A prospective clinical study of regenerative endodontic treatment of traumatised non-vital immature teeth using bi-antibiotic paste.

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Introduction:

Despite the growing use of revitalisation endodontic technique (RET) in the management of immature teeth with necrotic pulps in the last decade, these techniques have unpredictable outcomes especially with regards to continuation of root development and thickening of root dentinal walls (Moreno-Hidalgo et al. 2013, Kontakiotis et al. 2014). Although a RET protocol has been published by the American Association of Endodontics (American Association of Endodontics 2016, European Society of Endodontology 2016), several RET techniques, medicaments and success criteria have been reported (Kontakiotis et al. 2014).

With the use of this approach, successful apical healing has been consistently reported in the literature (79 -100%), while continuation of root development and thickening of root dentinal walls have only been achieved in 27.3-47% and 20-57% respectively of cases reported in the literature (Nazzal & Duggal 2017). Furthermore, early studies have reported outcomes of RET regardless of the reason for pulpal necrosis, in other words combining outcomes for traumatised teeth, carious teeth and those with dental anomalies. This could be one of the reasons that has contributed to these conflicting results as damage to the Hertwig Epithelial Root Sheath (HERS) following trauma could result in failure in continuation of root development and thickening of dentinal walls (Nazzal & Duggal 2017). Recently published studies on the use of RET in managing traumatised immature teeth with necrotic pulps have reported the least successful results mainly in terms of continuation of root development (Nagata et al. 2014, Saoud et al. 2014). These results suggest that traumatised teeth might be less likely to respond to RET than those teeth pulps that have become necrotic due to developmental anomalies such as dens- invaginatus/evaginatus.

Several RET protocols have been proposed (Kontakiotis et al. 2015) making it difficult to compare the results of various studies. The use of a tri-antibiotic mixture, in particular those containing Minocycline, is associated with tooth discolouration as is the use of Mineral Trioxide Aggregate (MTA) which is often used in achieving a coronal seal in this technique (Reynolds et al. 2009, Kim et al. 2010). Alternatives to Minocycline have been proposed, including omitting Minocycline altogether from the antibiotic mixture (Thibodeau & Trope 2007). This is supported by some data that describes similar antibacterial effects of the bi-antibiotic paste consisting of a mixture of 100 mg Metronidazole and 100 mg Ciprofloxacin to that of tri-antibiotic paste (Twati et al. 2011).

The aim of this prospective study was to assess the success of RET using bi-antibiotic paste for the management of traumatised immature teeth with necrotic pulps.

Methods:

Ethical approval was obtained from the National Research Ethics Service (NRES) Committee, Yorkshire & the Humber - Leeds East, UK. The legal guardians of fifteen healthy children (age range=7-10 years, mean=8.3 years) with traumatised immature maxillary incisors with necrotic pulps consented to take part. Patients were considered for the study if they met the following inclusion criteria:

- Fit and healthy (ASA I and II);
- Cooperative enough to allow treatment under local analgesia;
- Had a minimum of one immature traumatised permanent incisor with necrotic pulp;.

Revitalisation endodontic treatment using a mixture of two antibiotics (ciprofloxacin and metronidazole), was performed by one operator (HN) as follows:

First treatment visit

1. Local analgesia was given when indicated using lidocaine 2%+epinephrine 1:80,000.
2. The tooth was isolated using dry dam, accessed and the necrotic pulp extirpated using barbed broaches.
3. The canal was then negotiated with minimal or no filing to prevent further weakening of dentinal walls.
4. The root canal system was then irrigated slowly with 0.5% sodium hypochlorite (NaOCl) with the needle introduced into the root canal at a point 2 mm short of the apical foramen.
5. The canal was then dried using paper points.
6. Metronidazole (100 mg) and Ciprofloxacin (100 mg) (TriBiodent, Royal Victoria Infirmary, Newcastle, UK), were mixed with distilled water and introduced into the canal

using the plastic part of a green cannula (VasofixR Safety, B Braun, Melsungen, Germany).

7. A cotton pellet was then placed to cover the root canal orifice and the access filled with a glass ionomer cement to prevent coronal leakage or contamination of the root canal with oral microorganisms.

Second treatment visit: (after 2 weeks)

1. Where needed, plain local analgesia (no vasoconstrictor) was administered and the tooth isolated and re-accessed as described above.
2. The antibiotic mixture was then flushed out of the root canal by irrigation with copious amounts of normal saline.
3. Following this the root canal was dried thoroughly with paper points.
4. This was then followed by insertion of a sterile sharp instrument (finger spreader) with a length of 2 mm beyond the working length, past the confines of the root canal, into the periapical tissues to intentionally induce bleeding into the root canal. The bleeding was then allowed to fill the root canal.
5. Once the root canal was filled with blood, a cotton pledget was placed in the pulp chamber and a clot was allowed to form in the root canal.
6. Once the clot had formed, the pulp chamber in the coronal part was thoroughly cleaned to remove remnants of blood, which could cause discolouration in the future.
7. The access cavity was then hermetically sealed with three layers of material to prevent coronal leakage and contamination: Portland cement (Medcem, Weinfelden, Switzerland), followed by glass ionomer (Fugi IX, GC Corporation, Tokyo, Japan), and composite resin (SpectrumTPH Compules®, Dentsply, Weybridge, UK).

Patients were reviewed clinically and radiographically post treatment by the same assessor (HN). Clinical assessment involved evaluation of the presence of signs of infection such as pain; abscess formation; presence of a sinus tract; tenderness to palpation and percussion; and sensibility testing using ethyl chloride cold test (Axongesic ®, BTC Invest, Praha, Czech Republic) and electric pulp testing (Electric Pulp tester, SybronEndo, Sybron Dental Specialties, California, USA).

Radiographs were taken, by the same operator, and standardised using a paralleling device (Dentsply Rinn, Elgin, IL), the same x-ray machine and technique (Jadhav et al. 2012, McTigue et al. 2013, Nagy et al. 2014, Narang et al. 2015). Radiographic images were viewed and measurements were performed using Infinitt digital radiograph software, Seoul, Korea. Radiographic assessment was performed by two calibrated paediatric specialists using two techniques; digital measurement and visual assessment.

Using visual assessment methods (McTigue et al. 2013, Narang et al. 2015), the assessors reported on continuation of root development, thickening of dentinal root walls and closure of apical foramen. Any discrepancy between the two assessors was resolved by discussion and agreement. Assessment of inter examiner reliability was performed using Kappa scores.

Using the digital measurement method, a modification of the line measurement technique (Jeeruphan et al. 2012, Nagy et al. 2014) was used with the assessors recording the following (Figure 1):

1) Root length: The distance between the mesial cemento-enamel junction and the mesial and distal ends of the radiographic root apex. The average of the mesial and distal root length measurements was recorded, to the nearest 0.01, as root length (Figure 1a)

2) Apical foramen width: The distance between the mesial and distal apical root ends (Figure 1b).

3) Dentinal wall width: The difference between the outer root thickness (Figure 1c) and the inner pulp canal width (Figure 1d), at two thirds root length measured from the cemento-enamel junction.

An average of the measurements performed by both assessors was used as the final measurement and inter examiner reliability was measured using intra-class correlation.

Comparison between root lengths, root dentinal wall widths and apical foramen widths was performed in order to assess root development between baseline radiographs, taken immediately after RET, and those taken at the 2 year follow-up (Nagy et al. 2014)(mean 22 months).

Crown colour changes were measured based on the method described by Day *et al.* (2011) using an objective digital camera system (IKAM). IKAM photographs were taken at baseline (immediately after treatment) and 2 years (18-27 months, mean= 24 months). Using MATLAB software, colour changes over time using CIELAB scores (L*, a*, b* and ΔE) were measured using a validated objective method (Day et al. 2011) by one assessor (AA). At 2 years, patients were asked to report their perception of traumatised tooth colour change following treatment and whether it affected them. Where a change of colour was reported, the parent's opinion regarding need for intervention was sought. The assessor also reported on tooth colour in comparison to the contralateral non-traumatised tooth.

Statistical analysis

Wilcoxon signed ranks test was used to assess the differences in root lengths, root dentinal wall widths and apical foramen widths between baselines and follow up radiographs. McNemar's Exact test was used to assess the difference in tooth pulpal response to electric pulp testing between baseline and follow up results. Descriptive statistics were used in reporting clinical findings at follow-up appointment. Statistical analysis was performed using SPSS software version 20 (SPSS, IBM Corp. © USA).

Results

A summary of the participant demographics, type of trauma resulting in loss of pulp vitality, pre-operative clinical and radiographic signs of infection, amount of pulpal bleeding during RET procedures, pre-operative root development stage, and reason for exclusion are presented in Table 1.

Twelve patients (mean age=8.3 years, range 7-10) remained in the study and were followed up for approximately two years (mean=22 months, range=18-27 months). Three patients were excluded: a patient was lost to follow up despite several attempts at contacting them; a second patient developed a calcific barrier probably due to long term use of calcium hydroxide while the tooth was orthodontically repositioned following a severe intrusion injury; and the third patient sustained a second trauma after 7 months of treatment which lead to recurrence of abscess and failure of treatment.

Most teeth in the study had required endodontic intervention following enamel/dentine crown fractures, except for two teeth that had been replanted following avulsion. Six patients had initially presented with clinical and/or radiographic signs of infection in the form of apical abscess associated with sinus formation and pus discharge in most cases. Eleven patients presented with 2/3-root formation with over half showing parallel apical dentinal walls. During RET, adequate bleeding was achieved in most cases with only 3 cases resulting in minimal bleeding, evident on paper points.

Table 2 shows the clinical and radiographic outcomes of RET treatments. Healing, with no clinical or radiographic evidence of infection, was observed in all cases before proceeding with second stage RET and this status was maintained throughout the study period. Although none of the teeth were responsive to cold testing after 2 years, one third of the teeth were responsive to electric pulp testing. McNemar's Exact test using binomial distribution, however, showed no significant effect of RET on eliciting an EPT pulpal response after 2 years (p value = 0.508), due to small sample size (n=12).

Table 3 shows the results of inter-examiner reliability test between both assessors, when using the visual and digital measurement techniques before and after treatment. These results indicate that continuous digital measurements were a better indicator of agreement between assessors with high intra-class correlation (ICC) scores and significant p-values indicating strong consistency between measurements. The kappa scores were low in comparison to ICC for agreement, with a negative value for dentinal wall width indicating complete randomness of visual assessment. Therefore, the results for the digital measurement of continuation of root development, thickening of root dentinal walls and apical foramen widths rather than the visual assessment results were reported in this study (Table 3).

The Wilcoxon signed rank test revealed no significant difference in root lengths nor root dentinal wall widths (Table 4). There was, however, a significant difference in apical foramen widths after 2 years (Table 4).

Over 2 years, IKAM recorded a global colour change of ΔE= 7.39 (SD = +/-3.25) with a mean change in L*= 0.79 (lighter), a*= 1.76 (redder) and b*=5.19 (yellower) (Table 5). Operator and software measurement error were calculated at ΔE=0.62. Despite the noticeable change reported by global colour change of ΔE=7.39, Tables 6 and 7 report patient and assessor perceptions of the crown colour change and patients' satisfaction. Most patients reported their teeth to be darker in colour after treatment which was similar to that reported by the assessor. However, despite the noticeable change, only one patient and their parent were not happy with the darker colour of their tooth and requested an intervention to lighten the tooth with most patients and parents being happy with the final outcome (Table 7).

Discussion

The use of RET has gained popularity with several published case reports/series (Kontakiotis et al. 2015), a growing base of randomised controlled trials (Nagata et al. 2014, Nagy et al. 2014, Bezgin et al. 2015), several systematic reviews (Moreno-Hidalgo et al. 2013, Kontakiotis et al. 2014) and a meta-analysis (Nicoloso et al. 2016).

Despite agreeing that the success of this technique in achieving regeneration rather than revascularisation/repair/mutagenisis is dependent upon achieving four essential tissue engineering principles mainly; 1) availability of suitable stem cells, 2) a scaffold for the stem cells to populate and orient in 3 dimensional manner, 3) appropriate signalling molecules promoting stem cell differentiation into the desired odontoblasts and 4) disinfection of the root canal system without damage to stem cells, (Wigler et al. 2013, Galler 2015); several RET have been used and reported in the literature (Kontakiotis et al. 2015).

Consequently, both the American Association of Endodontics (2016)and the European Society of Endodontics (2016) have published guidelines/position statement to provide guidance on an evidence based protocol for use in RET.

The RET protocol used in this study was based on the available evidence when this study was conducted, the results of local studies conducted at the Leeds Dental Institute (Twati et al. 2009, 2011), and successful cases treated in the department using the same protocol used in this study (Figure 2). The case presented in Figure 2 and Figure 3 e-f highlight the ability of the protocol used in this study to promote continuation of root development and thickening of dentinal walls. However; it should be highlighted that the current AAE and ESE recommendations are based on the available evidence to date and should be consulted in conjunction with the available literature in any future studies.

There are several sources of stem cells in the oral cavity (Hargreaves et al. 2013) with some researchers implicating that stem cells of the apical papilla (SCAP) as having a major role in RET (Huang et al. 2008). Our study involved promoting stem cell population of the root canal system through induction of bleeding from the periapical area. Lovelace et al. (2011) reported 400-600 fold increase in mesenchymal stem cells markers in blood collected from the root canal, similar to the technique used in the present study, in comparison to levels found in systemic blood samples.

In tissue engineering, a scaffold is an endogenous or transplanted material that provides a 3 dimensional microenvironment promoting cell growth, differentiation, adhesion, and migration (Nakashima & Akamine 2005). Several synthetic and natural scaffold for use in RET have been reported in the literature (Murray et al. 2007, Gathani & Raghavendra 2016).

Promoting a blood clot in RET has the advantage of transporting stem cells from the apical area into the root canal system while allowing these cells to differentiate in a 3 dimensional manner. However, referring to a blood clot as a scaffold is controversial. Although popular in the literature (Huang 2009, Kontakiotis et al. 2015) and seems to fulfil the criteria of a scaffold, a blood clot is considered an uncontrolled environment that promotes healing rather than regeneration. Promoting bleeding and clot formation in the root canal system was performed in this study in order to promote stem cell migration into the root canal system (Lovelace et al. 2011).

In order to disinfect the root canal system, minimal negotiation of the canals was performed to prevent damage to the thin enamel walls followed by irrigation with 0.5% sodium hypochlorite and application of a bi-antibiotic paste containing 100 mg Ciprofloxacin and 100 mg Metronidazole to the root canal system.

Sodium hypochlorite has been shown to be an effective root canal irrigant due to its bactericidal, bacteriostatic effect in addition to its tissue dissolution properties (Martin 1975). Sodium hypochlorite has been used as the only irrigant or in combination with other irrigants in 97% of RET studies published till May 2014 with concentrations ranging from 1-6% (Kontakiotis et al. 2015). Some laboratory studies have investigated the effect of sodium hypochlorite concentration on stem cells (Trevino et al. 2011, Martin et al. 2014). Martin et al. (2014) assessed the effect of several sodium hypochlorite concentrations (0.5%, 1.5%, 3%, and 6%) followed by either 17% EDTA or normal saline and reported the negative effects of high concentration of sodium hypochlorite on the survival and differentiation of stem cells of the apical papilla (SCAP) and recommended the use of 1.5% sodium hypochlorite followed by 17% EDTA.

Therefore, the AAE (2016) and ESE (2016) recommend using sodium hypochlorite concentrations of 1.5% and 1.5–3%, respectively, followed by the use of 20 mL gentle irrigation with 17% EDTA in RET.

The RET protocol used in this study involved the use of 0.5% sodium hypochlorite with no use of 17% EDTA irrigation as the evidence of the effect of sodium hypochlorite concentration and EDTA effect was only evident following the start of this study (Trevino et al. 2011). Initial work by Trevino et al. (2011) suggested the possible positive effect of 17% EDTA irrigation on the viability of stem cells following irrigation with high concentrations of sodium hypochlorite. However, such an effect was only established after the work of Martin et al (2014) whereby the use of 17% EDTA irrigation following different sodium hypochlorite concentrations (0.5%, 1.5%, 3%, or 6%) had shown a protective effect on stem cell viability.

Never the less, the use of 0.5% sodium hypochlorite irrigation without EDTA, in that study, reported a reduction on SCAP cell viability of 37% rather than completely diminishing SCAP viability (Martin et al. 2014).

The availability of signalling molecules capable of promoting stem cell differentiation is crucial for successful regeneration of pulp tissues. EDTA is a chelating agents able to demineralise the superficial dentine layer, therefore releasing dentinal growth factors such as transforming growth factor-β, and bone morphogenetic protein 2, consequently promoting stem cell differentiation into the desired odontoblasts (Galler et al. 2011).

The RET protocol used in this study did not involve the use of EDTA following sodium hypochlorite irrigation. This protocol was based on the available evidence at the start of this study where EDTA was not used before 2012 (Kontakiotis et al. 2015). Successful cases treated at the department using this protocol (Figure 2) coupled with continuation of root formation in some of the cases treated as part of this study (Figure 3 e-f) indicate stem cell differentiation.

The use of sodium hypochlorite (1.5-3%) followed by 17% EDTA is currently recommended in order to reduce effect of sodium hypochlorite on stem cells (Martin et al. 2014) and promote release of dentinal signalling molecule (Galler et al. 2011), therefore those irrigants should be used in future RET studies.

The use of antibiotic paste has been reported in 88% of studies (Jeeruphan et al. 2012, Nagy et al. 2014, Bezgin et al. 2015, Kontakiotis et al. 2015) with few researchers using the same antibiotic mixture used in the present study (Iwaya et al. 2001, Hargreaves et al. 2013) while others replaced Minocycline with other antibiotics such as Cephaclor (Bezgin et al. 2015), Amoxicillin (Kahler et al. 2014) or Clindamycin (McTigue et al. 2013). The decision to eliminate Minocycline from the mixture in the present study was due to it's potential to discolour teeth (Kim et al. 2010) which was further supported by recent work showing similar antimicrobial effect of the tri-antibiotic and bi-antibiotic pastes (Twati et al. 2011). This antibiotic mixture is currently recommended by the AAE (2016) as one of the potential intracanal medicaments used in RET. On the other hand, the ESE currently recommends the use of calcium hydroxide instead of an antibiotic mixture ESE (2016). Although there are currently few studies comparing the effect of calcium hydroxide versus antibiotic mixture (Bose et al. 2009, Nagata et al. 2014), this recommendation is mainly based on recent studies showing cytotoxicity to stem cells, and inability to remove antibiotic mixtures from root canal systems.

Ruparel et al. (2012) reported a reduction in the survival of stem cells of the apical papilla (SCAP) when subjected to several antibiotic mixtures including tri-antibiotic and bi-antibiotic mixtures in the high concentrations used for RET. This effect was not shown with lower concentrations of the antibiotic mixtures (0.1-1mg/ml) and calcium hydroxide use (Althumairy et al. 2014).

Furthermore, Berkhoff et al. (2014) reported the inability of the current irrigation systems, including the system used in this study, in removing antibiotic mixtures in comparison to calcium hydroxide. Eighty-eight percent of the antibiotic mixture was retained in the root canal system regardless of the irrigation system used while 98% of calcium hydroxide was removed.

The problems of antibiotic resistance, risk of sensitisation or even development of an allergic reaction to the antibiotics used were other drivers to such recommendations.

Achieving a coronal seal is also crucial in maintaining a suitable root canal environment. The use of MTA in achieving a coronal seal, hence preventing future contamination, has been associated with crown discolouration. Most commercially available MTA contains agents, such as bismuth oxide, which is used to enhance its radio-opacity. Bismuth Oxide is an agent known to cause discolouration of teeth, therefore Portland cement, which has been shown to cause less tooth discolouration than MTA (Lenherr et al. 2012), was used in the present study.

The present study reports complete periodontal healing of all teeth evident by the lack of clinical and radiographic signs of infection, which is consistent with the results published in the literature (Nazzal & Duggal 2017). However the effect on continuation of root development, thickening of dentinal walls and apical foramen widths was inconsistent and warrants further discussion. Figure 3 shows radiographic results of three of the patients treated in this study highlighting different outcomes achieved in terms of continuation of root development, thickening of dentinal walls and apical foramen widths.

The lack of continuation of root development, shown in the present study, is consistent with those reported recently (Nagata et al. 2014, Saoud et al. 2014). With regards to thickening of dentinal walls, the literature shows inconsistent results with thickening generally reported between 20-57% with a minimum of 41% of traumatised teeth (Nagata et al. 2014, Saoud et al. 2014).

In our view, the lack of continued root development in our study sample could be attributed to damage to Hertwig's epithelial root sheath (HERS) during trauma. It is a known biological process of embryology that without the organising influence of the epithelium, undifferentiated mesenchymal cells cannot differentiate into specialised cells, such as odontoblasts and cementoblasts.

While we feel it is appropriate to hypothesise the viability of the HERS in discussing the results of this study and the huge variability of the outcome of this technique in managing immature traumatised teeth reported in the literature, further research is needed to test this hypothesis. In order to reduce post treatment crown colour change, Minocycline was omitted from the protocol used in the present study and Portland cement (a non-bismuth oxide containing cement) was used instead of the more widely used MTA. Although changes in colour were still observed using CIELAB scores, patients and parents were mostly satisfied with their tooth colour suggestive of minimal non-significant changes.

Conclusions:

The results of this study indicate that the use of bi-antibiotic revitalisation endodontic technique, as described in this paper, in managing immature traumatised teeth with necrotic pulps is not successful in promoting continuation of root development or thickening of root dentinal walls over a period of 2 years. Survival of the teeth with no signs of infection, on the other hand, was achieved successfully over the same period of time. Omitting Minocycline and using Portland cement resulted in a measurable crown colour change using an objective colour measurement system although most patients were satisfied with the aesthetic outcome.

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Figure Legends:

Figure 1 Radiographs showing root measurements (using Infinitt digital radiograph software, Seoul, Korea), a) Root length: the distance between the cemento-enamel junction and the radiographic apex measured on mesial and distal sides of the root. The average of the mesial and distal root length measurements was recorded as the root length. b) Apical foramen width: The distance between the mesial and distal apical ends. c, d) Dentinal width: the outer root thickness and the inner pulp canal width at two thirds root length were measured. The difference between the outer root thickness (c) and the inner pulp canal width (d) was recorded as the root dentinal wall width.

Figure 2 Long cone radiographs of non vital immature UR2 with loss of vitality secondary to dense invaginatus treated with RET using the same protocol used in this study. Radiographs showing complete root formation and thickening of dentinal root walls over 32 months. (Published in (Nazzal & Duggal 2017), approved for republication by European Archives of Paediatric Dentistry).

Figure 3 Long cone periapical radiographs of three patients treated in this study showing periapical healing and different outcomes in terms of continuation of root development, thickening of root dentinal walls and apical foramen widths. (a, b) show healing of PA area with no continuation of root development, minimal thickening of dentinal walls and minimal closure of apical foramen in immature UL2 with necrotic pulp following enamel/dentine crown fracture (Case # 6) (Published in (Nazzal & Duggal 2017), approved for republication by European Archives of Paediatric Dentistry). (c, d) show healing of PA area with minimal continuation of root development, minimal thickening of dentinal walls, but an Apical barrier with apical foramen closure is evident in immature UR1 with necrotic pulp following enamel/dentine crown fracture (Case # 13) (e, f) show healing of PA area with continuation of root development, thickening of dentinal walls and closure of apical foramen in immature UL1 with necrotic pulp following replantation (case #15) (Published in (Nazzal & Duggal 2017), approved for republication by European Archives of Paediatric Dentistry).

Table 1 Showing participant demographics, type of trauma resulting in loss of vitality, pre-operative clinical and radiographic signs of infection, amount of pulpal bleeding during RET procedure, pre-operative root development stage, and reason for exclusion.

	Sex	Age* Years	Trauma type	Infection Pre-op	Periapical lesion	Bleeding**	Root development stage		Inclusion
							Root length	Apex Morphology	
1	M	8	E/D 11	No	Yes	(++)	3	Parallel	Included
2	M	7	E/D C 11	Sinus+ pus	No	(+++)	3	Unclear	Included
3	M	8	Avulsion 11	Sinus+ pus	Unclear	(+)	3	Parallel	Included
4	M	10	E/D/R 11	Sinus+ pus	Yes	(+)	4	Convergent	Included
5	M	9	E/D 22	No	Yes	Missing	3	Convergent	Excluded: Second trauma
6	M	9	E/D 22	No	Yes	(++)	3	Divergent	Included
7	M	9	E/D 11	Sinus tract	Yes	(+)	3	Divergent	Excluded: Failed to return
8	M	7	E/D 21	No	Yes	(++)	3	Parallel	Included
9	M	8	E/D 21	Sinus+ pus	Yes	(+)	3	Convergent	Included
10	F	8	E/D 11	Sinus+ pus	No	Missing	3	Parallel	Included
11	F	7	Intrusion	No	Yes	(-)	3	Parallel	Excluded: Apical stop
12	M	9	E/D11	No	No	(++)	4	Parallel	Included
13	M	10	E/D C 11	No	Yes	(++)	4	Parallel	Included
14	M	9	E/D 11	No	No	(++)	3	Parallel	Included
15	F	7	Avulsion 21 (2h in milk)	No	No	Missing	2	Parallel	Included

*at start of treatment, **Bleeding (+: minimal-evident on paper point, ++: minimal bleeding-evident clinically, +++: perfuse bleeding), E=Enamel, D=Dentine, root length: 1= ¼ root length, 2= ½ root length, 3=2/3 root length, 4=complete root length with open apex, 5=complete root length with closed apex.

Table 2 showing pre and post-operative clinical (signs of infection, response to cold and EPT tests) and radiographic (root lengths, root dentinal wall widths, and apical foramen widths) outcomes of RET.

	Infection Pre-op	Infection 2 years	Cold BL	Cold 2 Y	EPT BL	EPT 2 Y	Root length 22m	Root Width 22m	Apical foramen 22m
1	No	No	(-)	(-)	(+)	(-)	N	N	↓
2	Sinus+Pus	No	(-)	(-)	(-)	(-)	↑	↑	↓
3	Sinus+Pus	No	(-)	(-)	(-)	(+)	Res.	Res.	↑
4	Sinus+Pus	No	(-)	(-)	(-)	(-)	N	N	↑
5									
6	No	No	(-)	(-)	(-)	(+)	N	↑	↓
7									
8	No	No	(-)	(-)	(-)	(-)	N	N	↓
9	Sinus+Pus	No	(-)	(-)	(-)	(-)	↑	N	↑
10	Sinus+Pus	No	(+)	(-)	(+)	(-)	↑	↑	↓
11									
12	No	No	(-)	(-)	(-)	(+)	↑	↑	↓
13	No	No	(-)	(-)	(-)	(+)	↑	↑	↓
14	No	No	(-)	(-)	(+)	(-)	↑	N	↓
15	No	No	(+)	(-)	(-)	(+)	↑	↑	↓

(-): Negative response, (+): Positive response, ↑: increased, ↓: decreased, N: No change

Table 3 showing results of inter examiner reliability test of visual assessment and digital measurement methods using kappa and Intraclass correlation respectively.

	Root length	**Dentinal wall width**	**Apical foramen width**
Kappa score (Visual assessment method)	0.333 p=0.116	-0.032 p=0.849	0.243 p=0.04
ICC score (Digital measurement method)	0.87 95% CI = 0.75-0.93 p <0.001	0.53 95% CI = 0.26-0.73 p <0.001	0.60 95% CI = 0.34-0.77 p <0.001

Table 4 Results of radiographic measurements showing median values at baseline (immediately after RET) and follow up (average 22 months) showing no statistical significant differences using Wilcoxon signed rank test ($p > 0.05$).

Radiographic Measurement	Stage	Median (mm)	Wilcoxon signed rank test (*p* value)
Root Length	Baseline	14.93	0.79
	Follow up	14.20	
Root Width	Baseline	2.51	0.93
	Follow up	2.67	
Apical foramen width	Baseline	1.86	0.013
	Follow up	1.20	

Table 5 Shows results of tooth crown colour change between baseline and 2 year photographs measured by CIELAB scores. Mean and standard deviation of CIELAB scores (L*, a*, b* and ΔE) are reported over time indicating colour change based on CIELAB colour space.

	Mean (n=9)	**SD**	**Colour**
ΔL*	0.79	3.98	Lighter
Δa*	1.76	2.8	Redder
Δb*	5.19	3.34	Yellower
ΔE	7.39	3.25	

Table 6 shows the results of the subjective patient and dentist colour perception after 2 years. The results were reported for all patients (n=12) and for those who had CIELAB scores (n=9).

	Compared to CIELAB scores (n=9)		All sample (n=12)	
	Patient colour perception	**Dentist opinion**	**Patient perception**	**Dentist opinion**
Yellower	1	1	1	1
Whiter	1	0	1	0
Yellow grey	1	1	1	1
Darker	4	5	4	7
No change	2	2	3	2
Unable to comment	0	0	1	0
Missing Data	0	0	1	1

Table 7 shows patients' satisfaction of crown colour change post operatively after 22 months.

	Patient colour perception	Compared to CIELAB scores (n=9)	All sample (n=12)
Happy with colour	-	6	7
Not affected	Patient deemed tooth yellow	1	1
	Patient deemed tooth darker	1	1
	Patient was unable to comment	0	1
Wants it lighter	Patient deemed tooth darker	1	1
Missing	-	0	1

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