
Article:
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Introduction:

The management of a necrotic immature permanent teeth in children has always been a challenge in paediatric dentistry due to the compromised crown root ratio, thin root dentine walls and wide-open apices. Traditionally, calcium hydroxide apexification or MTA apical plug techniques have been the treatment of choice for these teeth. However, it is well known that not only do these traditional methods fail to qualitatively improve root dentinal wall dimensions, teeth are further weakened by the materials used in such techniques[1-3]. Studies have shown that despite receiving endodontic treatment, over 50 percent of such teeth are lost in the first 5-10 years following injury[3-5], thus leaving the child with a treatment burden for the rest of their lives.

In order to overcome these problems, regenerative endodontic therapy (RET) has been advocated as an alternative treatment option, for treating necrotic immature permanent teeth. The paradigm shift towards management of necrotic immature permanent teeth using RET is not a novel concept in pulp biology, and has previously been discussed in the dental scientific literature by Nygaard-Østby as early as 1961[6]. Currently, RET has received significant attention in the scientific community, with numerous studies and guidelines of various specialist organisations advocating its use as the foremost approach for treatment of non-vital immature permanent teeth[7-9]. Although, healing of periapical pathology is a prerequisite of any successful technique, the main aim of RET is to facilitate an increase in root length and promote thickening of dentinal walls, which is essential in preventing long term root fractures [4]

Several prospective studies, describing different techniques of RET, have been published in the last decade[10]. However, the analysis of treatment outcomes have revealed conflicting results [11],with majority showing clinical signs of soft tissue healing and radiographic

resolution of periapical pathology, but without the desired increase in root length, apex closure or dentinal wall thickening [12].

Assessment of the available data suggest that these conflicting results possibly stem from the fact that different RET techniques were used, and there was a significant lack of data which differentiate between traumatised and non-traumatised teeth, thus resulting in difficulty for the comparisons of outcomes across studies[13, 14]. The removal of ischemic damaged Hertwig Epithelial Root Sheath (HERS) of autotransplanted teeth in monkeys have been found to lead to compromised or arrested root formation with subsequent invasion of bone, periodontal ligament (PDL) and cementum cells into the pulp canal [15]. Therefore, it is possible that, depending on the type of traumatic injury sustained, there may be different degrees of irreversible damage to HERS in traumatised non-vital immature teeth, which in turn may influence the outcome of RET treatment resulting in some cases showing favourable results while others fail to regenerate.

In the UK, the majority of young individuals below the age of 16, are referred to paediatric dental specialists for management of dental conditions such as non-vital immature teeth. These patients are also treated by paediatric dental speciality trainees under the supervision of paediatric dental specialists or practitioners with advanced training in paediatric dentistry working in the capacity of paediatric dental specialists.

As RET gains popularity, as an alternative treatment option for management of this condition in young individuals, it is timely that information about paediatric dental specialists' and specialty trainee's experiences and opinions on performing RET be evaluated. Therefore, this study aimed to assess the knowledge, experience and the opinion of UK paediatric dental specialists and speciality trainees on the use of RET for the treatment of immature non-vital teeth in children and adolescents.

Methods:

This was a cross sectional study, with a 22-item questionnaire used to evaluate UK paediatric dental specialists' and trainees' experiences of using RET for the management of non-vital immature teeth. Institutional ethical approval was obtained from the University of Leeds Research Ethics Committee prior to commencement of the study (220216/HN/190). The questionnaire was developed using the Bristol Online Survey tool and piloted on a small group of specialist dentists, for ease of understanding and reduction of ambiguity of questions, prior to administration. An invitation email explaining the survey and the questionnaire was circulated electronically to members of the British Society of Paediatric Society (BSPD) between 16th August and 9th of November 2016 with a reminder email sent on 30th of October 2016. Individual follow-up with non-respondents was not carried out due to the anonymity of the survey.

Information collected in the questionnaire included the following:

1. Demographics – Data on positions held, dentists' type of practice (primary care, community service etc), and region of work.
2. Whether respondents use RET and/or alternative methods for managing non-vital immature teeth.
3. Perceived barriers for the use of RET.
4. Experience in using RET for treatment of non-vital immature teeth
5. RET technique used: disinfection technique, intracanal medicament, type of scaffold, and coronal seal material used.
6. Their opinions of what they considered as a successful outcome for RET in terms of continuation of root development, periapical healing,
7. Factors affecting RET success and any post treatment sequelae encountered in their own cases.

Data were coded and analysed using the Bristol Online survey tool. Descriptive statistics analysing participant's responses was computed.

Results:

Participants:

A total of 111 participants completed the survey, of which 13 responses were excluded as the respondents were neither UK General Dental Council (GDC) registered, UK based paediatric dental speciality trainees, nor working in the capacity of a UK specialist in paediatric dentistry. A total of 98 responses were included in the final analysis. These consisted of UK paediatric dental specialists or trainee (UKPDS/Ts), of which 61 were UK based specialists (GDC registered specialists working in a UK based specialist post), 21 were UK based paediatric dental speciality trainees and 16 were dental practitioners working in the capacity of paediatric dental specialists. The responses of UK based paediatric dental speciality trainees were included in this survey as they carry out paediatric dental specialist level work under the direct supervision of UK GDC registered paediatric dental specialists. The responses of dental practitioners working in the capacity of a paediatric dental specialist, such as staff grade paediatric dentists, were also included as such positions require higher education/training in paediatric dentistry delivered by approved training posts.

The majority of respondents worked at university based dental institutes (n=37, 37.7%) with almost equal number working in community services and hospitals (Figure 1 A). There was a good distribution of UKPDS/T representation across the country (Figure 1 B).

Techniques used for the management of non-vital immature teeth:

Close to a quarter of respondents (n=24, 24.5%) reported using RET for the management of non-vital immature teeth. Of these, only 2 respondents reported having treated more than 20 cases in the last 5 years, with the majority (n=15; 62.5%) reported treating between 1-5 cases in a 5-year period (Figure 2).

Other respondents reported the use of calcium hydroxide apexification (54, 48.6%) or Mineral Trioxide Aggregate (MTA) apical plug technique (47, 42.3%) as the more commonly

used techniques in management of non-vital immature teeth. Furthermore, 10 participants (9%) reported using Biodentine® apical plug technique.

Different barriers to the use of RET have been reported by respondents as summarised in Figure 3.

Use of RET for the management of non-vital immature teeth

Respondents who reported using RET for the management of non-vital immature teeth (n=24), were then also required to respond to questions on case selection, RET technique/protocol used, perceived factors affecting RET success, and if they encountered any post-operative complications following RET.

Case selection:

Most participants reported using RET for immature permanent teeth which had become non-vital as a result of dental trauma (n=22, 54%), followed by dental anomalies (n=14, 34%) and dental caries (n=5, 12%). With regards to the effect of trauma on success of RET, more than half of respondents were unsure as to its effect (n=13, 54%). Seven respondents (29%), reported that they felt RET treatment on traumatised teeth was less successful than other teeth treated with RET, whereas 2 other respondents (9%) reported the opposite effect.

In response to the question regarding whether participants thought that the success of RET was affected by the aetiology for the loss of vitality, the majority of respondents (n=12; 50%) reported being unaware of such relationship.

RET Technique/Protocol used:

Canal Disinfection:

Most participants reported using sodium hypochlorite (NaOCl) as the primary disinfectant solution (n=19, 89.2%) with almost equal number of participants (one third) using

concentrations of <1.5% (n=8) and 1.6-6% (n=9). Only 2 participants (8.3%) reported using a concentration of 1.5% NaOCl, while 5 (20.8%) did not use NaOCl in disinfection. Chlorhexidine use on the other hand was much less prevalent with only 5 (20.8%) participants using it as the sole disinfection solution, while 2 other respondents (8.3%) reported using it in combination with sodium hypochlorite. With regards to EDTA use, 16 participants (66.7%) reported using 17% EDTA in their RET protocol.

Intracanal medicament:

The majority of respondents reported using calcium hydroxide paste as intra canal medicament (n=10, 41.7%), followed by bi-antibiotic paste (Ciprofloxacin and Metronidazole) (n=9, 37.9%) and tri antibiotic paste (n=5, 20.8%).

Scaffold and coronal seal:

Eighteen participants (75%) reported using a blood clot as a scaffold with the remaining participants not using any other scaffold system.

Coronal seal:

MTA was reported as the most commonly used coronal seal materials over the blood clot (n=14, 58.3%), followed by Biodentine (n=5, 20.8%), glass ionomer (n=4, 16.7%) and composite (n=1, 4.2%).

Success of RET:

A successful outcome (>70% success rate) was reported by 66.6% (n=16) of participants, whereas 7 participants (29.2%) reported the technique to be successful (50-70% success rate) and/or unpredictable in terms of healing (Figure 4).

In terms of promoting continuation of root development, slightly more than 1/3rd (n=10, 41.6%) reported the technique to be successful in majority (>70%) of their cases. Twelve

participants reported the technique to be unpredictable while one participant reported the technique to be unsuccessful (Figure 4).

With regards to promoting thickening of dentinal walls, more than half (n=16, 66%) reported the technique to be unpredictable, with only 3 participants (12.5%) reporting the technique to be successful in 70-90% of cases (Figure 4).

Respondents were largely undecided, with a 50% split in opinion, when asked whether they felt that healing of periapical pathology with absence of continuation of root development and thickening of dentinal walls was considered a successful outcome of RET.

Factors affecting success of RET:

Participants were also asked whether stage of root development, aetiology of loss of vitality, and degree of infection had any impact on the success of RET. In general, a large number of participants were not aware of such effect, especially in relation to root development stage (n=16, 66.7%) and reason for loss of vitality (n=12, 50%). Some participants reported that teeth with more immature roots (n=5, 20.8%) and those non-traumatised (n=9, 29.2%) appeared to have better outcomes.

RET post-operative complications:

With regards to post-operative RET complications, most respondents reported post-operative discolouration of teeth (n=21, 78%) (Figure 5). Other complications such as root fractures and ankylosis as a result of RET were much less common (Figure 5).

Discussion:

The distribution of the questionnaire survey was through the BSPD electronic mailing list, which includes most UK registered paediatric dental specialists and speciality trainees in paediatric dentistry. However, the authors acknowledge that it is possible that few UK-based

specialists are non BSPD members. In addition, the mailing list also includes non-UK based members, which resulted in the need to discard some data sets. Nevertheless, the results included participation of a large number of UK based specialists/trainees and practitioners working in the capacity of paediatric dental specialists, with an acceptable representation of paediatric dental specialists across the country.

Management of non-vital immature teeth remains a challenge in paediatric dentistry with different endodontic management techniques available. This survey showed different techniques used by UKPDS/T in managing non-vital immature teeth including calcium hydroxide apexification, apical plug technique using MTA/Biodentine and RET.

Although calcium hydroxide has been used for several decades in apexification, long term use of calcium hydroxide as intracanal medicament has been shown to reduce dentinal fracture resistance, therefore, contributing to long-term cervical tooth fractures[2-4]. Consequently, the prolonged use of calcium hydroxide in apexification technique has been discouraged as a first line management technique in non-vital immature teeth[8, 9, 13].

The use of MTA apical plug technique is currently considered the standard technique in managing non-vital immature teeth with a similar success rate to that of calcium hydroxide apexification[16, 17]. The most recent systematic review and meta-analysis comparing success rates between calcium hydroxide apexification and MTA apical plug technique, reported high success rate for both techniques of 91% and 93%, respectively [17]. The follow up period, however, was only 34.8±4.4 months. The high level of cervical fractures following calcium hydroxide apexification of 28-77% was reported after 4 year follow up [4]. More recently, MTA has been shown to reduce dentinal fracture resistance[1, 18, 19] which raises concern regarding the long-term success and survival of MTA root treated teeth. This effect is, however, controversial, as other researchers have shown an opposite effect[20-22].

Consequently, promoting qualitative and quantitative increase in root dimensions through pulpal regeneration is ultimately the desired outcome hence the need for RET. Unfortunately, despite the plethora of published studies including randomised controlled trials the outcomes of this treatment modality remain unpredictable at best [11-13].

A recent systematic review and meta-analysis has recommended the use of MTA apical plug technique over RET and calcium hydroxide apexification [23]. However, MTA apical plug technique is not suitable in immature teeth with very short roots as such teeth have very compromised crown root ratios, therefore, poor long-term prognosis. Therefore, despite the unpredictability of the outcomes of the RET currently used; this treatment modality is a viable option of management in those teeth where MTA apical plug is judged to be unsuitable[9].

In the UK, unlike many other countries, UKPDS/T usually manage non-vital immature permanent teeth with the management of such teeth being taught in all paediatric dentistry programmes across the country. It is, therefore, important to evaluate whether the adoption of a newer technique, such as RET, has been utilised by this group of practitioners. Despite the current recommendations that RET should be considered as one of the options for management of non-vital immature teeth, only 24.5% of respondents reported using RET in managing these teeth. The main perceived barrier reported included lack of training, lack of suitable case indications, and lack of evidence supporting the technique. Others reported barriers included work-related limitations, e.g. lack of necessary medicaments, limited budget for RET and inability to obtain institution approval to perform RET. It is understandable that clinicians might feel cautious about using a new technique especially one that they were not trained to use. However, this technique is currently well recognised as one of the treatment modalities in the management of certain non-vital immature teeth and should be considered when managing these teeth as recommended by the American Association of Endodontics

(AAE), the European Society of Endodontology (ESE) and the European Academy of Paediatric Dentistry (EAPD)[7-9]. The authors, therefore, would like to highlight that an evidence based, free access, step by step description of the recommended RET protocol is published by the AAE and could be accessed electronically on the AAE website (http://www.aae.org/uploadedfiles/publications_and_research/research/currentregenerativeendodonticconsiderations.pdf.) [7]. This should help improve clinicians' confidence in performing this technique and gain institutional approval in their places of work.

With regards to the necessary antibiotic mixture, obtaining the required antibiotic mixture (Metronidazole (100 mg) and Ciprofloxacin (100 mg)) is one of the barriers reported in this survey as access to TriBiodent (Royal Victoria Infirmary, Newcastle, UK, supplied as three capsules of Metronidazole (100 mg), Ciprofloxacin (100 mg) and Minocycline (100 mg)) can be challenging. The use of calcium hydroxide paste rather than antibiotics mixture has been suggested by the AAE and recommended by the ESE and EAPD[8, 24-26]. This recommendation is based on few clinical studies showing similar success rates when using Calcium Hydroxide to that of antibiotic mixtures [27], laboratory studies showing less effect of Calcium Hydroxide on stem cell survival in comparison to the antibiotic mixtures [28, 29], and difficulties in removing the antibiotic mixture from root canal systems in comparison to calcium hydroxide [30].

Tooth discolorations have been linked to the use of tetracycline group antibiotics, which has been cited as one of the undesirable effects of RET and certainly consistent with the results reported in this survey. The use of calcium hydroxide is unlikely to cause any significant discolouration.

Most participants reported using sodium hypochlorite in canal chemical disinfection while the recommended 1.5% concentration [7] was only used by 2 participants. Sodium hypochlorite is the most commonly used irrigant in RET studies, with concentrations ranging

from 0.5-6%. Decreasing NaOCl concentration reduces its stem cell toxicity, antibacterial effect and tissues dissolving abilities, while increasing its volume or temperature potentiates its effectiveness[31] and stem cell toxicity. The recommendation of using 1.5% hypochlorite is based on the work of Martin et al. (2014) [32] who assessed the effect of different sodium hypochlorite concentrations (0.5, 1.5, 3, and 6%) followed by either 17% EDTA or normal saline and reported negative effects of high concentration of sodium hypochlorite on the survival and differentiation of stem cells of the apical papilla (SCAP). They recommended the use of 1.5% sodium hypochlorite followed by 17% EDTA. Only16 participants (66.7%), however, reported using 17% EDTA in their RET protocol. The use of EDTA following irrigation with sodium hypochlorite is now widely recommended[7-9]. The effect of different combinations of irrigants on SCAP viability has been reported showing the best outcome, in terms of cell survival, when using 17% EDTA following sodium hypochlorite. Therefore the use of 1.5% sodium hypochlorite followed by 17% EDTA is currently the recommended irrigation protocol in RET and should be implemented.

The use of chlorhexidine as the sole disinfection irrigant was reported by a fifth of respondents, while another 8% reported using this in conjunction with other solutions such as sodium hypochlorite. The use of chlorhexidine has been shown to be toxic to the survival of SCAP cells [33]. Furthermore, chlorhexidine combined with either EDTA or NaOCl has been found to be antagonistic causing precipitations that increases the risk of tooth discoloration, as well as potential leaching of unidentified chemicals into the periradicular tissues [34] hense precluding the use of chlorhexidine as an irrigant in RET protocols.

Interestingly, a quarter of respondents did not induce bleeding nor used any other scaffold system in their RET protocol especially that the use of a scaffold system is recommended in all RET published studies. Although a blood clot is not biologically the best scaffold and possibly results in repair than regeneration, in the absence of other viable clinically available

scaffolds, inducing bleeding into the root canal remains the easiest way in allowing stem cell population of the root canal system while orienting themselves in 3 dimensional manner. Therefore, although a clot is not the best tissue engineering scaffold, this remains the most commonly used method [10, 13, 23, 35].

Other types of scaffolds which have been reported in the literature include the use of platelet rich plasma (PRP), which has been shown to result in similar outcomes to using blood clots [12, 36]. This scaffold involves obtaining a sample of patient's intravenous blood, therefore, requires patient cooperation as well as additional equipment such as a centrifuge machine precluding its availability for routine chair side treatment.

The use of MTA in creating a coronal seal has been reported as the most commonly used material in RETs. The use of this material is linked to crown discolouration as a result of MTA's bismuth oxide content. To circumvent this, other material including Portland cement has been shown to have the same properties of MTA with much less discolouration effect due to the lack of bismuth oxide [37] and currently being used in some RET protocols [13]. Other AAE recommended coronal seal materials include bioceramics or tricalcium silicate cements such as Biodentine.

Several participants questioned the success of this technique in terms of root development as an outcome and felt it was unpredictable which is consistent with that reported in the literature as although the predictability of periapical healing remains high [9], continuation of root development and thickening of dentinal walls, remains unpredictable[27, 36, 38, 39].

Conclusions:

This survey highlights a low uptake of RET by current UKPDS/T with several barriers identified. Deviations from the current evidence based RET guidelines were identified. Recommendations addressing the management of immature non-vital teeth especially related to the use of RET in light of the findings of this survey were made as follows:

1) The prolonged use of calcium hydroxide in apexification techniques has been discouraged as a first line management technique in non-vital immature teeth[8, 9, 13].
2) The use of MTA apical plug technique is currently considered the standard technique in managing non-vital immature teeth. However, MTA apical plug technique is not suitable in immature teeth with very short roots as such teeth have very compromised crown root ratios.
3) Despite the unpredictability of RET outcomes especially in promoting continuation of root development and thickening of dentinal walls, this treatment modality is a viable option of management in teeth with wide open apices where MTA apical plug is judged to be unsuitable[9].
4) Several international guidelines have recently been published by AAE, ESE and EAPD in support of RET as one of the viable management options of immature non-vital teeth. These guidelines should help clinicians gain institutional approval in their places of work, therefore, overcoming one of the barriers reported in this survey.
5) The recent recommendation of using calcium hydroxide in root canal disinfection instead of antibiotic mixtures may help overcome the barrier of antibiotic availability, therefore facilitating a wider use of RET by UKPDS/T.
6) The use of chlorhexidine is not advised as a root canal irrigant in current RET protocols. Instead the use of 1.5% sodium hypochlorite followed by 17% EDTA is recommended.
7) Although not the best tissue engineering scaffold, creation of a blood clot in the root canals remains the most commonly used method [10, 13, 23, 35]. The development of a scaffold system with specific signaling molcules targeted at promoting stem cell differentiation into the desired odontoblasts is currently ongoing[40].

8) Creation of an UK national RET database similar to that established by the AAE is crucial in gathering data with regards to different RETs used, share best practices, and facilitate future research.

Declaration of interests:

The authors declare no conflicts of interest nor any funding attained in the conduct of this questionnaire study.

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Figure Legends:

Figure 1 Respondent demographics represented using A) Bar chart showing respondent's type of practice, B) Bar chart showing respondents region of work.

Figure 2 Bar chart showing respondent's experience in using RET.

Figure 3 Bar chart showing barriers preventing participants from using RET in managing non-vital immature teeth.

Figure 1 Bar chart showing participant's experience with RET success in terms of continuation of root development, thickening of root dentinal wall and healing.

Figure 5 Bar chart showing post-operative RET complications.

References:

1. Twati W, Wood D, Liskiewicz T, Duggal M. Effect of non-setting calcium hydroxide and MTA on human dentine following long term application. Int J Paediatr Dent 2009;**19:S1(Abstract O16-117)**:43.

2. Twati W, Wood D, Liskiewicz T, Willmott N, Duggal M. An evaluation of the effect of non-setting calcium hydroxide on human dentine: a pilot study. Eur Arch Paediatr Dent 2009;**10**:104-109.

3. Andreason J, Farik B, Munksgaard E. Long-term calcium hydroxide as a root canal dressing may increase risk of root fracture. Dent Traumatol 2002;**18**:134-137.

4. Cvek M. Prognosis of luxated non-vital maxillary incisors treated with calcium hydroxide and filled with gutta-percha. A retrospective clinical study. Dent Traumatol 1992;**8**:45-55.

5. Al-Jundi S. Type of treatment, prognosis, and estimation of time spent to manage dental trauma in late presentation cases at a dental teaching hospital: a longitudinal and retrospective study. Dent Traumatol 2004;**20**:1-5.

6. Östby B. The Role of the Blood Clot in Endodontic Therapy an Experimental Histologic Study. Acta Odontol Scand 1961;**19**:323-353

7. American Association of Endodontics. AAE Clinical Considerations for a Regenerative Procedure. American Association of Endodontics; 2016; Available from: http://www.aae.org/uploadedfiles/publications_and_research/research/currentregenerativeendodonticconsiderations.pdf. (Accessed 7.7.17).

8. European Society of Endodontology. European Society of Endodontology Position Statement: Revitalisation Procedures. Int Endod J 2016;**49**:717-723.

9. Duggal M, Tong H J, Al-Ansary M, Twati W, Day P F, Nazzal H. Interventions for the endodontic management of non-vital traumatised immature permanent anterior teeth in children and adolescents: a systematic review of the evidence and guidelines of the European Academy of Paediatric Dentistry. Eur Arch Paediatr Dent 2017;**18**:139-151.

10. Kontakiotis E G, Filippatos C G, Tzanetakis G N, Agrafioti A. Regenerative endodontic therapy: a data analysis of clinical protocols. J Endod 2015;**41**:146-154.

11. Kontakiotis E G, Filippatos C G, Agrafioti A. Levels of Evidence for the Outcome of Regenerative Endodontic Therapy. J Endod 2014;**40**:1045–1053.

12. Tong H J, Rajan S, Bhujel N, Kang J, Duggal M, Nazzal H. Regenerative Endodontic Therapy in the Management of Nonvital Immature Permanent Teeth: A Systematic Review-Outcome Evaluation and Meta-analysis. J Endod 2017;**43**:1453-1464.

13. Nazzal H, Duggal M S. Regenerative endodontics: a true paradigm shift or a bandwagon about to be derailed? Eur Arch Paediatr Dent 2017;**18**:3-15.

14. Nazzal H, Kenny K, Altimimi A, Kang J, Duggal M S. A prospective clinical study of regenerative endodontic treatment of traumatised immature teeth with necrotic pulps using bi-antibiotic paste. Int Endod J 2017. doi: 10.1111/iej.12808.

15. Andreasen J, Kristerson L, Andreasen F. Damage of the Hertwig's epithelial root sheath: effect upon root growth after autotransplantation of teeth in monkeys. Endod Dent Traumatol 1988;**4**:145-151.

16. Chala S, Abouqal R, Rida S. Apexification of immature teeth with calcium hydroxide or mineral trioxide aggregate: systematic review and meta-analysis. Oral Surg Oral Med Oral Pathol Oral Radiol Endod 2011;**112**:e36-e42.

17. Lin J C, Lu J X, Zeng Q, Zhao W, Li W Q, Ling J Q. Comparison of mineral trioxide aggregate and calcium hydroxide for apexification of immature permanent teeth: A systematic review and meta-analysis. J Formos Med Assoc 2016;**115**:523-530.

18. Aksel H, Askerbeyli-Ors S, Deniz-Sungur D. Vertical root fracture resistance of simulated immature permanent teeth filled with MTA using different vehicles. J Clin Exp Dent 2017;**9**:e178-e181.

19. Forghani M, Bidar M, Shahrami F, Bagheri M, Mohammadi M, Attaran Mashhadi N. Effect of MTA and Portland Cement on Fracture Resistance of Dentin. J Dent Res Dent Clin Dent Prospects 2013;**7**:81-85.

20. Tuna E B, Dinçol M E, Gençay K, Aktören O. Fracture resistance of immature teeth filled with BioAggregate, mineral trioxide aggregate and calcium hydroxide. Dent Traumatol 2011;**27**:174-178.

21. Bortoluzzi E A, Souza E M, Reis J M, Esberard R M, Tanomaru-Filho M. Fracture strength of bovine incisors after intra-radicular treatment with MTA in an experimental immature tooth model. Int Endod J 2007;**40**:684-691.

22. Milani A S, Rahimi S, Borna Z, Jafarabadi M A, Bahari M, Deljavan A S. Fracture resistance of immature teeth filled with mineral trioxide aggregate or calcium-enriched mixture cement: An ex vivo study. Dent Res J (Isfahan) 2012;**9**:299-304.

23. Nicoloso G F, Potter I G, Rocha R O, Montagner F, Casagrande L. A comparative evaluation of endodontic treatments for immature necrotic permanent teeth based on clinical and radiographic outcomes: a systematic review and meta-analysis. Int J Paediatr Dent 2016;**27**:217-227.

24. Cehreli Z C, Isbitiren B, Sara S, Erbas G. Regenerative endodontic treatment (revascularization) of immature necrotic molars medicated with calcium hydroxide: a case series. J Endod 2011;**37**:1327-1330.

25. Bose R, Nummikoski P, Hargreaves K. A retrospective evaluation of radiographic outcomes in immature teeth with necrotic root canal systems treated with regenerative endodontic procedures. J Endod 2009;**35**:1343-1349.

26. Alobaid A S, Cortes L M, Lo J, et al. Radiographic and clinical outcomes of the treatment of immature permanent teeth by revascularization or apexification: a pilot retrospective cohort study. J Endod 2014;**40**:1063-1070.

27. Nagata J Y, Gomes B P, Rocha Lima T F, et al. Traumatized immature teeth treated with 2 protocols of pulp revascularization. J Endod 2014;**40**:606-612.

28. Diogenes A R, Ruparel N B, Teixeira F B, Hargreaves K M. Translational science in disinfection for regenerative endodontics. J Endod 2014;**40**:S52-57.

29. Ruparel N B, Teixeira F B, Ferraz C C R, Diogenes A. Direct Effect of Intracanal Medicaments on Survival of Stem Cells of the Apical Papilla. J Endod 2012;**38**:1372–1375.

30. Berkhoff J A, Chen P B, Teixeira F B, Diogenes A. Evaluation of triple antibiotic paste removal by different irrigation procedures. J Endod 2014;**40**:1172-1177.

31. Spencer H, Ike V, Brennan P. Review: the use of sodium hypochlorite in endodontics--potential complications and their management. Br Dent J 2007;**202**:555-559.

32. Martin D E, De Almeida J F, Henry M A, et al. Concentration-dependent effect of sodium hypochlorite on stem cells of apical papilla survival and differentiation. J Endod 2014;**40**:51-55.

33. Trevino E G, Patwardhan A N, Henry M A, 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;**37**:1109-1115.

34. Rossi-Fedele G, Dogramaci E, Guastalli A, Steier L, de Figueiredo J. Antagonistic interactions between sodium hypochlorite, chlorhexidine, EDTA, and citric acid. J Endod 2012;**38**:426-431.

35. Moreno-Hidalgo M C, Caleza-Jimenez C, Mendoza-Mendoza A, Iglesias-Linares A. Revascularization of immature permanent teeth with apical periodontitis. Int Endod J 2013;**47**:321-331.

36. Jadhav G, Shah N, Logani A. Revascularization with and without platelet-rich plasma in nonvital, immature, anterior teeth: a pilot clinical study. J Endod 2012;**38**:1581-1587.

37. Lenherr P, Allgayer N, Weiger R, Filippi A, Attin T, Krastl G. Tooth discoloration induced by endodontic materials: a laboratory study. Int Endod J 2012;**45**:942-949.

38. Nagy M M, Tawfik H E, Hashem A A, Abu-Seida A M. Regenerative potential of immature permanent teeth with necrotic pulps after different regenerative protocols. J Endod 2014;**40**:192-198.

39. Bezgin T, Yilmaz A D, Celik B N, Kolsuz M E, Sonmez H. Efficacy of platelet-rich plasma as a scaffold in regenerative endodontic treatment. J Endod 2015;**41**:36-44.

40. Matoug-Elwerfelli M, Nazzal H, Graham J, Duggal M, Raif E. Decellularisation of dental pulp for use as a scaffold in regenerative endodontics. Int J Paediatr Dent 2015;**25(S1)**:Abstract number PZ04.04.