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Comparison of the Subcutaneous Tissue Reactions Caused by Chloroform and Halothane Dipped Gutta-Percha

Kloroform ve Halotana Daldırılan Güta-Perka ile Oluşan Subkutanöz Doku Reaksiyonlarının Karşılaştırılması

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ABSTRACT

Aim: The flat canals don't lend themselves to the easy adaptation of a standardized gutta-percha cone. The adaptation of a master cone to these canals may be achieved with a customized cone, prepared by a solvent dip technique. The purpose of this study was to evaluate the biocompatibility of chloroform and halothane, which are used in "solvent dip fitted gutta-percha technique".

Materials and Methods: Twenty one Spraque Dawley rats were used. Teflon tubes containing gutta-percha softened with chloroform or halothane were implanted into the dorsal subcutaneous connective tissue of rats. Gutta-percha and an empty teflon tube were implanted to each rat for control. Four implants from different groups were placed to each rat. After the observation periods (1, 4 and 8 weeks) rats were sacrificed for histopathological analysis.

Results: The results of the study demonstrated that the inflammatory reactions for all implant types were moderate or severe after one week. The degree of reactions was subsided after 4 and 8 weeks. There were minimal or mild inflammatory reactions for all implant types after 8 weeks.

Conclusions: The severity of inflammatory reactions for all implant types was decreased over time. It was concluded that there were no obvious microscopic differences among the histopathological tissue reactions caused by chloroform-dipped gutta-percha, halothane-dipped gutta-percha, control gutta-percha or teflon.

KEYWORDS
Biocompatibility, Gutta-percha Solvents, Chloroform, Halothane

ÖZET

Amaç: Yassı Kanallar standart bir güta-perka konun kolay adaptasyonuna izin vermezler. Bu tip kanallara bir ana konun uyumu çözücüye daldırma tekniği ile hazırlanan şekillendirilmiş bir konla sağlanabilir. Bu in vivo çalışmanın amacı çözücüye daldırılarak sıkıştırılan güta-perka tekniğinde kullanılan chloroform ve halotanın biouyumluluğunu değerlendirmekti.

Gereç ve Yöntem: 21 adet Spraque Dawley cinsi rat kullanıldı. Kloroform veya halotana daldırılan güta-perka içeren teflon tüpler ratların sırt bölgesindeki deri altı bağ dokusuna implante edildi. Güta-perka ve boş teflon tüp her rata kontrol amacıyla implante edildi. Her rata farklı gruplardan dört implantasyon yapıldı. Gözlem dönemlerinden sonra (1, 4 ve 8 hafta) ratlar histopatolojik analiz için sakrifiye edildiler.

Bulgular: Çalışmanın sonuçları 1 hafta sonra tüm implant tipleri için inflamatuar reaksiyonların orta ve şiddetli derecede olduğunu gösterdi. Reaksiyonların derecesi, 4 ve 8. haftalardan sonra azaldı. 8 hafta sonunda tüm implant tipleri için minimal veya hafif inflamatuar reaksiyonlar vardı.

Sonuç: Tüm implantlar için inflamatuar reaksiyonların şiddeti zamanla azaldı. Chloroform ve halotana daldırılan güta-perka, kontrol güta-perka ve teflonla oluşan histopatolojik doku reaksiyonları arasında belirgin bir farklılık olmadığı görüldü.

ANAHTAR KELİMELER
Biouyumluluk, Güta-perka Çözücüler, Kloroform, Halotan

INTRODUCTION

Lateral condensation is the most commonly used method of root canal obturation[1,2]. Adaptation of gutta-percha to the prepared root canal has been largely facilitated by the introduction of standardized endodontic instruments and standardized master cones[1-3]. However some canals don't lend themselves to the easy adaptation of a standardized master cone. These include flat "ribbon-shaped" and "lance-shaped" canals[4-6]. In addition, some instrumentation studies have demonstrated that it was difficult to produce a perfectly round apical preparation[7,8]. In these canals; a tug back sensation may be misleading. Closer adaptation of a master cone to these canals may be achieved with a customized cone, prepared by a solvent dip technique, which creates impression of the apical part of the canal[6]. In addition, thermoplastized gutta-percha techniques can provide good apical sealing in these canals. However, warm techniques often do not allow length control and the extrusion of material beyond the apical limits of the root canal space is a common finding[9,10]. Therefore the solvent dip technique can be an alternative in these canals.

Chloroform is the most commonly used gutta-percha solvent because it can dissolve gutta-percha rapidly. However The Food and Drug Administration[11] (FDA) designated chloroform as a potential carcinogen.

Because of the concerns about chloroform, clinicians and researchers have developed a renewed interest in finding alternative solvents[12,13]. In addition, the effect of solvents on dentin has been investigated in many researches[14-16]. Wourms et al.[12] were the first to suggest the possibility of using of the general anesthetic agent halothane in endodontics. Halothane ($C_2HBrClF_3$) was chosen because of its chemical similarity to chloroform ($CHCl_3$). Although in vitro sealing ability results have been good for the solvent-dipped technique,[17-20] the possible toxicity of solvents which are used in solvent-dipped technique should also be investigated.

Chloroform and halothane were found to be a cytotoxic agent in vitro[22-24] and in vivo[22,25-28]. But in these studies gutta-percha was dissolved in solvents. Therefore solvent concentrations were presumably very high. In the solvent dip technique however, relatively little solvent is used.

The purpose of this in vivo study was to evaluate the biocompatibility of gutta-percha dipped either chloroform or halothane.

MATERIALS and METHODS

The protocol of this study was approved by the local Ethics Committee on animal experimentation. A total of 21 female adult Spraque-Dawley rats, weighing 250-300g each, were obtained from Selcuk University Experimental Medicine and Application Center and experiments were performed in the same center. Animals were divided into three groups, each containing 7 rats, in accordance with three observation periods, 1, 4 and 8 weeks respectively.

Rats were anesthetized by intramuscular administration of Ketamine HCl (Ketalar, Parke-Davis, Pontypool, England), 0.001g per 1g body weight. Each animal was secured to a rat board with an elastic band. The backs of rats were shaved and disinfected with 5% iodine in alcohol. Four incisions were made in the dorsum (right and left scapular and pelvic sites) and subcutaneous pockets were carefully prepared to a depth of approximately 20mm.

In total 84 teflon tubes, each 7 mm in length with an inner diameter of 1.3 mm, were used in this study.

Size #100 gutta-percha (Diadent, Korea) was dipped into the chloroform (Merck, D-6100, Germany) for 1 second and then it was placed into a teflon tube. Finally it was implanted to the left pelvic site of each rat.

Size #100 gutta-percha was softened with halothane (Fluothane, Abdi İbrahim İlaç AS, Turkey) for 5 seconds and then it was placed into a teflon tube. Finally it was implanted to the right pelvic site of each rat. The tubes were placed into the pockets as the frontal sites of them dipped solvents were as far from the suture area.

Size #100 gutta-percha was placed into the left scapular site of each rat and empty teflon tube was implanted into the right scapular site of each rat for control.

At the end of each experimental period (1, 4 and 8 weeks) animals were killed by ether inhalation. After implants and their surrounding tissues were surgically removed, the ends of the implants most distant from the suture area were marked for histopathological examination, in order to minimize affect from the wound healing. Tissue samples containing implant were fixed in 10% neutral formalin solution. After fixation, the tissues were processed for paraffin embedding and then longitudinally sectioned through the implants. Serial sections approximately 5-6µm thick were obtained from each specimen and stained with haematoxylin and eosin. The thickness of any fibrous capsule formed at the ends of the implants was measured using an ocular micrometer in a light microscope.

In the histopathological evaluation, the following aspects were evaluated: tissue reaction at the material-tissue interface, the various types of inflammatory cells, vascular changes, presence and characteristics of a capsule adjacent to the implant and the occurrence of necrosis. Inflammatory reactions were classified according to the criteria described by Tavares et al.[29]: none or minimal (grade1)- none or minimal inflammation and presence of a well-defined fibrous capsule; mild (grade2)- small number of inflammatory cells and well-defined fibrous capsule; moderate (grade3)- greater accumulation of inflammatory cells and a thick capsule with cells and capillaries and severe (grade4)- large numbers of inflammatory cells including granulocytes, macrophages, lymphocytes, plasma cells and giant cells, absence of a collagenous fibrous capsule, and areas of tissue necrosis.

RESULTS

Macroscopic examination showed that wound healing was satisfactory in all animals, and no complication was seen.

Microscopic findings were arranged in accordance with experimental periods. The frequency of ratings (none or minimal, mild, moderate and severe) for the two test materials, gutta-percha dipped chloroform and gutta-percha dipped halothane, and for the two controls, gutta-percha and empty teflon tube, in each period are presented in Table I.

TABLE I

Frequency of rating (none or minimal, mild, moderate and severe grades) for all implants in each experimental period. (Distribution of tissue reaction grades to all implants in respect to each group)

Experimental period	Material	None or minimal	Mild	Moderate	Severe
Group 1 (1 Week)	Teflon	-	1	2	4
	Gutta-percha	-	-	3	4
	Halothane	-	-	3	4
	Chloroform	-	-	2	5
Group 2 (4 Weeks)	Teflon	2	3	2	-
	Gutta-percha	1	4	2	-
	Halothane	-	5	2	-
	Chloroform	-	5	2	-
Group 3 (8 Weeks)	Teflon	6	1	-	-
	Gutta-percha	4	3	-	-
	Halothane	3	4	-	-
	Chloroform	2	5	-	-

Group 1 (1 week period):

The reactions in this group were commonly classified as grade 4. The lesions were in general characteristic of a foreign body reaction and resembled each other in all specimens. The tissue reactions were composed of many inflammatory cells, foreign body giant cells, a thick and vascular fibrous tissue and sometimes tissue necrosis between capsule and implant (Fig. 1). Most of the inflammatory cells were identified as granulocyte, and there were also macrophages with engulfed material in their cytoplasm, lymphocytes and plasma cells. Extensive areas of necrosis were observed adjacent to implants in this group and it was classified as grade 4.

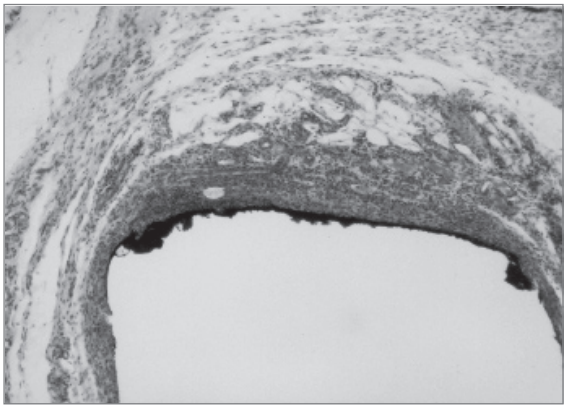

FIGURE 1

Group 1 (1-week period), gutta-percha dipped chloroform: Moderate grade inflammation, vascular proliferation, edematous connective tissue and infiltration of inflammatory cells (orig mag x 100).

Group 2 (4 weeks period):

The severity and grade of lesions was noticeably diminished in comparison with Group 1, and foreign body reactions and tissue necrosis absolutely disappeared at four-week duration. The majority of the specimens exhibited a mild inflammatory reaction, consisting of a thin fibrous capsule, a small number of mononuclear cells and a few granulocytes. There were no severe inflammatory reactions. The cellularity, edema and vascularity near and within the capsule had fairly decreased. The thickness of fibrous capsule and the intensity of inflammatory cells slightly increased in moderate lesions. In the reactions to empty teflon tubes, a granulation tissue was growing from fibrous capsule into the empty lumen of tube, and therefore the capsule was seen as slightly thicker than the others, however inflammatory cells were less in teflon's reaction. So these lesions were evaluated as mild.

Group 3 (8 weeks period):

In general, the eight-week specimens exhibited the least severe lesions between the three observations groups. Although there were no moderate or severe reactions, a trend in the severity of inflammation appeared to be emerging among the control and experimental groups: From the least severe to the most severe; this trend was teflon tube (control), gutta-percha (control), gutta-percha dipped in halothane, and gutta-percha dipped in chloroform. A thin and well-defined fibrous capsule consisting of fibrocytes and collagen fibres was observed at the frontal edges of implants. Inflammatory cells were not discovered in most cases, whereas in some mild grade lesions a few mononuclear cells, granulocytes and/or macrophages were observed (Fig. 2 and 3). The fibrous capsule over the gutta-percha was generally the

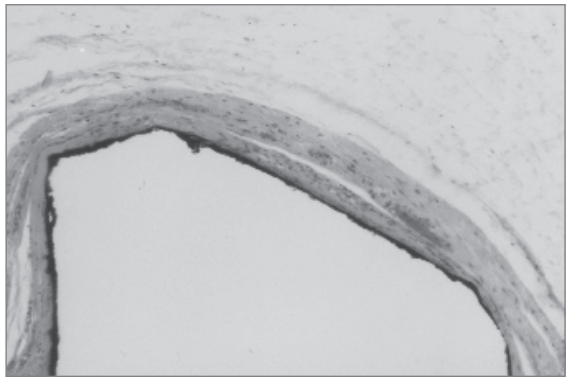

FIGURE 2

Group 3 (8-week period), gutta-percha dipped halothane: Mild inflammation, a well-defined and thin fibrous capsule and small number of mononuclear cells (orig mag x 100).

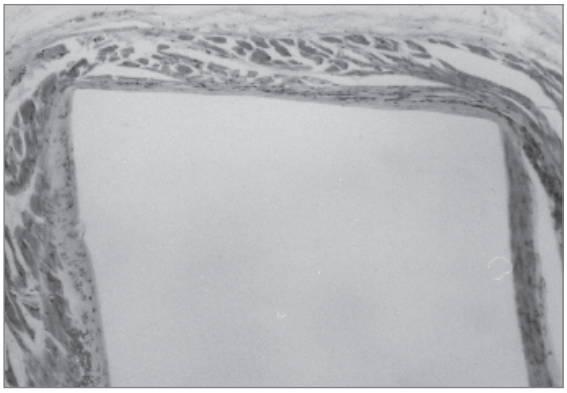

FIGURE 3

Group 3, (8-week period), teflon tube: No inflammation, a well-defined and thin fibrous capsule and small number of mononuclear cells and healthy muscle tissue were seen (orig mag x 100).

same thickness, but sometimes thinner than the capsule thickness over the long side of the teflon tubes.

After eight weeks of implantation, all inflammatory tissue reactions had reduced, compared to the 1 and 4 weeks groups.

DISCUSSION

The procedure of obturating the root canal is of utmost importance in root canal therapy. It involves the introduction of a foreign body into the mesenchyme of the jaw and its placement in direct contact with viable periapical tissues. The outcome of such treatment will depend on the reaction of the connective tissues to this implanted material.

If such potentially irritating materials are injudiciously allowed to contact the periapical tissue, pain associated with inflammation and necrosis may result. The regenerative power, repair and function of the area are lowered and chances of success are decreased. Therefore, endodontic filling materials and their diffusible components should be critically evaluated for their level of tissue irritability. Hence, it can be stated that the study of the biological properties of root canal sealers is at present one of the major means of evaluating their suitability for clinical application.

Many different methods have been described for assessing tissue toxicity.[30-34] In vitro studies are very helpful to increase our knowledge about basic biological effects. However there are numerous factors which come to play in more complex animal model and can not be studied in vitro. Therefore, in vivo experimental models must be used to obtain answers to some important questions[35].

The majority of implantation studies have been performed in soft tissues. Spangberg[35] commented that such implantation was easy to do and required minor surgical skills.

Investigators usually use teflon, silicon or polyethylene tubes which allow precise control of the amount of the material that will be in contact with the tissues. In this study we used teflon tubes to simulate the root canal and to standardize tissue-material contact area.

Keane & Harrington[36] and Smith & Montgomery[17] showed that a 1-second dip for the chloroform-dip technique and a 5-second dip for the halothane-dip technique gave the best results for clinical success. Therefore we used these time periods for softening gutta-percha in this study.

Metzger et al.[5] showed that chloroform evaporated most rapidly during the first 3 minutes of drying (62%) and suggested that gutta-percha which had been dipped to chloroform should be allowed to air dry, before the cone was inserted to the canal. But in our study gutta-percha was not allowed to dry for evaluating biocompatibility of solvents. Gutta-percha cones were placed directly to teflon tubes after they were dipped to solvents. Additionally the teflon tubes were implanted rapidly into the connective tissues of each surgical site.

Tissue reactions are three dimensional processes surrounding the implants. The precise characterization of such tissue reactions in measurable terms is difficult, as light microscopic observations must be performed layer by layer in rather thin sections. Serial analysis permits a detailed, subjective summary, however[35].

Because of the differences among animal species, implantation sites, methods, observation periods, and the criteria used for actual evaluation, an objective comparison of the findings of these studies is difficult.

The observation period after inserting implants must be established in such a way that both short-term reactions as well as long-term healing can be observed. It is naturally important to obtain information about the initial effect on tissues of endodontic materials. Surgical implantation, however, introduces such tissue trauma and subsequent surgical inflammation that it becomes practically impossible to distinguish this tissue response from that created by the material being investigated[35]. This study supported these findings. The samples in group 1 (1 week) showed significant inflammatory tissue reaction primarily due to the surgical trauma.

The results of this study showed that the implanted materials led to moderate or severe inflammatory responses initially, but the response decreased in time with an increasingly thickening fibrous connective tissue capsule forming around the tube. Moderate at the beginning and decreasing in magnitude with time, these reactions satisfied American National Standards Institute/American Dental Association technical requisites[37] in determining a material as biologically acceptable.

Barbosa et al.[24] have shown that gutta-percha dissolved by chloroform, halothane or turpentine in L929 mouse fibroblast cells were toxic. In addition, Chang & Chou[28] have examined the cytotoxicity of gutta-percha dissolved halothane, and demonstrated that it was toxic to human gingival fibroblast cells.

Taking into account the variations between our study and other studies, it is difficult to compare our data with previous results. Gutta-percha dissolved in solvents has been examined in previous studies[22-28]. But the biocompatibility of gutta-percha and solvent, when used in solvent dip techniques, has not been previously investigated. However more extensive investigations concerning the biocompatibility of gutta-percha and solvent when used in solvent dip technique will be necessary in the future.

CONCLUSIONS

-The tissue samples examined at the 1-week period showed significant inflammatory tissue reactions, primarily due to surgical trauma. It therefore seems impractical to use one-week, post-subcutaneous implantation observations to evaluate tissue responses to endodontic materials.

-The severity of inflammatory reactions to implanted chloroform-dipped gutta-percha and halothane-dipped gutta-percha decreases over time. There is no obvious difference among the histopathological tissue reactions caused by these materials and reactions caused by gutta-percha or teflon controls.

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REFERENCES

1. Ingle JI, Leubke R, Zidell JD, Walton RE, Taintor JF. Obturation of the radicular space. In: Ingle JI, Taintor JF, editors. Endodontics. 3rd ed. Philadelphia: Lea & Fabiger; 1985: 223-307.
2. Nguyen NT. Obturation of the root canal system. In: Cohen S, Burns RC, editors. Pathways of the Pulp. 5th ed. St. Louis: Mosby Year Book, Inc; 1991: 193-282.
3. Weine FS. Canal filling with semisolid materials. In: Weine FS, editors. Endodontic Therapy. 5th ed. St. Louis: Mosby Year Book, Inc; 1996: 423-77.
4. Kerekes K, Tronstad L. Morphometric observations on root canals of human premolars. J Endod 1977; 3: 74-9.
5. Metzger Z, Assif O, Tamse A. Residual chloroform and plasticity in customized gutta-percha master cones. J Endod 1988; 14: 546-9.
6. Metzger Z, Nissan R, Tagger M, Tamse A. Apical seal by customized versus standardized master cones: a comparative study in flat and round canals. J Endod 1988; 14: 381-4.

7. Jungman CL, Uchin RA, Bucher JF. Effect of instrumentation on the shape of the root canal. J Endod 1975; 1: 66-9.

8. Weine FS, Kelly RF, Lio PJ. The effect of preparation procedures on the original canal shape and on apical foramen shape. J Endod 1975; 1: 255-62.

9. Siqueira Jr JF, Rocas IN, Favieri A, Abad EC, Castro AJR, Gahyva SM. Bacterial leakage in coronally unsealed root canals obturated with 3 different techniques Oral Surg Oral Med Oral Pathol Oral Radiol Endod 2000; 90: 587-90.

10. Gilhooly RMP, Hayes SJ, Bryant ST, Dummer PMH. Comparison of lateral condensation and thermomechanically compacted warm α-phase gutta-percha with a single cone for obturating curved root canals. Oral Surg Oral Med Oral Pathol Oral Radiol Endod 2001; 91: 89-94.

11. Food and Drug Administration (FDA). Chloroform, use as an ingredient (active or inactive) in drug products. Federal Register Number. 26845. Washington DC: Government Printing Office; 1976.

12. Wourms DJ, Campbell A.D, Hicks ML, Pelleu GB. Alternative solvents to chloroform for gutta-percha removal. J Endod 1990; 16: 224-6.

13. Schafer E, Zandbiglari T. A comparison of the effectiveness of chloroform and eucalyptus oil in dissolving root canal sealers. Oral Surg Oral Med Oral Pathol Oral Radiol Endod 2002; 93: 611-6.

14. Dogan H, Tasman F, Cehreli ZC. Effect of gutta-percha solvents at different temperatures on the calcium, phosphorus and magnesium levels of human root dentin. J Oral Rehabil 2001; 28: 792-6.

15. Kaufman D, Mor C, Stabholz A, Rotstein I. Effect of gutta-percha solvents on calcium and phosphorus levels of cut human dentin. J Endodon 1997; 23: 614-5.

16. Erdemir A, Eldeniz AU, Belli S. Effect of gutta-percha solvents on mineral contents of human root dentin using ICP-AES technique. J Endodon 2004; 30: 54-6.

17. Smith JJ, Montgomery S. A comparison of apical seal: chloroform versus halothane-dipped gutta-percha cones. J Endod 1992; 18: 156-60.

18. Wong M, Peters DD, Lorton L, Bernier WE. Comparison of gutta-percha filling techniques: three chloroform gutta-percha filling techniques, part 2. J Endod 1982; 8: 4-9.

19. Narracott P. An in vitro comparison of the single cone and lateral condensation techniques using frictioned-fitted and solvent-dip-fitted primary gutta-percha cones. Aust Dent J 1989; 34: 49-51.

20. Erdemir A, Üngör M. Çözücüye daldırılarak şekillendirilmiş guta-perka tekniğinde kloroform ve halotan ile elde edilen apikal sızdırmazlıkların karşılaştırılması. Hacettepe Dişhek. Fak. Derg. 2002; 26: 39-44.

21. Adanır N, Erdemir A, Eldeniz AÜ, Belli S. Comparison of apical leakage in root canals obturated with various gutta-percha techniques. Türkiye Klinikleri Dişhek. Bil. Derg, 2005; 11: 33-8.

22. Wennberg A. Biological evaluation of root canal sealers using in vitro and in-vivo methods. J Endod 1980; 6: 784-7.

23. Pascon EA, Spangberg LSW. In vitro cytotoxicity of root canal filling materials. 1. Gutta-percha. J Endod 1990; 16: 429-33.

24. Barbosa SV, Burkard DH, Spangberg LSW. Cytotoxic effects of gutta-percha solvents. J Endod 1994; 20: 6-8.

25. Olsson B, Sliwkowski A, Langeland K. Intraosseous implantation for biological evaluation of endodontic materials. J Endod 1981; 7: 253-65.

26. Olsson B, Sliwkowski A, Langeland K. Subcutaneous implantation for the biological evaluation of endodontic materials. J Endod 1981; 7: 355-69.

28. Olsson B, Wennberg A. Early tissues reaction to endodontic filling materials. Endod Dent Traumatol 1985; 1: 138-41.

28. Chang YC, Chou MY. Cytotoxicity of halothane on human gingival fibroblast cultures in vitro. J Endod 2001; 27: 82-4.

29. Tavares T, Soares LJ, Silveira NL. Reaction of rat subcutaneous tissue to implants of gutta-percha for endodontic use. Endod Dent Traumatol 1994; 10: 174-8.

30. Spangberg LSW. Correlation of in vivo and in vitro screening tests. J Endod 1978; 4: 296-9.

31. Pissiotis E, Spangberg LSW. Reaction of bony tissue to implanted silver glass ionomer and a reinforced zinc oxide-eugenol cement. Oral Surg Oral Med Oral Pathol Oral Radiol Endod 2000; 89: 623-9.

32. Bernath M, Szabo J. Tissue reaction initiated by different sealers. Int Endod J 2003; 36: 256-61.

33. Ozbas H, Yaltirik M, Bilgic B, Issever H. Reactions of connective tissue to compomers, composite and amalgam root-end filling materials. Int Endod J 2003; 36: 281-7.

34. Saidon J, He J, Zhu Q, Safavi K, Spangberg LSW. Cell and tissue reaction to mineral trioxide aggregate and portland cement. Oral Surg Oral Med Oral Pathol Oral Radiol Endod 2003; 95: 483-9.

35. Spangberg LSW, editors. Experimental endodontics. 1st ed. Florida: CRC Press Inc; 1990: 173-210.

36. Keane KM, Harrington GW. The use of a chloroform-softened gutta-percha master cone and its effect on the apical seal. J Endod 1984; 10: 57-63.

37. American National Standards Institute / American Dental Association (ANSI/ADA). Document No. 41 for recommended standard practices for biological evaluation of dental materials. Washington DC: Government Printing Office; 1982.

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