



ORIGINAL ARTICLE

A prospective case series in regenerative endodontics: The effective use of diluted antibiotic hydrogels in endodontic regeneration procedures

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Abstract *Objective:* to investigate the effectiveness of diluted antibiotic hydrogels in endodontic regeneration procedures.

Materials and Methods: One conventional treatment (calcium hydroxide) and two experimental treatments containing 1 mg/mL antibiotic in gel (MC-TAP and MC-DAP) were prepared for this study. The effect of these medicaments on the proliferation and differentiation potential of dental pulp stem cells (DPSCs) was examined before their clinical use, using WST and ALP assays. Twenty-two teeth diagnosed with necrotic immature roots were treated with calcium hydroxide, MC-TAP or MC-DAP using the endodontic regeneration protocol as described by the American Association of Endodontists (AAE). Teeth were examined after application of the medicament, at the time of regeneration and after 12–24 months.

Results: All treated teeth showed resolution of signs and symptoms shortly after treatment with the different medicaments. However, signs and symptoms returned a few months after initial treatment in clinical cases treated with MC-DAP. All successfully treated teeth examined after 12–24 months were free of signs and symptoms, but vitality was not achieved.

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Conclusion: Low concentration antibiotic gel containing triple antibiotic paste (MC-TAP) can be successfully used for endodontic regeneration procedures.

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1. Introduction

Double antibiotic paste (DAP), triple antibiotic paste (TAP), and calcium hydroxide have been successfully used to disinfect the root canal system during endodontic regeneration procedures (Diogenes et al., 2014). However, in vitro studies suggest that all intracanal medicaments used (TAP, DAP and calcium hydroxide) are directly toxic to dental stem cells (Yadlapati et al., 2014, Althumairy et al., 2014, Labban et al., 2014) in the concentrations actually used in endodontic regeneration (500–1000 mg/mL) (Yadlapati et al., 2014, Althumairy et al., 2014, Labban et al., 2014).

The use of diluted intracanal antibiotics (TAP and DAP) has been suggested (Diogenes et al., 2013) to enhance the survival rate of human stem cells present in the apical papillae. Diluted intracanal formulation of 1 mg/mL of DAP or TAP had a significant antibacterial effect against early bacterial biofilm formation (Sabrah et al., 2013) and established bacterial biofilm (Sabrah et al., 2015a). Furthermore, the residual antibacterial activity of the 1 mg/mL formulation was comparable to 1000 mg/mL (Sabrah et al., 2015b).

One of the challenges associated with the use of diluted antibiotics in treating immature infected teeth is that they do not have a viscous consistency. The combination of diluted antibiotics with a viscous vehicle may be a good strategy to efficiently deliver the recommended concentration of antibiotics into the root canal system. Methylcellulose (MC), a synthetic polymer of cellulose, is commonly used as a vehicle to deliver different intracanal medicaments (i.e. UltraCal™ XS and Pulpdent) and can be a good vehicle to combine with diluted antibiotics.

Recently, the American Association of Endodontists (AAE Clinical Considerations for a Regenerative Procedure, 2016) recommended the use of diluted antibiotics in a concentration of 0.1–1 mg/mL for endodontic regeneration procedures. However, there are no clinical case reports on the use of these low concentrations of antibiotics (0.1–1 mg/mL) in endodontic regeneration procedures. Furthermore, high concentrations of TAP (500–1000 mg/mL) was still used in recently published clinical case reports (Becerra et al., 2014, Nagata et al., 2014). Therefore, the aim of this study is to investigate the effectiveness of MC gels, combined with 1 mg/mL of TAP or DAP antibiotics, in clinical endodontic regeneration treatment of necrotic immature teeth.

2. Materials and methods

2.1. Study design

This study was designed as a prospective case series. The required ethical approval was obtained from the IRB committee at the University of Jordan Hospital. Treatment groups were calcium hydroxide (UltraCal™ XS, Ultradent, UT, USA) and two MC gels (MC-TAP and MC-DAP). The CARE

Checklist (2013) for writing a case report was used in this article.

2.2. Study setting and selection criteria

Participants of this study were chosen from a list of patients attending the university of Jordan hospital with signs and symptoms caused by the presence of necrotic permanent immature teeth. Periapical radiographs (Dental Imaging Software, 6.14.0) were taken to ensure the presence of a *peri*-apical lesion around the root apex of the involved teeth. The selected teeth had a pulp space that was not needed for retaining final restoration. Only compliant patients with no known allergy to antibiotics or any medicaments were included. Informed consent forms were signed by parents. The study was conducted between July 1, 2016 and July 31, 2019.

2.3. Preparation of the intracanal medicaments

The first medicament gel consisted of 2% (w/v) MC (MW 88,000 g/mol, Acros Organics, New Jersey) and 1 mg of DAP (Hikma Pharmaceuticals, Amman, Jordan) mixed in 1 mL of distilled water at room temperature (MC-DAP). The second medicament gel consisted of 2% (w/v) MC and 1 mg of TAP (Hikma Pharmaceuticals) mixed in 1 mL of distilled water at room temperature (MC-TAP). A magnetic stirrer (300 rpm) was used for 24 h to ensure proper mixing of the different components. Sterile containers and stir bars were used and all mixing procedures were carried out in a laminar flow cabinet.

Before using the medicaments for the treatment of patients, we studied the proliferation and osteogenic differentiation ability of dental pulp stem cells (DPSCs) after direct exposure to MC based medicaments containing 1 mg/mL of antibiotics (MC-TAP, MC-DAP) following a previously published protocols (Labban et al., 2014, Sabrah et al., 2015a, b, Phumpatrakom and Srisuwan, 2014, McIntyre et al., 2019). Furthermore, antibacterial properties were confirmed against intracanal bacterial samples according to previously published protocol (Sabrah et al., 2013).

2.4. Intervention

A periapical radiograph was taken to determine the extent of the lesion, root length and width. A vitality test was performed by cold testing. Further periapical and periodontal assessments were also performed. Local anesthesia, dental dam isolation and access were carried out, followed by copious, gentle irrigation with 1.5% NaOCl (20 mL/canal, 5 min) using an irrigation needle with a closed end and side-vents. The irrigation needle was positioned about 1 mm from the root end, to minimize cytotoxicity to stem cells in the apical tissue. This was followed by irrigation with saline (20 mL/canal, 5 min), with the irrigation needle positioned about 1 mm from the root

end. The canal was dried with paper points, and the intracanal medicaments were placed inside the canal space below the cemento-enamel junction, using an irrigation needle (to minimize crown staining). The access cavity was finally sealed with 3–4 mm of a temporary restorative material (Glass Ionomer, Riva Self Cure, SDI, Victoria, Australia). All procedures were carried out by the same endodontist.

The second appointment took place 2 to 4 weeks later to assess the response to the initial treatment. If there were signs/symptoms of persistent infection, additional treatment time with antimicrobials or calcium hydroxide was considered. Anesthesia with 3% mepivacaine without vasoconstrictor was administered and dental dam isolation was placed. After removing the GIC restoration, the canals were irrigated with

Table 1 Case specific data (age, tooth, sex (M or F), etiology of the lesion, signs/symptoms, presence of periapical lesions, the medicament used and the results) of the teeth treated with regenerative endodontics.

Case number	Sex and age	Tooth	Periapical radiograph	Signs and symptoms	Etiology	Periapical lesion	Last follow up*	Intracanal medicaments	Degree of success
1	10, M	LL5	Fig. 1-a	Pain/ swelling	Dental caries	yes	24	MTAP	S2
2	12, M	UR1	Fig. 1-b	Sinus tract/ fracture	trauma	yes	12	MTAP	S1
3	12, M	UL1	Fig. 1-b	Sinus tract/ fracture	trauma	yes	12	MTAP	S1
4	12F	UR5	Fig. 2-a	Cavitated/ pain on eating	Dental caries	yes	5	MDAP	Failed after 5 months, RCT was done
5	8, M	LL6	Fig. 2-b	Pain/ swelling	Dental caries	yes	3	MDAP	Failed after 3 months. Retreated with calcium hydroxide
6	10, F	UR6	Fig. 1-c	pain	Dental caries	yes	24	MTAP	S2
7_1	8, F	UL1	Fig. 2-c	pain	Dentinogenesis imperfect	yes	1	MDAP	Failed after 1 month, symptoms persist, re-treated with calcium hydroxide
7_2	8, F	LR1	Fig. 3-a	pain	Dentinogenesis imperfect	Yes	12	Calcium hydroxide	S1
7_3	8, F	UR4	Fig. 3-b	pain	Dentinogenesis imperfect	Yes	12	Calcium hydroxide	S1
7_4	8, F	LL5	Fig. 3-c	pain	Dentinogenesis imperfect	yes	12	Calcium hydroxide	S1
7_5	8,F	LR4	Fig. 3-d	pain	Dentinogenesis imperfect	yes	12	Calcium hydroxide	S1
8	13, F	LL7	Fig. 4-e	Pain	Dental caries	yes	24	Calcium hydroxide	S2
9	11, M	UL1	Fig. 2-e	Pain/ fracture	trauma	yes	12	MDAP	S2 Failed after 12 months cause of loss of coronal tooth structure, RCT was done to fabricate post and core
10	13, M	LR6	Fig. 2-d	pain	Dental caries	yes	7	MDAP	S1 Failed after 7 months, symptoms returned, retreated with calcium hydroxide
12	13, M	UR1	Fig. 4-f	fracture	trauma	yes	4	Calcium hydroxide	S1, RCT was done after 4 months cause of loss of coronal tooth structure.
13	11, M	UR1	Fig. 4-g	Fracture	trauma	yes	12	Calcium hydroxide	S2
14	11, M	UL1	Fig. 4-g	Fracture	trauma	yes	12	Calcium hydroxide	S2
15	9, M	UR1	Fig. 4-h	fracture	trauma	yes	15	Calcium hydroxide	S2
16	14, F	UR7	Fig. 1-d	pain	Dental caries	yes	12	MTAP	S2

* All successful cases that were followed up less than 12 months was affected by Covid-19 pandemic. Parents/patients refuse to visit the hospital during that time.

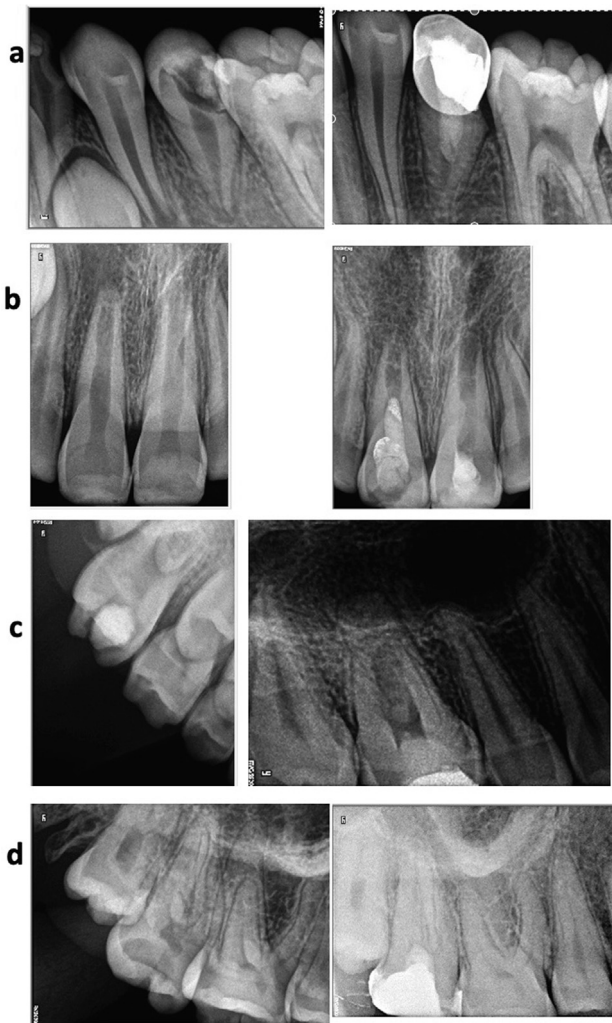


Fig. 1 Periapical radiographs of teeth treated with MTAP medicaments at the first and final follow up, a represent case 1, b represent case 2 and 3, c represent case 6 and d represent case 16. Images on the left are pre-treatment and images on the right are the post-treatment.

copious, gentle irrigation of 17% EDTA (20 mL), then dried with paper points. Bleeding into the canal system was created by over-instrumenting with a sterile endo file up to the level of the cemento-enamel junction. Finally, a white MTA capping material and a 3–4 mm layer of glass ionomer (Riva Light Cure) was gently placed over the capping material and light-cured for 40 s.

Participants were followed up after treatment, and again 12–24 months after the regeneration procedure. At each follow-up visit, a clinical and radiographic exam was performed.

3. Results

The prepared medicaments were shown to be effective and safe to use in clinical regenerative endodontics according to the antibacterial, proliferation and the ALP activity assays.

Twenty-two necrotic teeth with open apices were selected and treated with the regenerative endodontics protocol. Case

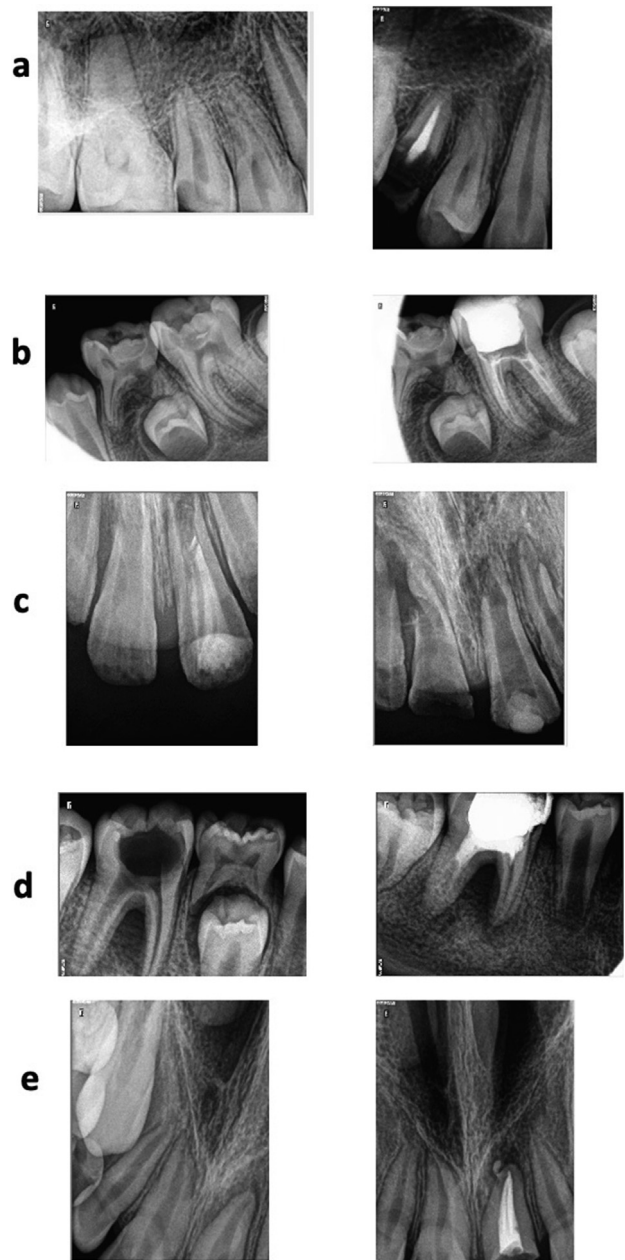


Fig. 2 Periapical radiographs of teeth treated with MDAP medicaments at the first and final follow up, a represent case 4, b represent case 5, c represent case 7_1, d represent case 10, and e represent case 9. Images on the left are pre-treatment and images on the right are the post-treatment.

specific data (age, tooth, sex, etiology of the lesion, signs/symptoms, presence of periapical lesions, the medicament used and the results) of the teeth treated with regenerative endodontics are presented in [Table 1](#).

Teeth with treatment failure were re-treated with the conventional treatment (calcium hydroxide) according to AAE recommendations. As stated by the AAE: “The degree of success of regenerative endodontic procedures is largely measured by the extent to which it is possible to attain primary, secondary, and tertiary goals; primary goal (S1): The elimination

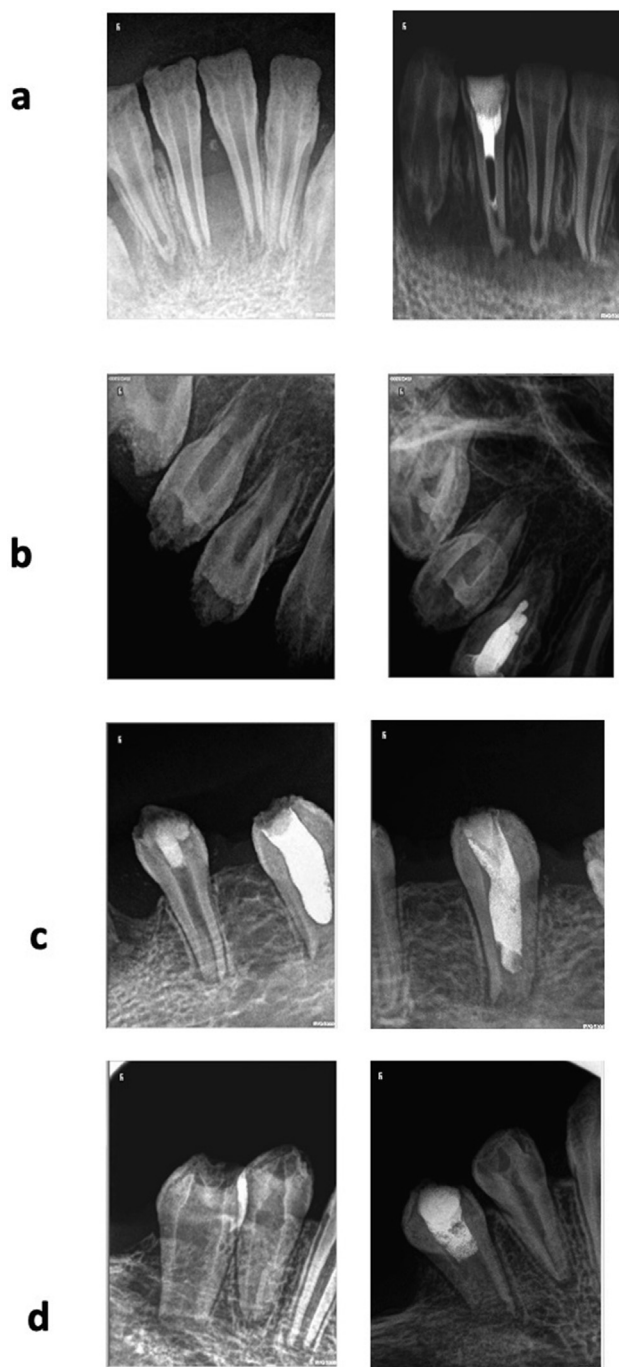


Fig. 3 Periapical radiographs of teeth treated with calcium hydroxide medicaments at the first and final follow up, a represent case 7_2, b represent case 7_3, c represent case 7_4, d represent case 7_5. Images on the left are pre-treatment and images on the right are the post-treatment.

of symptoms and the evidence of bony healing, secondary goal (S2): Increased root wall thickness and/or increased root length (desirable, but perhaps not essential), and tertiary goal (S3): Positive response to vitality testing (which, if achieved, could indicate a more organized vital pulp tissue” (AAE *Clinical Considerations for a Regenerative Procedure*, 2016).

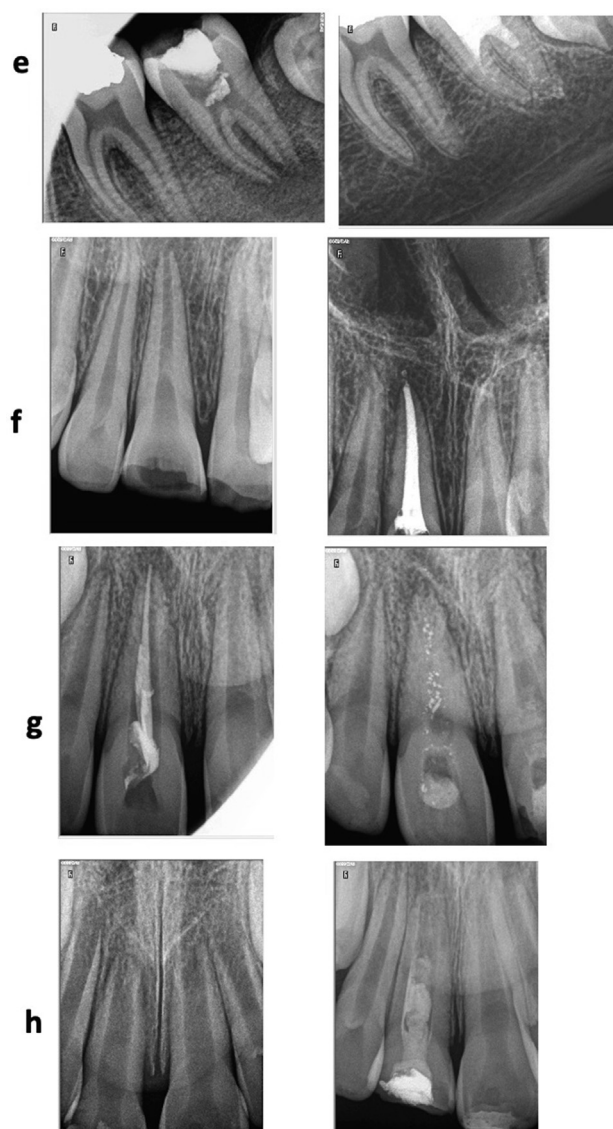


Fig. 4 Periapical radiographs of teeth treated with calcium hydroxide medicaments at the first and final follow up, e represent case 8, f represent case 12, g represent case 13 and 14, and h represent case 15. Images on the left are pre-treatment and images on the right are the post-treatment.

Pre-operative and post-operative radiographs for MC-TAP, MC-DAP, and calcium hydroxide are present in [Figs. 1, 2, 3 and 4](#).

4. Discussion

Regenerative endodontics is one of the most exciting developments in dentistry. It uses tissue engineering to restore the root canal to a healthy state, allowing for continued development of the root and surrounding tissue. Previous clinical reports on endodontic regeneration have used both TAP and DAP in a concentration of approximately 500–1000 mg/mL (Diogenes et al., 2013). Due to the significant role of stem cells in endodontic regeneration, their viability in the existence of

antibiotic paste is fundamental for success. Recent studies have found that high concentrations of intracanal medicaments (TAP, DAP) are directly and indirectly toxic to stem cells, and different types of cells present in the periodontium (Labban et al., 2014, Phumpatprakom and Srisuwan, 2014, Althumairy et al., 2014). However, concentrations of 0.1–2 mg/mL of TAP or DAP have no cytotoxic effect on dental pulp cells (Labban et al., 2014, Phumpatprakom and Srisuwan, 2014, Sabrah et al., 2015^a).

One of the challenges associated with the use of diluted antibiotic concentrations in immature infected teeth is that they do not have a viscous consistency. To efficiently deliver the intended concentration of antibiotics into the root canal, incorporation of the diluted antibiotics into a viscous vehicle may be a good option. The objectives of using different medicament vehicles are mainly to maintain therapeutically active concentrations and increase the residence time of the medicament inside the root canal system. In this study we used a MC vehicle in a concentration that produced 4000 cp viscosity in order to be able to insert the medicaments inside the root canal.

In this case series, five teeth were treated with MC-TAP gel. Of these, three teeth achieved root closure (S2) and two teeth achieved resolution of signs and symptoms (S1) (Fig. 1). Unfortunately, we were unable to follow the latter two teeth for more than 12 months. The parent confirmed that his child had not had any problems since the treatment. However, he refused a follow-up visit due to Covid-19 pandemic and the fear of getting infected when visiting the hospital.

Another five teeth were treated with MC-DAP gel. Treatment failed in three teeth, as symptoms returned after initiating the regeneration process. In two teeth, it failed due to loss of coronal tooth structure (Fig. 2). All teeth with treatment failure were re-treated with calcium hydroxide, and regeneration procedures were repeated. MC-DAP has been shown to be effective against different endodontic pathogens in vitro (Sabrah et al., 2013, Sabrah et al., 2015a, b, Tagelsir et al., 2016, Jacobs et al., 2017). However, MC-DAP did not lead to a successful regeneration outcome. Previous studies have shown that MC-DAP gels have a good antibacterial effect and long residual antibacterial activity (Sabrah et al., 2015a, b, Jenks et al., 2016). However, these studies reported the residual antibacterial effect against a single bacterial species grown in the laboratory. The complexity of the root canal system and the complex bacterial biofilm that grows inside it require the use a mixture of antibiotics in order to kill as much as possible of the bacterial biofilm. TAP was first developed by Hoshino and his colleagues (Hoshino et al., 1996) and reported to be effective against the pathogens commonly found inside the root canal system. Therefore, it is expected to do better than DAP in endodontic regeneration procedures.

Case seven in this study was diagnosed with dentinogenesis imperfecta. Dentinogenesis imperfecta is an autosomal dominant disease characterized by severe hypomineralization of dentin and altered dentin structure. In the first treatment of this patient, MC-DAP gel was used. However, the symptoms returned after one month, and we had to use calcium hydroxide for re-treating this tooth and all other teeth of this patient. Calcium hydroxide was effective in controlling symptoms. However, the immature roots were not fully closed at the time of the last follow-up attended by the patient. A mutation in the

dentin sialophosphoprotein (DSPP) gene present in dentinogenesis imperfecta patients, which affects the normal dentinogenesis process (La Dure-Molla et al., 2015) might play a role in the absence of an increase in the root wall thickness and/or length. All other cases treated with calcium hydroxide showed successful growth of the immature roots except one case that failed due to loss of coronal tooth structure.

5. Conclusion

Both calcium hydroxide and MC-TAP gel were used successfully in treating immature necrotic teeth using a regenerative endodontic protocol. The use of MC-DAP gels containing 1 mg/mL should be avoided if possible.

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Ethical statement

The required ethical approval was obtained from the IRB committee at the University of Jordan Hospital. Informed consent and full explanation of the study were given to each participant prior recruitment.

CRedit authorship contribution statement

Alaa H.A Sabrah: Conceptualization, Methodology, Investigation, Data curation, Writing – original draft. **Mohammad M. Hammad:** Investigation. **Fouad K Wahab:** Supervision. **Abeer AlHadidi:** Investigation. **Nesreen A Salim:** Formal analysis. **Ayman F. Alelaimat:** Validation. **Ibrahim Khatib:** Writing – review & editing.

Declaration of Competing Interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

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