

Regenerative Endodontic Treatment: Report of Two Cases with Different Clinical Management and Outcomes

Mehrfam Khoshkhounejad¹, Noushin Shokouhinejad², Salma Pirmoazen³✉

¹Assistant Professor, Dental Research Center, Dentistry Research Institute, Tehran University of Medical Sciences, Tehran, Iran; Department of Endodontics, School of Dentistry, Tehran University of Medical Sciences, Tehran, Iran

²Associate Professor, Dental Research Center, Dentistry Research Institute, Tehran University of Medical Sciences, Tehran, Iran; Department of Endodontics, School of Dentistry, Tehran University of Medical Sciences, Tehran, Iran

³Assistant Professor, Department of Endodontics, School of Dentistry, Tehran University of Medical Sciences, Tehran, Iran

Abstract

Endodontic intervention in necrotic immature permanent teeth is usually a clinical challenge. With appropriate case selection, regenerative treatment can be effective, providing a desirable outcome. However, there is still no consensus on the optimal disinfection protocol or the method to achieve predictable clinical outcome. This article presents two cases of regenerative treatment in necrotic immature teeth, using mineral trioxide aggregate (MTA) and Biodentine™ as coronal barriers and different irrigants, which led to different clinical outcomes.

Key Words: Regeneration; Endodontics; Dental Pulp Necrosis; Stem Cells

Journal of Dentistry, Tehran University of Medical Sciences, Tehran, Iran (2015; Vol. 12, No. 6)

✉ Corresponding author:

S. Pirmoazen, Department of Endodontics, School of Dentistry, Tehran University of Medical Sciences, Tehran, Iran

S_pirmoazen@yahoo.com

Received: 7 March 2015

Accepted: 26 June 2015

INTRODUCTION

Endodontic treatment of necrotic immature teeth is challenging. Proper preparation and obturation of the apical portion of immature teeth are difficult to achieve because of the thin, fragile dentinal walls and the blunderbuss anatomy [1]. Multiple-session apexification using calcium hydroxide used to be the treatment of choice for such cases [2]. Later-proposed one-step apexification by the induction of artificial barriers using materials such as MTA greatly decreased the frequency of sessions and duration of treatment [3]. However, both these techniques suffer a major limitation; they do not allow the continuation of root development and consequently result in the formation of a fragile root [4].

A recently suggested approach is based on creation of an environment that induces root

maturation. This approach includes disinfection of the root canal system and use of antibiotic paste as an intracanal medicament. Revascularization is a biological alternative approach for treatment of immature necrotic teeth. In contrast to the conventional apexification and artificial formation of an apical barrier, revascularization enables root maturation [5,6]. Vital apical pulp tissue or Hertwig's epithelial root sheath might have remained in necrotic, open-apex teeth. If present, these tissues may proliferate and result in root development when the canal has been well disinfected and the inflammatory process has been reversed [7].

Modern era of regenerative endodontics was started by a case report by Banchs and Trope [8] in 2004. The proposed regenerative treatment generally starts with chemical

disinfection of the root canals. Different concentrations of sodium hypochlorite (NaOCl) including 6% [9, 10], 5.25% [5,8,11], 2.5% [12,13], and 1.25% [14] and different concentrations of chlorhexidine (CHX) including 2% [9] and 0.12% [11] have been successfully used for this purpose. The procedure continues by application of triple antibiotic paste as an intracanal medicament, which is composed of ciprofloxacin, metronidazole and minocycline, as suggested by Hoshino et al [15]. In the absence of clinical signs and symptoms of periradicular diseases, the treatment continues with removing the paste and inducing bleeding into the canals by irritating the periapical tissues. After formation of a blood clot, the orifice of the canal is sealed with MTA, which is placed over the clot as a biocompatible sealing material. Finally, the crown is permanently restored.

It must be mentioned that revascularization has certain practical limitations. There is no agreement on the methods to produce predictable clinical outcomes or optimal disinfection protocols. A range of clinical protocols have been used to treat these cases, with various irrigants, medicaments, clinical procedures and follow-up times. Criteria for predictable revascularization are still lacking. It is difficult to select the appropriate non-vital teeth with residual vital apical cells, which are believed to be necessary for a successful regenerative procedure.

This article describes two different revascularization treatment protocols in necrotic immature teeth, which led to different clinical outcomes.

CASE REPORT

Case one: A healthy 16 year-old male patient was referred to the Department of Endodontics at the School of Dentistry of Tehran University of Medical Sciences. The patient's chief complaint was occasional pus discharge from a gumboil in the anterior region of the

upper jaw. The patient recalled a history of an impact trauma and crown fracture of the left maxillary incisor eight years earlier. Clinical examination revealed crown fracture and a composite filling on tooth #21. Tooth mobility was within the normal limits. All the teeth in the maxillary anterior region were responsive to cold test, using Endo-Frost cold spray (Roeko; Coltene Whaledent, Langenau, Germany) except for tooth #21. There was no traceable sinus tract at the time. Radiography revealed immature root of tooth #21 with a radiolucent periapical lesion (Fig. 1A). The diagnosis of necrotic pulp with asymptomatic apical periodontitis was made. Informed consent was obtained. After local infiltration anesthesia with 1.8 mL of 2% lidocaine with 1:80,000 epinephrine (Daroupakhsh, Tehran, Iran), rubber dam isolation, and access cavity preparation, the working length was determined by placing a large file in the canal and taking a periapical radiograph. The root canal system was irrigated with 20 mL of 5.25% NaOCl followed by 20 mL of 0.2% CHX [8,16]. Triple antibiotic paste (ciprofloxacin, metronidazole, doxycycline) was used as intracanal medicament for three weeks. In the next visit, the patient was asymptomatic. Local infiltration anesthesia was performed with 3% plain mepivacaine, without vasoconstrictor to facilitate bleeding as suggested by Petrino et al [11]. The antibiotic intracanal medicament was gently removed and flushed out of the canal with copious irrigation with 5.25% NaOCl. After drying the canal, bleeding was induced inside the canal with a sterile #50 hand file (MANI Inc., Utsunomiya, Japan), which was inserted one millimeter beyond the apical foramen and the coronal part of the canal was sealed with ProRoot MTA (Dentsply Tulsa Dental, Tulsa, OK, USA) over the blood clot. A moist cotton pellet was placed over the MTA in the access cavity, and the tooth was temporarily restored with Coltosol (Asia Chemi Teb Co., Tehran, Iran) (Fig. 1B).

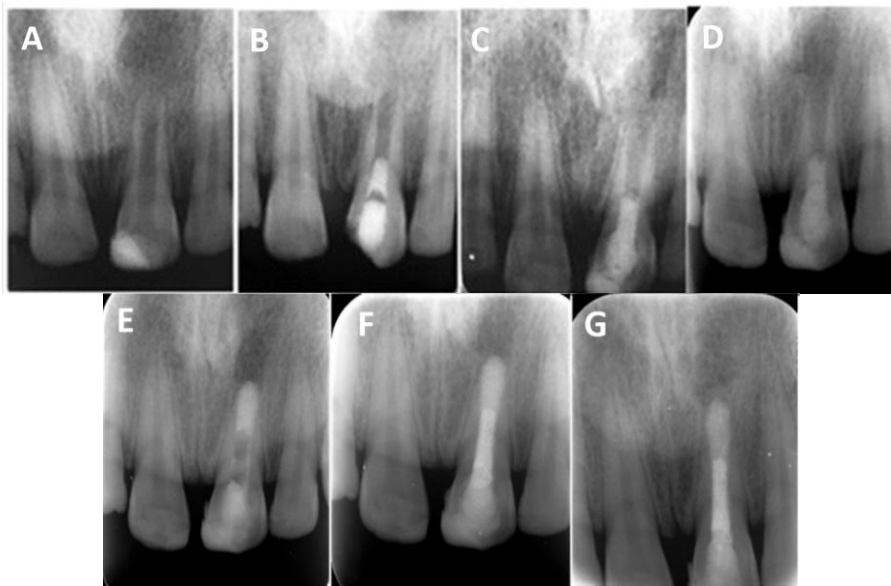


Fig. 1. (A) Preoperative periapical radiograph of tooth #21. Note the immaturity of root. (B) After placing MTA on blood clot and temporary filling. (C) After completion of regenerative endodontic procedure and permanent coronal restoration. (D) Six-month follow-up. Note the periapical radiolucent lesion. (E and F) After placing MTA plug as apical barrier and obturation of coronal part of the root. (G) One-year follow-up. The healing of periapical lesion was observed.

One week later, the patient was recalled to ensure the setting of MTA, and permanent restoration of the tooth was performed with composite resin (Dentsply International, Milford, DE, USA) (Fig. 1C).

Three months later, at the first follow-up appointment, no signs or symptoms were recorded. At the six-month recall, recurrence of pus discharge was reported by the patient. No evidence of thickening of the canal walls or continuation of root development was noted on radiographs and apical rarefaction was seen (Fig. 1D). According to the American Association of Endodontics (AAE) guidelines [17], the regenerative treatment was considered not successful. Therefore, apical closure with MTA plug was scheduled.

After preparing the access cavity, the coronal MTA was removed with a diamond-coated straight tip (E32D, NSK, Tokyo, Japan) attached to an ultrasonic scaler (Varios 970, NSK, Tokyo, Japan) under copious irrigation. A five-millimeter thick ProRoot MTA plug was placed in the apical part of the canal (Fig. 1E).

After one week, the MTA setting was ensured and the coronal part was filled with gutta-percha (Meta Bio-med Co., LTD, Seoul, Korea) and AH-26 sealer (Dentsply, De Trey, Konstanz, Germany). The crown was restored with composite resin (Dentsply International, Milford, DE, USA) (Fig. 1F).

The patient was recalled every three months for radiographic examination and evaluation of clinical signs and symptoms. In the follow up sessions and after one year, the patient had no signs or symptoms. At the one-year follow up, the radiolucent lesion had resolved (Fig. 1G).

Case two: A healthy 17 year-old female patient was referred to the Department of Endodontics at the Dental Faculty of Tehran University of Medical Sciences. The patient complained of slight swelling and pus discharge adjacent to one of her lower teeth. Clinical examination revealed an opening on the occlusal surface of the mandibular right second premolar (tooth #45) that seemed to cause pulpal exposure (Fig. 2).

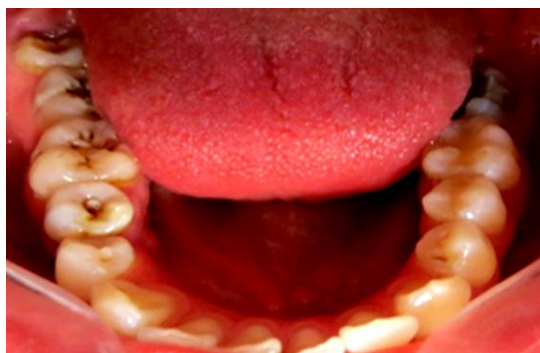


Fig. 2. Cavity on the occlusal surface of tooth #45 and a sinus tract in the lingual side of the tooth.



Fig. 3. Panoramic radiograph. Note the immaturity of tooth #45.

The tooth was not responsive to cold test using Endo-Frost cold spray. A swelling and sinus tract were seen in the lingual side of the tooth. The sinus tract was not traceable. On radiographs, tooth #45 had a blunderbuss short root with thin dentinal walls and a radiolucency embracing the root (Figs. 3 and 4A).

At the first appointment, informed consent was obtained. After injection of local anesthetic agent comprising of 3% plain mepivacaine and rubber dam isolation, the access cavity was prepared. The working length was determined by placing a large file in the canal and taking a periapical radiograph. Then, the root canal system was copiously and gently irrigated with 20 mL of 1.5% NaOCl followed by 20 mL of normal saline [14]. Triple antibiotic paste (ciprofloxacin, metronidazole, doxycycline) was used as intracanal medicament for three weeks (Fig. 5). In the next visit, the patient was asymptomatic and the sinus tract had resolved. Local infiltration anesthesia was performed using 3% plain mepivacaine. The antibiotic intracanal medicament was gently removed from the canal via irrigation with 20 mL of normal saline and 20 mL of 17% EDTA. After drying the canal, bleeding was induced inside the canal with an overextended #70 hand file (MANI Inc., Utsunomiya, Japan), and the coronal part of the canal was sealed with

Biodentine™ (Septodont, St. Maur-des-Fossés, France).

One week later, the tooth was permanently restored with composite resin (Dentsply International, Milford, DE, USA) (Figs. 4B and 6). The patient was recalled every three months. No clinical signs or symptoms were recorded. At the six-month follow-up, no evidence of thickening of the canal walls or continuation of root development was seen on the radiographs, but obvious intracanal calcification was noted (Fig. 4C). The patient had no signs or symptoms. At the one-year follow-up, clinical findings were the same. On radiographs, diffuse intracanal calcification was seen in the vicinity of the coronal Biodentine™, and radiopaque calcified bridges were noted in the middle and apical parts of the canal (Fig. 4D). The tooth was not responsive to cold test.

DISCUSSION

In this case report, we evaluated two immature necrotic teeth with periapical radiolucencies. The patients were the same age. Pulp necrosis in the first case was due to a traumatic event. In the second case, pulp exposure was assumed to be due to caries or dens evaginatus, which had led to necrosis before tooth maturation. Dens evaginatus may occur in any tooth but is most commonly seen in premolars, especially mandibular premolars.

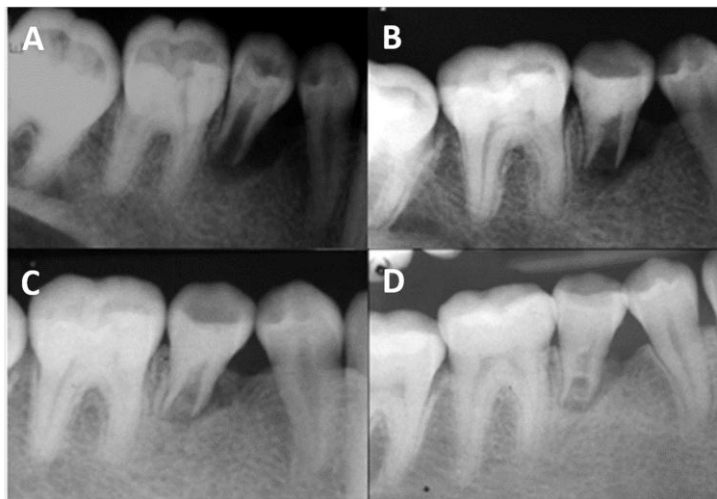


Fig. 4. (A) Blunderbuss short root of tooth #45 with thin dentinal walls and a radiolucency embracing the root. (B) After regenerative endodontic treatment and permanent coronal restoration. (C) Six-month follow-up. (D)

Dens evaginatus is one of the most prevalent tooth anomalies, and following attrition during normal tooth function, it usually leads to pulp exposure and devitalization at an early age [8,11,18]. If necrosis occurs, these cases are usually asymptomatic, and development of periapical lesions and formation of sinus tract may pursue.

Pulp revascularization was considered to be the treatment of choice in order to save the teeth and promote root development. The primary goal of regenerative endodontic procedures is healing of apical periodontitis as stated in the revised AAE guidelines (July 2013). According to the guidelines, the secondary goal is to increase root wall thickness and/or root length. The tertiary goal is to regain a positive response to pulp testing. Both the secondary and tertiary goals are desirable but possibly not essential to determine the clinical success [17]. According to the guidelines, the first case did not fulfill the essential requirements of a successful treatment and consequently, the patient received an alternative treatment plan.

Root canal disinfection in regenerative endodontic treatment is challenging and debatable.

One of the critical points is to choose the most effective antimicrobial agent for maximum cleaning of the root canal system. On the other hand, in teeth with an immature apex, the root canal is large in size, permitting easier permeation of antimicrobial agents into the root canal system and towards the periapical region.

In fact, the concern is how to prevent relatively toxic antimicrobial agents from gaining access to the periapical tissues, which may contain stem cells and vasculature that are necessary for the regeneration process. Attempts were made to find an antimicrobial agent with the least toxic effects in terms of both chemical composition and concentration. The use of Endo-Vac has been mentioned as a good strategy to avoid periapical extrusion of irrigants such as NaOCl [19].

The different concentrations of NaOCl including 6% [9, 10], 5.25% [5, 8, 11], 2.5% [12,13] and 1.25% [14] and different concentrations of CHX (0.12% to 2%) [9,15] have been successfully used for this purpose. In our two cases, different irrigation protocols were used. In the first case, the tooth was irrigated with 5.25% NaOCl followed by 0.2% CHX [8,16].



Fig. 5. End of first appointment. Note the non-traceable sinus tract in the lingual side of tooth #45.



Fig. 6. After permanent restoration.

For removing the triple antibiotic paste in the second session, irrigation was performed using 5.25% NaOCl.

In the second case, the root canal system was irrigated with 1.5% NaOCl followed by normal saline [14]. In the next visit, the antibiotic dressing was removed from the canal using normal saline. Next, 17% EDTA was used as the final irrigant because the use of EDTA is beneficial for providing dentin-derived growth factors [20,21].

The half- or full-strength (3% and 6%, respectively) concentrations of NaOCl have been shown to prevent stem cell attachment to dentin surfaces, and are toxic to stem cells of the apical papilla (SCAP) [20, 22]. It has also been shown that 2% CHX was the most toxic irrigant for SCAP. It is worth noting that in some of the published successful revascularization cases, full-strength NaOCl has been used for irrigation, at least in the first appointment [8,11]. Previous studies showed that the use of 17% EDTA significantly increased attachment of newly formed mineralized tissues to dentinal canal walls [21]. Growth factors are released from the root canal dentinal walls following EDTA irrigation. In the second case (eliminating CHX from the rinsing protocol), adding %17 EDTA and using NaOCl in a lower concentration might have had a positive effect on the treatment outcome.

In the second case, Biodentine™ was used as the coronal barrier instead of MTA. Biodentine™ is a bioactive tooth-colored calcium silicate-based cement. A previous study showed the bioactivity of Biodentine™ as it increased pulp cell proliferation and biomineralization [23]. Therefore, Biodentine™, as a suitable material, has been suggested for the purpose of dentin-pulp complex regeneration in the clinical setting [1,23]. Only one previous study used Biodentine™ in the revascularization process and reported resolution of the associated periapical pathology in a mandibular incisor of a 15 year-old patient [1]. In the aforementioned study, the tooth was irrigated with 6% NaOCl and the triple antibiotic paste was applied as the intracanal medicament with no instrumentation. Biodentine™ was then applied to achieve a coronal seal. The tooth was then permanently restored with bonded resin. The lesion resolved after 18 months. The authors stated that Biodentine™ was suitable for maintaining the vitality of dental pulp stem cells and creating a suitable environment for revascularization of dental pulp and consequent completion of root maturation [1].

The success of revascularization/revitalization therapy depends on efficient disinfection of the root canal system [24]. If infection persists in the root canal, not only regeneration but

also repair will not occur in the pulp-periapical tissue complex [24]. There may be a relation between time of trauma and quality of root development; the longer the duration of pulp necrosis, the lower the quality of root development after regenerative treatments [4]. Such association has also been discussed in previous studies reporting decreased or no root development and failure of the procedure [11, 25].

Lenzi and Trope [25] discussed the possibility of longstanding infection destroying the cells capable of pulp regeneration. However, considering the successful outcomes of regenerative endodontic treatments in cases with long-lasting apical periodontitis, they concluded that this might not be the reason. Another possible explanation is the maturation of bacterial biofilm, which results in more difficult elimination by conventional protocols. Perhaps this can be assumed as one of the underlying factors for the unfavorable outcome in our first case.

In the second case, intra canal calcification was seen with no increase in root length or thickness of dentinal walls. The patient was asymptomatic. According to the AAE guidelines, healing of the apical periodontitis is the only essential requirement for clinical success [17]. Deposition of a cementum-like tissue on root canal dentinal walls following regenerative endodontic treatment was reported in an animal study. This tissue was irregular and assumed to be responsible for root development. In the same study, the authors reported the formation of cementum bridges in the root canal system and reported that it might be due to the MTA potential of hard-tissue induction [26].

Nosrat et al. [4] discussed the drawbacks and unfavorable outcomes of regenerative endodontic treatments and mentioned that formation of a hard-tissue barrier inside the canal between the coronal MTA plug and the root apex was among the unfavorable outcomes.

However, in a more recent article, Fouad and Nosrat [27] suggested that clinicians and the research community must reach a consensus about the clinically acceptable outcome. They suggested re-defining the clinical success and proposed that the clinical success must be re-defined as when calcification occurs in the absence of any signs and symptoms and the infection is completely resolved. Despite the fact that formation of intracanal mineralized tissue and pulp canal obliteration are inconsistent with the concept of regeneration, it seems logical to compromise the idealistic expectations in this field and overlook some of the shortcomings.

CONCLUSION

There is considerable debate on the ideal outcome of regenerative endodontic treatment. With respect to the nature of regeneration, it seems that there is a gap between the expected histological outcomes and what actually happens in the root canal system, at least in many instances. Since many of these teeth achieve acceptable clinical outcomes i.e. being infection-free, asymptomatic and clinically functional, it seems logical to reframe our perspectives in this field and expand the scope of definition of success in endodontic regenerative treatment, and consider the aforementioned clinical outcomes as success.

REFERENCES

- 1- Khetarpal A, Chaudhary S, Talwar S, Ravi R, Verma M. Revascularization of immature permanent tooth with periapical lesion using a new biomaterial - A case report. *Int. J. Dent. Sci. Res.* 2013 Sep;1(1):20-2.
- 2- Rafter M. Apexification: a review. *Dent Traumatol.* 2005 Feb;21(1):1-8.
- 3- Torabinejad M, Chivian N. Clinical applications of mineral trioxide aggregate. *J Endod.* 1999 Mar;25(3):197-205.
- 4- Nosrat A, Homayounfar N, Oloomi K. Drawbacks and unfavorable outcomes of regenerative endodontic treatments of necrotic

- immature teeth: a literature review and report of a case. *J Endod.* 2012 Oct;38(10):1428-34.
- 5- Ding RY, Cheung GS, Chen J, Yin XZ, Wang QQ, Zhang CF. Pulp revascularization of immature teeth with apical periodontitis: a clinical study. *J Endod.* 2009 May;35(5):745-9.
- 6- Huang GT. A paradigm shift in endodontic management of immature teeth: conservation of stem cells for regeneration. *J Dent.* 2008 Jun;36(6):379-86.
- 7- Chen MY, Chen KL, Chen CA, Tayebaty F, Rosenberg PA, Lin LM. Responses of immature permanent teeth with infected necrotic pulp tissue and apical periodontitis/abscess to revascularization procedures. *Int Endod J.* 2012 Mar;45(3):294-305.
- 8- Banchs F, Trope M. Revascularization of immature permanent teeth with apical periodontitis: new treatment protocol? *J Endod.* 2004 Apr;30(4):196-200.
- 9- Reynolds K, Johnson JD, Cohenca N. Pulp revascularization of necrotic bilateral bicuspid using a modified novel technique to eliminate potential coronal discoloration: a case report. *Int Endod J.* 2009 Jan;42(1):84-92.
- 10- Shin SY, Albert JS, Mortman RE. One step pulp revascularization treatment of an immature permanent tooth with chronic apical abscess: a case report. *Int Endod J.* 2009 Dec;42(12):1118-26.
- 11- Petrino JA, Boda KK, Shambarger S, Bowles WR, McClanahan SB. Challenges in regenerative endodontics: a case series. *J Endod.* 2010 Mar;36(3):536-41.
- 12- Chueh LH, Huang GT. Immature teeth with periradicular periodontitis or abscess undergoing apexogenesis: a paradigm shift. *J Endod.* 2006 Dec;32(12):1205-13.
- 13- Chueh LH, Ho YC, Kuo TC, Lai WH, Chen YH, Chiang CP. Regenerative endodontic treatment for necrotic immature permanent teeth. *J Endod.* 2009 Feb;35(2):160-4.
- 14- Thibodeau B, Trope M. Pulp revascularization of a necrotic infected immature permanent tooth: case report and review of the literature. *Pediatr Dent.* 2007 Jan-Feb;29(1):47-50.
- 15- Hoshino E, Kurihara-Ando N, Sato I, Uematsu H, Sato M, Kota K, 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 Mar;29(2):125-30.
- 16- Hargreaves KM, Cohen S, Berman LH. Cohen's pathways of the pulp expert consult: 10th ed. Mosby, St. Louis; 2011: 614.
- 17- Considerations for Regenerative Procedures. [Internet]. American Association of Endodontics. 2013. [cited October 1, 2013]. Available at: <http://www.aae.org>
- 18- Levitan ME, Himel VT. Dens evaginatus: literature review, pathophysiology, and comprehensive treatment regimen. *J Endod.* 2006 Jan;32(1):1-9.
- 19- da Silva LA, Nelson-Filho P, da Silva RA, Flores DS, Heilborn C, Johnson JD, et al. Revascularization and periapical repair after endodontic treatment using apical negative pressure irrigation versus conventional irrigation plus triantibiotic intracanal dressing in dogs' teeth with apical periodontitis. *Oral Surg Oral Med Oral Pathol Oral Radiol Endod.* 2010 May;109(5):779-87.
- 20- Trevino EG, Patwardhan AN, Henry MA, Perry G, Dybdal-Hargreaves N, Hargreaves KM, et al. Effect of irrigants on the survival of human stem cells of the apical papilla in a platelet-rich plasma scaffold in human root tips. *J Endod.* 2011 Aug;37(8):1109-15.
- 21- Yamauchi N, Yamauchi S, Nagaoka H, Duggan D, Zhong S, Lee SM, et al. Tissue engineering strategies for immature teeth with apical periodontitis. *J Endod.* 2011 Mar;37(3):390-7.
- 22- Ring KC, Murray PE, Namerow KN, Kuttler S, Garcia-Godoy F. The comparison of the effect of endodontic irrigation on cell adherence to root canal dentin. *J Endod.* 2008 Dec;34(12):1474-9.
- 23- Zanini M, Sautier JM, Berdal A, Simon S.

Biodentine induces immortalized murine pulp cell differentiation into odontoblast-like cells and stimulates biomineralization. *J Endod.* 2012 Sep;38(9):1220-6.

24- Lin LM, Shimizu E, Gibbs JL, Loghin S, Ricucci D. Histologic and histobacteriologic observations of failed revascularization/revitalization therapy: a case report. *J Endod.* 2014 Feb;40(2):291-5.

25- Lenzi R, Trope M. Revitalization procedures in two traumatized incisors with

different biological outcomes. *J Endod.* 2012 Mar;38(3):411-4.

26- Wang X, Thibodeau B, Trope M, Lin LM, Huang GT. Histologic characterization of regenerated tissues in canal space after the revitalization/revascularization procedure of immature dog teeth with apical periodontitis. *J Endod.* 2010 Jan;36(1):56-63.

27- Fouad AF, Nosrat A. Pulp regeneration in previously infected root canal space. *Endod Topics.* 2013 May;28(1):24-37.