root closure scores improved statistically in Group A, when compared to Group B, at the end of 6th and 12th months of intervention. There are several factors that could have helped the immature teeth in the experimental arm achieve continued root development. First, the immature tooth has a wide root canal, an open apex and short root. In the ideal case, there is pulp necrosis with an immature apex opening more than 1 mm in a mesiodistal dimension radiographically.[26] Murray et al.,[7] have even suggested that the revascularization of necrotic pulp in a tooth with a closed apex may require instrumentation to approximately 1–2 mm in apical diameter to allow systemic bleeding into the root canal system. So the new tissue has easy access to the root canal system and a relatively short distance for proliferation to reach the coronal pulp horns. Secondly, these patients are young (7–16 years old), and so have greater healing capacity or stem cell regenerative potential. Thirdly, all root canals of diseased teeth were treated as conservatively as possible. No instrumentation was done in the root canals whereas all of the studies used sodium hypochlorite (NaOCl) and chlorhexidine as irrigants. Minimum instrumentation and disturbance of the root canal system also preserved more viable pulp tissues. Though both the antibiotic paste combination showed changes in the root development, yet the results were variable. This could be due to their difference in the canal disinfection potential. Finally, the formation of a blood clot might have served as a protein scaffold, permitting 3-dimensional in growth of tissue. It is suggested that all these mentioned favorable factors finally could lead to a clinically successful outcome with regards to root closure.

The results of the present study have to be interpreted after considering the following limitations. The assessment of root closure in the present study was done using objective methods, in which the root length was assessed in millimeter from the CEJ to the midpoint of intersection of the root apex using the standard IOPA film, as suggested by Bose et al.[27] Another limitation of the present study was the loss of follow-up of some subjects in both the treatment and control arms.

6. Conclusion

The results to this study show that the revascularization potential of triple antibiotic paste is superior to that of the double antibiotic group. The increase in the root length is statistically superior over the study period in the triple antibiotic arm when compared to that of the double antibiotic arm. There existed no difference in the subjective perception of pain and assessment of root closure among the various test and control groups. Thus, within the limitations of this study, it can be concluded that triple antibiotic paste shows better revascularization potential when compared to that of the double antibiotic paste.

Conflicts of interest

The authors have none to declare.

REFERENCES

1. Katebzadeh N, Dalton BC, Trope M. Strengthening immature teeth during and after apexification. *J Endod*. 1998;24:256–259.
2. Abbot P. Apexification with calcium hydroxide: when should the dressing be changed? The case for regular dressing changes. *Aust Endod J*. 1998;24:27–32.
3. Sheehy EC, Roberts GJ. Use of calcium hydroxide for apical barrier formation and healing in non-vital immature permanent teeth: a review. *Br Dent J*. 1997;183:241–246.
4. Andreasen JO, Farik B, Munksgaard EC. Long-term calcium hydroxide as a root canal dressing may increase risk of root fracture. *Dent Traumatol*. 2002;18:134–137.
5. Cehreli ZC, Sara S, Uysal S, Turgut MD. MTA apical plugs in the treatment of traumatized immature teeth with large periapical lesions. *Dent Traumatol*. 2011;27:59–62.
6. Sarris S, Tahmassebi JF, Duggal MS, et al. A clinical evaluation of mineral trioxide aggregate for root-end closure of non-vital immature permanent incisors in children: a pilot study. *Dent Traumatol*. 2008;24:79–85.
7. Murray PE, Garcia-Godoy F, Hargreaves KM. Regenerative endodontics: a review of current status and a call for action. *J Endod*. 2007;33:377–390.
8. Jung IY, Lee SJ, Hargreaves KM. Biologically based treatment of immature permanent teeth with pulpal necrosis: a case series. *J Endod*. 2008;34:876–887.
9. Hoshino E, Kurihara-Ando N, Sato I, 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;29:125–130.
10. 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;29:118–124.
11. Kim JH, Kim Y, Shin SJ, Park JW, Jung Y. Tooth discoloration of immature permanent incisor associated with triple antibiotic therapy: a case report. *J Endod*. 2010;36:1086–1091.
12. Kendall JM. Designing a research project: randomised controlled trials and their principles. *Emerg Med J*. 2003;20:164–168.
13. Nygaard-Ostby B. The role of the blood clot in endodontic therapy: an experimental histologic study. *Acta Odont Scand*. 1961;19:323–353.
14. Nygaard-Ostby B, Hjortdal O. Tissue formation in the root canal following pulp removal. *Scand J Dent Res*. 1971;79:333–349.
15. Iwaya SI, Ikawa M, Kubota M. Revascularization of an immature permanent tooth with apical periodontitis and sinus tract. *Dent Traumatol*. 2001;17:185–187.
16. Banchs F, Trope M. Revascularization of immature permanent teeth with apical periodontitis: new treatment Prot. *J Endod*. 2004;30:196–200.
17. 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;55:446–452.
18. Kontakiotis EG, Filippatos CG, Tzanetakis GN, Agrafioti A. Regenerative endodontic therapy: a data analysis of clinical protocols. *J Endod*. 2015;41:146–154.
19. Chang YC, Huang FM, Tai KW, Chou MY. The effect of sodium hypochlorite and chlorhexidine on cultured human periodontal ligament cells. *Oral Surg Oral Med Oral Pathol Oral Radiol Endod*. 2001;92:446–450.
20. Martin DE, De Almeida JF, Henry MA, et al. Concentration-dependent effect of sodium hypochlorite on stem cells of apical papilla survival and differentiation. *J Endod*. 2014;40:51–55.
21. Nayar S, Bishop K, Alani A. A report on the clinical and radiographic outcomes of 38 cases of apexification with mineral trioxide aggregate. *Eur J Prosthodont Restor Dent*. 2009;17:150–156.
22. Al Ansary MA, Day PF, Duggal MS, Brunton PA. Interventions for treating traumatized necrotic immature permanent anterior teeth: inducing a calcific barrier & root strengthening. *Dent Traumatol*. 2009;25:367–379.
23. 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;8:172–176.
24. Thibodeau B, Trope M. Pulp revascularization of a necrotic infected immature permanent tooth: case report and review of the literature. *Pediatr Dent*. 2007;29:47.
25. Trope M. Treatment of an immature tooth with a non vital pulp and apical periodontitis. *Dent Clin N Am*. 2010;54:313–324.
26. Kling M, Cvek M, Mejare I. Rate and predictability of pulp revascularization in therapeutically reimplanted permanent incisors. *Endod Dent Traumatol*. 1986;2:83–89.
27. Bose R, Nummikoski P, Hargreaves K. A retrospective evaluation of radiographic outcomes in immature teeth with necrotic root canal systems treated with regenerative endodontic procedures. *J Endod*. 2009;35:1343–1349.