Use of Mineral Trioxide Aggregate with or without a Collagen Sponge as an Apical Plug in Teeth with Immature Apices

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Objective: *This retrospective study aimed to evaluate the clinical outcomes of the apical plug performed using MTA with or without collagen sponge in immature anterior maxillary teeth with necrotic pulp.* ***Study design:*** *The study included apical obturation of 20 upper incisor teeth from 18 patients and outcomes of 12-month follow-up. The teeth were divided into 2 groups with 10 cases in each group according to the apexification protocol (Group 1; apical plug with MTA, Group 2; collagen sponge and apical plug with MTA). The artificial apical barrier, approximately 4-mm-thick, was created with MTA in each group. Based on clinical and radiographic criteria, the outcome was assessed using the periapical index (PAI) by 2 calibrated investigators.* ***Results:*** *In this study, 3 of the 6 teeth (50%) in Group 1 and 5 of the 8 teeth (62.5%) in Group 2 healed at the 12-month follow-up. However, there was no statistically significant difference between the groups at the post-treatment follow-up times.* ***Conclusion:*** *The use of collagen as an apical matrix prior to the MTA plug can be suggested due to favorable clinical outcomes.*

Keywords: *Apical plug, mineral trioxide aggregate, immature teeth, collagen sponge, internal matrix,*

INTRODUCTION

In immature teeth with necrotic pulp caused by trauma, caries, and other insults, the absence of a natural constriction in the apical of the root canal can produce difficulties of apical obturation and the root-filling procedure.[1] To stimulate the formation of hard tissue in the apical region or to form a barrier at the root apex with a biocompatible material have been recommended at the treatment of the teeth with immature apices.[2] The most widely accepted material used to stimulate apical barrier formation is calcium hydroxide [$Ca(OH)_2$].[3] However, in apexification with $Ca(OH)_2$, some disadvantages have been reported such as the requiring multiple visits, prolonged treatment time, increasing the fragility of the tooth, coronal leakage due to loss of the temporary filling material, insufficient apical hard tissue formation, and unpredictability of apical closure.[3,4] Therefore, to form a barrier at the root apex with a biocompatible material has recently been more preferred compared with apexification with $Ca(OH)_2$.[5]

Bioceramic-based materials show similarity with biological hydroxyapatite that gains them excellent biocompatibility properties, and they have an osteoconductive effect when in contact with the hard tissues.[6] Numerous bioceramics based on tricalcium silicate powder and commonly known as mineral trioxide aggregate (MTA) have been introduced to worldwide markets in recent years.[7] These MTA-type cements are similar in their elemental compositions, despite the differences in terminology.[8] It was aimed to improve factors such as handling, setting time, and surface structure and to eliminate tooth discoloration depend on using these materials in newly developed calcium silicate cements

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(Angelus MTA, Biodentine, Calcium enriched mixture cement, BioAggregate, etc.).[7]

The previous studies have presented that MTA could stimulate formation of the hard tissue and function as a scaffold for better biological sealing. [9,10] This was observed histologically as dental tissue formation in the pulp cavity and calcific barrier formation in periradicular tissues with low inflammatory response.[11] Even if this material is biocompatible, it is recommended not to over-extrusion to the apical region. In this case, the adverse effects of healing in the periapical tissues were reported.[12,13] Apical matrix materials such as calcium sulphate, hydroxyapatite, platelet-rich fibrin, and collagen, which have been used increasingly, have enabled more convenient and predictable placement of the MTA.[14]

Collagen is the most abundant protein in the human body, and it is known that mesenchymal cells easily adhere and integrate into collagen-based hydrogels.[15] A sponge made of collagen is naturally biocompatible and also quite porous to allow for cell infiltration and exchange of oxygen and nutrients.[16] By functioning as a scaffold, resorbable collagen sponges support platelet aggregation, stabilize clots, facilitate recovery by attracting fibroblasts to the wound area, and enable increased vascularization in the recovering tissue.[17,18] In 1992, Lemon introduced the "internal matrix concept" for the treatment of furcation perforations. In this concept, collagen was combined with hydroxyapatite and enabled to reach the necessary hardness so that the restorative material which was put on it could be condensed.[19] In 2005, Bargholz presented the "modified matrix concept" in which collagen could be used directly without being subjected to any procedure, by using MTA.[20] The use of collagen in the canal as an apical barrier is a new method. It has been reported that collagen sponge can stimulate healing in the alveolar bone in apexification treatments carried out via this method.[21] In the literature, there are case report studies presenting the success of this method.[22,23] However, few of them involve the follow-up of the healing.[20,21]

In this study, we aimed to evaluate the clinical and radiographic outcomes of the apical plug performed by using MTA with or without a collagen sponge in necrotic teeth with immature apices after the 12-month follow-up.

MATERIALS AND METHOD

The study included the apexification treatments of 20 upper incisor teeth with necrotic pulps and open apices belonging to 18 patients aged between 8 and 13, who had applied to the Department of Pediatric Dentistry at the Faculty of Dentistry from Gaziantep University. The study was planned as a retrospective one to include a 12-month follow-up of the apical plug application. To arrive at a periradicular and pulpal diagnosis for the tooth in question, clinical preoperative testing was performed. The testing included visual examination for any sinus tracts or swelling, evaluation of tooth mobility, periodontal probing, percussion, palpation, and pulpal tests such as electric pulp testing or application of refrigerant. To visualize the radiographic status of the periapical tissues, a preoperative radiograph was taken at least 1, or more when necessary. The root development stage of each participant was assessed according to modified Moorrees stages (Moorrees *et al*.1963a,b) (Table 1). Teeth with a diagnosis of necrotic pulp after testing were included in the

Table 1. Distribution and follow-up of the treated teeth

Case	Gender	Age	Tooth/teeth	Clinical symptom	RDS	PAI scores			
						İnitial PAI	6th PAI	12th PAI	Outcome
1	M	9	21	Fistula	3	5	3	2	Healed
2	F	8	21	Recurrent fistula	4	5	5	4	Healing
3	M	9	21	Swelling	4	4	4	3	Healing
4	M	10	21	Fistula	4	4	3	2	Healed
5	F	11	21	Fistula	4	5	4	-	Healing
6	M	12	11	Fistula	3	5	4	-	Healing
7	F	8	11	Acute pain, swelling	3	5	4	3	Healing
8	M	8	21	Asymptomatic	4	5	3	2	Healed
9	M	8	11	Swelling	4	5	4	-	Healing
10	M	11	21	Fistula	4	5	4	-	Healing
11	F	10	11/21	Recurrent fistula	4/4	3/4	3/3	2/2	Healed/ Healed
12	M	8	11	Swelling	4	4	3	-	Healing
13	M	9	11/21	Recurrent fistula	4/4	5/4	4/3	3/2	Healing/ Healed
14	M	9	11	Swelling	4	5	3	2	Healed
15	M	8	11	Fistula	4	5	4	3	Healing
16	F	9	11	Recurrent fistula	3	5	4	3	Healing
17	F	8	21	Asymptomatic	3	3	2	-	Healed
18	M	9	11	Fistula	4	4	3	3	Healing

F;female, M;male, RDS; root development stage (According to modified Moorrees stages [Moorrees et al., 1963a,b])

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study. The procedure to be applied was explained to the patients and their parents in detail, and their written consent was obtained. The anamnesis information of the patients and their clinical and radiographic evaluations in the initial and follow-up appointments were recorded in a case form. Following the clinical and radiographic evaluation, patients were divided into 2 groups:

Group 1: Apical plug with MTA (n=10),

Group 2: Collagen sponge + apical plug with MTA (n=10).

Treatment Protocol

The patient was anesthetized with 2% lidocaine and a rubber dam (optradam plus Ivoclar Vivadent AG, Schaan/Liechtenstein) was placed to produce a disinfected area. If present, caries were removed, the tooth was accessed. Instrumentation was performed using hand files, Gates-Glidden drills, or rotary instruments to remove pulp tissue remnants and debris and to shape root canal walls. The canals were irrigated with 2.5% sodium hypochlorite solution (NaOCl; ACE, Father SpA, Kadiköy, Istanbul) between each instrumentation. A 30-gauge open-ended needle (Kerr Hawe Sa, Bioggio, Switzerland) was used to prevent the overflow of the irrigation solution from the apical region. NaOCl and distilled water were used in final irrigation at the first visit and canals were dried with paper points (Diadent paper point, DiaDent Group International, Burnaby Canada). As the canal medicament, $Ca(OH)_2$ paste (Ultracal; Ultradent, South Jordan, Utah, USA) was placed by using spiral root filler and was condensed with paper-points in order to adapt it to the canal walls and root apex. After the application of a sterile cotton pellet or sponge, the access was closed with a provisional restorative material (3M ESPE AG, Seefeld, Germany), and an appointment was made for the second visit for 7 days later.

At the next appointment, reassessment was achieved after the placement of a rubber dam as described above. The $Ca(OH)_2$ paste was removed using irrigation solution and instrumentation. All the teeth were asymptomatic in the second visit, and no signs of infection were observed in the root canals. As final irrigation, 17% ethylenediaminetetraacetic acid (SybronEndo, CA, USA), 2.5% NaOCl, and distilled water were used, respectively, and the canals were dried up with the sterile paper-point.

In Group 1, Angelus MTA (Angelus Indústria de Produtos Odontológicos S/A; Londrina, PR, Brazil) was mixed in compliance with the manufacturer's recommendations. It was placed to the root canal using a sterilized MTA Carrier (Angelus Indústria de Produtos Odontológicos S/A; Londrina, PR, Brazil) and condensed to the apex with a plugger (Buchanan hand plugger, Kerr, Orange, California, USA). A minimum 4-mm thickness was obtained at the apex and was confirmed radiographically. After that, the sterile wet cotton pellet was placed in the canal and restored with a temporary filling material.

In Group 2, according to the manufacturer's recommendation, collagen sponges (CollaPlug, Zimmer Dental Inc., Carlsbad, CA, USA) were placed as orthograde in small pieces till the level of the apex and condensed. After applying MTA as described in Group 1, a wet cotton pellet was placed in the canal and restored with a temporary filling material.

The temporary filling material and cotton pellet were removed 24 hours later, the setting of the MTA was examined with an 80 K-type hand file, and the root canal was filled with canal paste (AH 26, Dentsply India) and gutta-percha, via the lateral condensation technique. The access was restored with a resin composite (3M ESPE Filtek Ultimate Seefeld, Germany), and an immediate post-treatment radiograph was then taken. The distribution of the treated teeth is shown in Table 1.

Follow-up procedure

Patients were invited for the post-treatment follow-up examination in the 3rd, 6th, and 12th months. In the 6th-month appointment, follow-up of 4 teeth in Group 1 and 2 teeth in Group 2 was completed as the individuals did not proceed to participate in the study. The presence of pain during percussion, palpation, or biting was evaluated by the principal researcher. Furthermore, symptoms such as swelling in the soft tissues or sinus tracts were visually examined. Radiography was taken to evaluate the current status of the periradicular tissues. Examples of the radiographs taken during the treatment protocols and follow-up procedure are given in Figure 1 according to the groups.

Radiographic Evaluation

All the cases were given random numbers, and their personal information was deleted by the principal researcher. Radiographs were taken by the principal researcher with the same contrast and brightness to ensure standardization and randomized through power points. Radiographs were calibrated according to the study of Trope *et al* [24] by two independent pediatric dentist observers, and periapical index PAI [25] scores were obtained. PAI includes scores from 1 to 5. Which score means what in defining the condition of periapical tissues were described in Table 2. Calibration was conducted by scoring the radiographs of 20 cases twice. After the first scoring, the true score among the observers was evaluated by the kappa analysis. The second scoring was done 7 days later. Observers were blinded while making an evaluation according to the PAI score. When disagreement appeared in the scoring of a particular radiograph, the observers established a consensus and made another evaluation.

Table 2. Definitions of PAI scores

1	Normal periapical structures
2	Small changes in bone structure
3	Changes in bone structure with some mineral loss
4	Apical periodontitis with well-defined radiolucent area
5	Severe apical periodontitis with exacerbating features

Statistical Analysis

SPSS Statistical Package version 22.0 (SPSS Inc., Chicago, IL, USA) was used to perform all the analyses. Cohen's kappa statistic was used to control the harmony between inter- and intra examiners. The chi-square test was performed to evaluate whether the results were influenced by gender, age, tooth number, and clinical symptoms. Groups were compared via the Mann-Whitney U test. A paired T-Test was used for time-based determination in each group. The descriptive statistics were stated as mean ± std. $p<0.05$ was considered significant.

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Figure 1. Examples of the radiographs taken during the treatment protocols and follow-up procedure in each group. (a) Initial, (b) apical plug with MTA, (c) immediate post-treatment, (d) 3, (e) 6, and (f) 12 months follow-up.

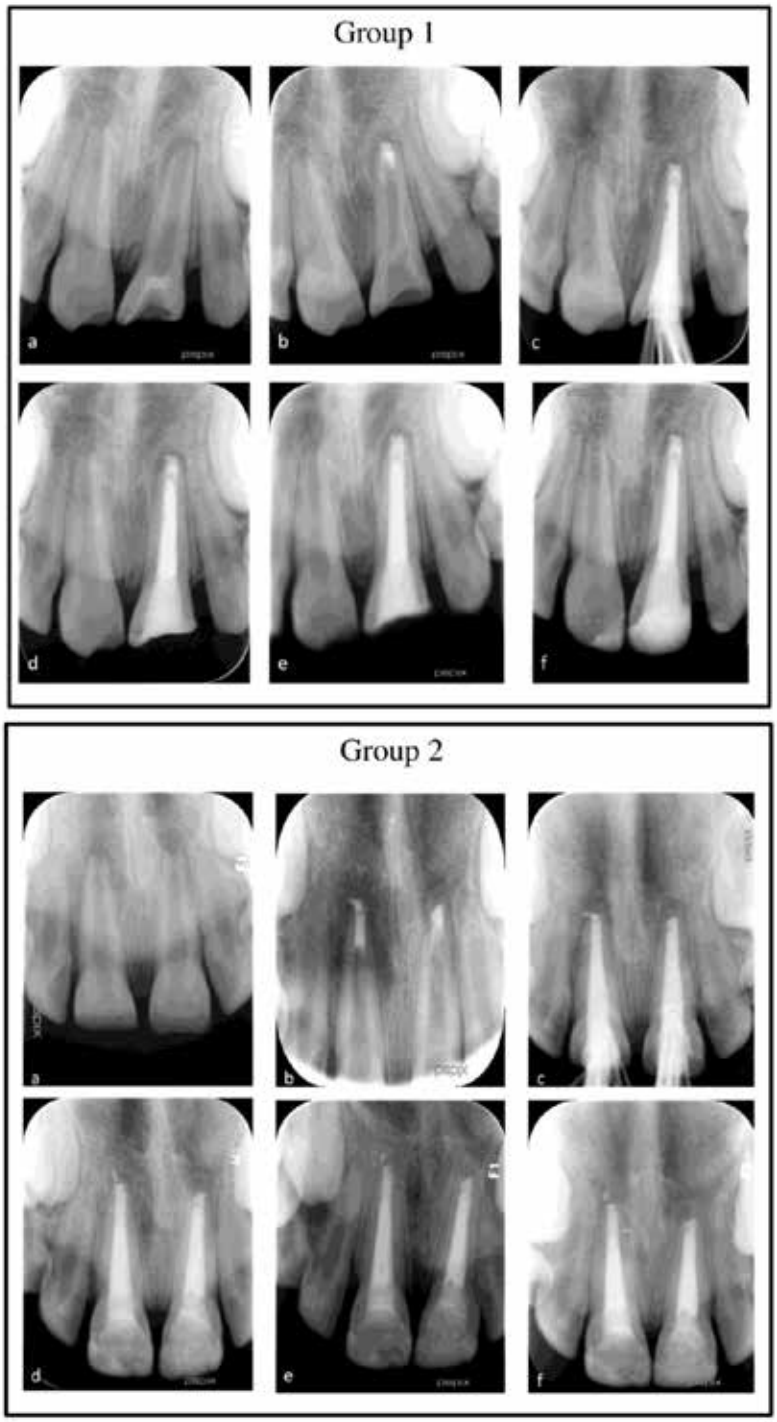

Table 3: Mean PAI values of groups in the different follow-up times

	Group 1 Mean (std.)	Sig.	Group 2 Mean (std.)	Sig.	ƀ
İnitial	4.80 (0.42)	-	4.20 (0.78)	-	0.105
3 months	4.40 (0.69)	0.104	4.10 (0.73)	0.343	0.393
6 months	3.80 (0.63)	0.001*	3.20 (0.63)	0.000*	0.089
12 months	2.66 (0.81)	0.004*	2.50 (0.53)	0.000*	0.852

ƀ; Significance value of the distribution between the groups, *; means a statistically significant difference between follow-up times in the same group (p≤0.05).

RESULTS

The weighted kappa statistic for calibration exercises varies between 0.60 and-0.80 between 2 observers. For postoperative reliability, Cohen's Kappa statistics vary between 0.65 and 0.80 for post-treatment and follow-up radiographs (intraobserver reliability). Interobserver reliability ranged between 0.67 and 0.83 between 2 observers. All the kappa statistics presented significant, compatible results.

The chi-square test showed that differences between age, sex, tooth number, and clinical symptoms did not affect clinical outcomes significantly.

The mean of PAI values according to the groups and follow-up times are given in Table 3. A statistically significant decrease in the PAI scores was observed in both groups in the 6th month initially ($p<0.05$). The decrease in the PAI scores obtained between the 6th and 12th months was also statistically significant for both groups ($p<0.05$). There was no statistically significant difference between the groups at the post-treatment follow-up times ($p>0.05$).

PAI scores were categorized to evaluate the clinical outcome: The teeth with a PAI score of 1 and 2 and without any clinical and radiographic symptoms were classified as "healed". The teeth with a PAI score of 1 and 2 and no signs and symptoms in radiographic follow-up were classified as "healing". The teeth with increased PAI scores regardless of whether they had symptoms were classified as " not healed". Accordingly, in this study, 3 of the 6 teeth (50%) in Group 1 and 5 of the 8 teeth (62.5%) in Group 2 healed at the 12-month follow-up. There were no teeth in the "not healed" category (Table 1).

DISCUSSION

This study aimed to evaluate the clinical and radiographic outcomes of using MTA as an apical plug with or without a collagen sponge following a 12-month follow-up in necrotic teeth with immature apices. The data obtained indicate that both methods stimulate the healing of periapical tissues. However, there was no statistically significant difference between the groups.

MTA is a biocompatible material that can be used to create a physical barrier in the root apex of the immature teeth and supports bone formation around the periodontium and at its interface.[26] Furthermore, it may induce apexogenesis by stimulating the mesenchymal root cells from the apical papilla to support the root maturation in the presence of periapical pathoses or abscesses.[27] Apical obturation with MTA, which is a simple and rapid technique that eliminates the need for intracanal dressing changes is less cytotoxic than other materials currently used in pulpal therapy and its setting time is approximately 4 hours in humid environments.[28] MTA obturation to apex can be performed in one or two-step visits. In the two-step apexification

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treatment, premedication of the canals with the $Ca(OH)_2$ during 7-10 days is recommended. At this point, the aim is not only to stimulate apical closure but also to control the bacterial populations in the root canal.[29] In the present study, two-step apexification was performed. However, there is no consensus in the literature regarding the use of $Ca(OH)_2$ as an intermediate medicament. Witherspoon et al.[30] reported outcomes of one or two-step apexification protocol with MTA in 144 teeth with no apical barriers. They concluded that MTA obturation performed without $Ca(OH)_2$ premedication in one-step was an appropriate alternative to the apexification treatment in two-step visits to support apical closure. However, Moore *et al* [31] reported similar clinical and radiographic results following apical plug treatment using both ProRoot and Angelus MTA after the initial $Ca(OH)_2$ dressing.

There are a limited number of studies showing the clinical outcomes of apexification with MTA,[32-35] the success rate of the treatment was reported as 76.5% and 91%.[33] In the available studies, PAI scoring was used to evaluate the success of the treatment. This method is considered a repeatable and objective method for confirming the healing. Simon *et al* [32] presented the clinical outcomes of the apexification with MTA and obtained decreased PAI scores in 27 of the 57 immature teeth (63%) after the 6 months follow-up. At least a 12-month follow-up of 43 cases, the researchers showed that 81% of them healed when considering the PAI score and the size of the apical lesion. In the study by Pace *et al* ,[34] it was reported that all of the 17 teeth became asymptomatic at the end of the 12-month follow-up, and 7 of them healed completely. In another study, healed of 17 of the 20 teeth (85%) with apexification of MTA was presented.[33] In the present study, PAI scores were decreased in 8 of 10 cases in Group 1at the 6-month follow-up, whereas the PAI scores remained stable in 2 cases. At the 12-month follow-up, decreased PAI scores were observed in all of the cases compared with their initial scores, and 3 of the 6 teeth (50%) healed completely. These data show that the apical plug with MTA is quite successful. Previous reports have indicated that this success has been similar compared to the apexification with $Ca(OH)_2$ which is also called the traditional method.[33,36] However, the use of MTA in apexification seems advantageous since it may be possible to eliminate the previously mentioned disadvantages of $Ca(OH)_2$.

The major problem in the presence of a wide-open apex is the need to limit the material to the apex; hence, the extrusion of a large amount of material into the periodontal tissue is avoided. A large amount of the extruded material may set before disintegrating and getting resorbed. This might lead to the persistence of the inflammatory process, possibly complicating or even preventing the repair of the tissue. The use of a matrix such as calcium hydroxide, hydroxyapatite, resorbable collagen, or calcium sulfate prevents the over-extrusion of the material into the periodontal tissues, decreases leakage in the sealing material, and provides for the positive response of the periodontal tissues.[37]

Collagen is a molecular structure that is characterized by a high diamino dicarboxylic amino acid content and carbohydrate moieties. Its hydrophilic nature provides a suitable surface geometry for cell adhesion. Collagen sponges create a 3-dimensional structure comprising large pores or canals, inter-canal communications, and macromolecule combinations of the connective tissue to increase scar tissue infiltration and cell growth in vivo.[38] Therefore, collagen sponges have been used in the treatment of teeth with immature apices in respect of both being structurally and functionally effective in wound repair and providing simplify of application. This material absorbs moisture and expands in addition to acting as a hemostatic.[22] It has been reported that the collagen particles increase vascularization and healing capacity of the pulp[39], and stimulate the formation of the dentin bridge by protecting the vitality of the pulp in vital pulp treatments.[40]

Grazielo Magro *et al* [21] reported that use of the collagen barrier with MTA could contribute to the reported that the use of the collagen barrier with MTA could contribute to trabecular bone formation by decreasing the acute inflammatory reaction in the alveolar bone socket. Furthermore, there are studies stating that the use of collagen sponge could prevent probable complications of the long-term application of $Ca(OH)_2$, and over-extrusion of MTA.[20,22,41]

In a study presenting the clinical outcomes of the use MTA with or without internal matrices in one-step apexification, similar to the present study, no statistically significant difference was found between the groups after 12-month follow-up.[42] In the present study, decreased PAI scores were obtained in Group 2 at the 6-month and 12-month follow-up similar to Group 1. At the 12-month follow-up, 5 of the 8 teeth (62.5%) were healed completely. Even if there were no statistically significant differences in the PAI scores between the groups, the percentage of the healed teeth was higher in Group 2 compared to Group 1. Due to the small number of cases and to include the outcomes of only a 12-month follow-up, it is not possible to come to a conclusion about the superiority of using the collagen sponge as an apical matrix in the apexification of immature teeth. Furthermore, although the collagen barrier seems to be a successful matrix, it has been reported that the stability of MTA cannot be predicted against physical forces that will occur during condensation.[43] However, the fact that the percentage of healed teeth was higher than apexification performed without the use of a collagen matrix might indicate that it stimulates healing and could increase the success of treatment.

One of the reasons for the clinical failure of root canal treatments is that the chemicals and materials used may cause coronal discoloration. It is recommended to remove these materials completely from the pulp chamber after obturation to avoid staining.[44] In the present study, this was taken into consideration when placing canal obturation materials, and after 12 months of follow-up, no clinically detectable coronal discoloration was encountered. However, the use of AH-26 sealer, a silver-containing material, constitutes a risk factor for discoloration in this study. The chromogenic potential of AH-26 sealer was attributed to the release of silver ions during or after setting reaction. Although manufacturers claim that new generation epoxy-based sealants are silver-free, potential risks of discoloration have not been overcome yet.[45]

CONCLUSION

The use of collagen as an apical matrix prevents the over-extrusion of the MTA and also stimulates healing of periapical tissues. Despite the limitations of this study, the clinical and radiographic outcomes of using the collagen matrix, which is a current concept, in the apexification of immature teeth were presented comparatively. This new concept can be suggested in the apexification of immature teeth because of its successful outcomes , however additional clinical and experimental studies are needed to determine the its reliability and applicability.

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