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Regenerative endodontic therapy in the management of non-vital immature permanent teeth: a systematic review - Outcome evaluation and Meta-analysis

Introduction: _Although the protocols in previously published studies appeared to be largely similar, there was inadequate evidence based guidelines to support a single protocol. This systematic review_aimed to summarize and quantitatively evaluate, using a meta-analysis, the outcomes for non-vital immature permanent teeth treated using regenerative endodontic technique (RET), as well as critically appraise the level and quality of evidence of existing publications.

Methods: Risk of bias assessment and level of evidence grading was carried out on all included studies. Meta-analyses using a random effects model were performed to combine the results of randomized controlled trials. The pooled success rate for each exposure was estimated for each outcome (event rates with 95% Confidence Intervals). Outcomes of all included studies were summarized.

Results: Success rates for tooth survival and resolution of periapical pathosis were excellent; however results for apical closure and continued root development were inconsistent. There are few well reported randomized prospective clinical studies. Reporting of long term outcomes and late stage effects was sparse. No study evaluated health economic outcomes and improvements to patient's quality of life.

Conclusions: Many knowledge gaps still exist within the studies published. Current published evidence is unable to provide definitive conclusions on the predictability of RET outcomes.

Introduction

Although several publications suggest that treatment using Regenerative Endodontic Technique (RET) has positive outcomes, the results of such studies should be interpreted with caution. The analysis of existing published protocols revealed that although these studies had largely similar reported RET protocols, there are inadequate strong evidence based guidelines to support a single protocol which can provide the most favorable outcome for treatment of infected immature permanent teeth. The majority of reported clinical protocols are largely formulated on methods published in case reports/series, with some modifications and improvements made based on in-vivo and in-vitro findings.

The field of regenerative endodontics in the management of non-vital immature teeth is constantly evolving with several published prospective studies including randomized controlled trials recently published. Given that RET is now considered as one of the viable treatment options for infected immature permanent teeth in young individuals, it is timely that the present literature be critically re-evaluated in light of this changing landscape.

The aim of the review was to critically appraise the quality of evidence of existing RET publications. The clinical and radiographic outcomes for non-vital immature permanent teeth treated using RET are summarized and evaluated using a meta-analysis.

Methods

Search strategy and outcome measures

A structured electronic search and reference list screening was undertaken until 25th March 2016 Electronic databases searched were MEDLINE (1st Jan 1946 to 25th March 2016), EMBASE and EMBASE classic (1st Jan 1947 to 25th Jan 2016), PubMed (1st Jan 1996 to 25th March 2016) and Cochrane Central Register of Controlled Trials (CENTRAL). Unpublished literature was electronically searched on ClinicalTrials.gov (www.clinicaltrials.gov) and the National Research Register (www.controlled-trials.com). Five randomized controlled trials were included for meta-analysis. A detailed systematic review protocol is available online on PROSPERO international prospective register of systematic reviews (1). The PRISMA flowchart summarizing the systematic review process is in **Figure 1**.

Quality Analysis and Level of Evidence (LOE):

Risk of bias assessment was applied to both study methodology and outcome measures of all included studies. Corresponding authors were contacted by email for clarifications of queries.

The quality of observational studies (cohort and case control studies) (2-4) were assessed using the Newcastle-Ottawa scale (NOS) (5). The Cochrane risk of bias tool (6) was applied to studies with randomized controlled trials (7-11), and uncontrolled prospective trial designs (12-17). For uncontrolled longitudinal studies, a modification including judgment of not applicable was introduced for domains such as randomization and allocation concealment.

The Scottish Intercollegiate Guidelines Network Grading System (SIGN)(18) was used to grade the level of evidence for all papers. All articles were assessed independently by two reviewers (HN and HJT), with information collected using standardized data collection proforma. In cases of disagreements, the overall risk of bias was achieved through consensus after discussions.

Outcome Measures

The data were analyzed based on guidance suggested in chapter nine of the Cochrane Handbook of Systematic Reviews of Interventions 5.0.2. (19). The principle outcome measures analyzed included: tooth survival, qualitative assessments of clinical and radiographic signs and symptoms of periapical healing, quantitative measurements of continued root development as evidence by closure or reduction in apical foramen width, root lengthening, root dentin thickening and/or relative radiographic area (RRA) calculations.

Synthesis of results

Meta-analyses were performed to combine the results of studies with similar exposures: Blood clot (BC), Platelet-rich Plasma (PRP) and control group (MTA apical plug technique); similar outcome measures (periapical healing, apical closure, root length, and dentine thickening); and a follow-up time of at least 6 months.

The pooled success rate for each exposure was estimated for each outcome (event rate with 95% Confident Intervals). The results were presented using forest plots. Values close to 1 implied an estimated success rate close to 100% indicative of an

optimal outcome while values close to 0 indicated a failure outcome. The analyses were carried out by a biostatistician (JK) using a random effects model which accounted for inter-study variations (20). The Cochran Q test and I^2 statistics were used to test heterogeneity among studies, with an error of p-value < 0.10 and I^2 of above 50 indicating heterogeneity. Subgroup analyses were performed for each of the exposure material (BC, PRP, and control). All analyses were performed using Comprehensive Meta-Analysis software (Version 2; Biostat, Englewood, NJ, USA).

Results

Study design:

Out of all comparative studies, only 2 studies (2, 10) evaluated outcomes based on different types of intracanal medicaments used. Four studies assessed outcomes of scaffolds used (7-9, 11); and 5 studies evaluated outcomes of RET against the treatment standard of $Ca(OH)_2$ apexification or MTA apical plug technique (2-4, 9, 11). Of these, 3 studies had both positive and negative controls (2, 9, 11).

Quality analysis and level of evidence:

Both cohort studies scored 6/9 (LOE=2+), while the case control study scored 3/9 (LOE=2-). A high level of bias was evident in all RCTs assessed (LOE=1-), and all uncontrolled prospective trials (LOE=3). The pooled analyses of the papers are in **Table 1.** **Figures 2 and 3** show the risk of bias summaries and classification of LOE.

Analysis of outcome measures

Type of teeth reported:

Traumatized non vital incisors were the most commonly RET treated teeth. Only 4 studies specified the primary trauma diagnosis of these teeth. Three studies reported the use of RET on molar teeth (2, 15, 17), but the numbers were low totalling only 9 out of 411 teeth. Eight studies had mixed aetiologies for loss of pulp vitality (caries, trauma, developmental anomaly). Outcome measures were not analysed based on etiology of vitality loss or trauma diagnosis, possibly related to the small sample sizes.

Recall period:

There was wide variability in follow up timings across the studies, with all except 1 study (17) having a minimum review of 12 months.

Primary outcomes:

Resolution of clinical signs and symptoms were high across studies, irrespective of intervention or control group. The clinical outcomes reported were tooth survival and clinical signs of healing. Tooth survival, was 100% in all but 2 studies (3, 4).

Radiographic outcomes included assessment of periapical pathology resolution, apical closure, increase in root length and root dentine thickening. In total, seven studies used computerized software to aid in image correction, as well as measurement analysis (e.g. SoPro, Image J software with Turboreg plug in, or Diagora). Software was utilized to mathematically model, transform and standardize dimensional changes between pre and post-operative radiographs prior to analysis. Of these, 2 studies evaluated radiographic results using RRA (4, 7), while others quantified increases in root length and dentine thickness using landmark identification and straight line measurement methods (2, 3, 9, 12, 14, 17). Only 1 study (9) quantified periapical bone density changes using Digora image analysis software.

Periapical healing:

Periapical healing was reported in all papers, and evaluated using both clinical and radiographic techniques. All papers reported radiographic assessments of periapical pathology resolution, but only 11 of 14 papers evaluated clinical signs of healing. Sensibility testing was evaluated in only 5 out of 14 studies, of which only 3 studies reported positive responses at low or inconsistent levels (7, 14, 17).

All studies included into the meta-analysis reported on periapical healing success rates (7-11). This was high across all comparison groups (BC = 91%, CI = 78% to 97%; PRP = 94%, CI = 74% to 99%; control = 91%, CI = 64% to 98%). For periapical healing, the results of BC, PRP and control group were comparable (I^2 =0.00, p=0.92) (Figure 3a).

Apical closure:

Apical closure was reported in 10 out of 14 papers, but closure rates were variable across studies. All studies included into the meta-analysis reported on apical closure success rates: BC = 76%, CI = 58% to 88%; PRP = 82%, CI = 57% to 94%; control = 6.4%, CI = 0.9% to 35%. There was a significant difference between control group compared with BC or PRP, but no significant differences between BC and PRP group (I^2=38.63, p=0.002).

Increase in root length and root dentine thickness:

Three studies in the meta-analysis reported on the success rates of root lengthening and root dentine formation (8, 10, 11). The comparison groups included BC scaffolds from all the 5 studies (7-11), PRP from 3 studies (7, 8, 11), and control groups consisting of MTA apical plug technique from 2 studies (9, 11). Results for root length and dentine thickening were identical as teeth with increased root lengths also had increased dentine thickening. The results of estimated success rate for both were: BC = 80%, CI = 48% to 95%; PRP = 94%, CI = 55% to 99%; control = 6.4%, CI = 0.5% to 46%. Similarly, there was a significant difference between control group compared with BC or PRP, but no significant differences between BC and PRP group (I^2= 70.32, p =0.006)

Secondary outcomes:

Late stage effect and side effects were reported inconsistently across papers. Apart from this, none of the other secondary outcome measures, which the authors intended for analysis, were reported. The most commonly reported late stage effects were pulp canal obliteration and tooth discoloration. Discoloration was reported in 50% of studies (4, 7,10, 13-16), and was correlated with tetracycline antibiotics or MTA use. More details of study characteristics and outcome measures evaluated can be found in **Tables 2 and 3.**

Forest plots **(Figures 4a-d)** show the estimated success rates of BC, PRP and control groups for (A) Periapical healing, (B) Apical closure, (C) Root lengthening and (D) Root dentine formation. **Table 4** shows the I^2 values for each subgroup.

Discussion

There are often deliberations on the definition of treatment "success" in RET studies. It can be argued that resolution of pain, infection and periapical pathology in the absence of continued root growth are considered "successful" cases as they reflect functional measures of healing with tooth retention in the longer term. However, the most desirable outcome of RET is to have clinically significant continuation of root development.

The majority of studies reported on clinical signs, while others additionally evaluated the recovery of sensibility test readings. This review found that positive

sensibility results were not consistent across studies, which is possibly due to difficulties in evaluating sensibility due to the presence of layered coronal seal over the blood clot scaffold.

Side effects or late stage effects documentation e.g. undesirable discoloration, pulp canal obliteration, atypical root morphology development, and loss of vitality following apical closure were sparse. Tooth discoloration following RET treatment was reported in 50% of studies. Aside from minocycline, bismuth oxide content in MTA has also been associated with coronal discoloration (21). Additionally, materials demonstrate greater color changes following contact with blood (21), which has implications in RET since they are placed in contact with the blood clot scaffold. To circumvent this, the use of dentine bonding agents and flowable composite in the coronal pulpal chamber has been proposed to minimize antibiotic and haemosiderin contact with dentinal walls (22). This method has been adopted in various studies, however its effectiveness is unpredictable (7).

Additionally, tissue repair mechanisms, the true nature of tissue formed within root canals and its long-term prognosis remain unknown. In animal models, ingrowth of PDL, bone and cementum have been found following RET (23). Similarly, histological observations of RET treated human teeth have revealed connective tissue ingrowth comparable with animal models (24, 25). The exact nature of the tissue repopulating the root canal system remains unclear and co-occupancy of both desirable tissues (e.g. fibroblasts, blood vessels and collagen) and undesirable tissues (e.g. cementoblasts and osteoblasts) within the canal spaces is likely (26). The literature documenting follow up of RET treated teeth beyond 18 months is limited, and its long term effects and full impact in a young patients remains unknown.

Results of the meta-analysis showed that success rates of periapical pathology resolution following RET was comparable to treatment with MTA or apexification. This suggests that both disinfection protocols have similar efficacy for bacterial elimination. Although the success rates for apical closure, increase in root length and dentine formation were greater for RET compared to the gold standard, the results of the meta-analysis (i.e. large confidence intervals and variability of I^2 values) caution of inconsistent outcomes and variable predictability for success. An arbitrary measure of 20% increase in root length has been suggested to be of clinical significant change (12); however, the number of published cases that actually attained this threshold cannot be

fully ascertained. Additionally, while some studies showed increases in post-operative root width or length, it is important to note that these were often subtle changes discernable only with the use of software aided quantification methods(4). As such, the clinical significance of these findings is uncertain.

In cases of long-standing infection, despite eradication of periapical pathology, arresting of root development can still occur. Chen et al. found that continued root development was independent of periapical pathology resolution (16). Root lengthening and apex formation are related to vitality and re-function of Hertwig Epithelial Root Sheath (HERS) and its interaction with SCAP cells. Under experimental conditions, it has been found that removal or ischemic damage of the Hertwig Epithelial Root Sheath (HERS) leads to compromised or arrested root formation and subsequent invasion of bone, periodontal ligament (PDL) and cementum cells into the pulp canal (27, 28). Therefore, teeth with severe trauma, coupled with the propensity for inaccurate tooth repositioning and/or delayed revascularization in emergent situations, are likely to suffer irreversible damage to HERS. In view of this, it is postulated that healing outcomes are different between traumatized teeth versus those without. Since a majority of RET treated teeth were due to traumatic injuries, it is possible that the results are reflective of this. Due to the heterogeneity of data present, the evaluation of the effect of etiology of pulpal necrosis or type of traumatic injury on healing outcomes was not possible. It would be interesting for future studies to evaluate this once more data is made available.

Taking radiographs is challenging in young patients due to behavioural issues or difficulties with structural differences, e.g. shallower floor of mouths. This can result in marked deviations in horizontal angulations, where the resultant radiographs are adequate for diagnosis of apical closure, but not for meaningful quantification of root growth. To circumvent problems with errors of angulation and to quantify changes in root development, a significant number of studies reported utilizing the NIH software Image J with TurboReg plug in for image transformation as proposed by Bose et al (2). While software imaging programs that control for angulation of two comparative radiographs appear to add validity to the biological changes after RET procedures, this method is not infallible. Among the studies using software for radiographic analysis, 3 studies reported having to discard data sets due to inability to select consistent landmarks for analysis (2, 4, 14). Furthermore, minimal data is available on the accuracy of this software.

There are several reasons for this. Firstly, there are inherent software limitations. TurboReg is not able to modify images with extreme deviations in the horizontal (bucco-lingual) angulation, nor does it correct for the 4%–8% magnification errors that are inherent in all periapical radiographs (2). As pre and post-operative radiographs are not always collected in a standardized manner, these discrepancies may not be correctable. Moreover, the lack of stable reference points, such as superimposition of teeth in the child population during the mixed dentition stage, can also affect TurboReg image correction (2). This demonstrates the necessity for standardizing the methods for radiograph angulation in future studies. Secondly, it is known that repeated image transformation tends to introduce inconsistencies, as evaluators are required to re-select landmarks prior to image transformation (14). This problem may be further amplified if repeat analysis is carried out on a new set of transformed images. Hence it is important to take into consideration intra-examiner agreement scores when evaluating reliability of radiographic methods.

The authors found substantial heterogeneity in the reporting of outcomes among studies, such as the report of pre and post-operative clinical factors, as well as the quantification and report of radiographic outcomes. All the above mentioned factors, in addition to variability between clinical protocols, have significant implications on the analysis of RET outcomes. In conclusion, the lack of standardized outcome sets of currently available data has greatly prevented the optimal use and combination of results required for in depth and accurate determination of success and prognostic factors affecting RET. Consequently, it was only possible to perform a meta-analysis comparing the types of scaffolds used (i.e. blood clot vs PRP). As for the other possible comparators, e.g. disinfection protocol, intracanal medicament used and etiology of non-vitality, only a narrative synthesis can be provided.

A high number of included studies in this review were uncontrolled longitudinal studies, and randomized controlled clinical trials with high levels of bias. While the authors acknowledge the value of well-documented case series for identification of important parameters that may guide the design of future prospective trials, the results should nevertheless be taken with caution as they are inherently predisposed to publication bias and lack of control groups for meaningful comparisons against other methods.

It should also be noted that none of the clinical trials included in this study reported sample size calculations. Results validity in clinical trials is influenced by sample sizes. Studies with small or insufficient sample sizes are at higher risk of being underpowered, thus giving rise to type II errors and null trial outcomes (29). As the quantification of increment in root development in RET studies is small, larger sample sizes are required to identify clinically significant changes, as well as compensate for the high tendency for patient attrition in clinical trial studies. However, the authors acknowledge that this should be adjusted for feasibilities such as funds, duration of study and availability of suitable and willing participants. Hence, collaborations across institutions and support for multi-center trials using single standardized protocols is critical.

The evaluation of oral health-related quality of life (OHRQoL) has gained prominence and has important implications for clinical practice and dental research (30). OHRQoL considers how oral health affects patients' social life, and helps researchers understand the associations between and among clinical variables, treatment processes, and its relation to a person-centered, self-reported health experience (30). In the context of RET, OHRQoL evaluation is paramount since non-vital immature permanent teeth often presents in young patients.

From a health economics perspective, risk-benefit and cost assessment of RET versus alternative treatment methods need to be evaluated. Additionally, other aspects such as relative risk of re-infection following treatment should be weighed against the periodicity of recall visits and radiographic evaluations. Last but not least, taking into consideration the voice of the child, some aspects which warrant evaluation are the young patient's perspective on treatment benefit in relation to self-esteem, acceptability of treatment methods including the need for adjunct pharmacological behavior interventions (i.e. need for treatment under sedation). Other evaluations include the feasibility of clinical protocols (e.g. drawing of intravenous blood for PRP preparation) in a regular clinic setting. All these have yet to be evaluated in any published RET study.

The results of this review revealed excellent success rates in terms of tooth survival and resolution of periapical pathology following RET. However, there were inconsistent results for more desirable outcomes such as continued root development. Currently, very few studies stand up to rigorous scrutiny normally applied to clinical

trials. There is a paucity of well documented long term prospective studies which report on long term outcomes beyond 18 months. Moreover, OHRQoL in a young patient has yet to be sufficiently evaluated.

Many variables essential to RET outcomes remain unsolved. Tissue engineering approaches and translational research are needed to understand the inter-relationship of all these factors, including appropriate delivery of each essential component in the right proportions, sequence and time. At this present status of this review, various gaps still exist in our knowledge. As more evidence becomes available, modification of RET techniques and its advocacy will evolve. It is the clinician's role to help ensure that the new protocols advocated are both clinically practical and acceptable to the young patient.

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Table 1: Newcastle-Ottawa Scale: For Case-control/ Cohort studies

Paper	Study Type	Selection				Comparability	Exposure			Total	Sign LOE
	Cohort studies	Representativeness of the exposed cohort	Selection of the non exposed cohort	Ascertainment of exposure	Demonstration that outcome of interest was not present at start of study	Comparability of cases and controls/cohorts on the basis of the design or analysis	Assessment of outcome	Was follow-up long enough for outcomes to occur	Adequacy of follow up of cohorts		
Jeeruphan et al 2012	Cohort	*	*	*	*			*		5/9	2+
Alobaid et al 2014	Cohort	*	*	*	*		*	*		6/9	2+
Paper	**Study Type**	**Selection**				**Comparability**	**Exposure**				
	Case Control studies	Is the case definition adequate?	Representativeness of the cases	Selection of Controls	Definition of Controls	Comparability of cases and controls/cohorts on the basis of the design or analysis	Ascertainment of exposure	Same method of ascertainment for cases and controls	Non-Response rate	**Total**	**Sign LOE**
Bose et al 2009	Case Control	*			*			*		3/9	2-

Table 2: Study characteristics and outcome measures evaluated

Study (sample size)	Follow up (months)	Tooth types and	Aetiology for pulp necrosis	Primary outcomes			Secondary outcomes
				Clinical evaluation	Radiographic evaluation		Late stage effects or Side effects
					Image evaluation method	Outcomes evaluated	
Cehrelli et al 2011 (n=6)	9-10	Tooth type: Molars	Not specified	▪ Tooth survival ▪ EPT and cold test	Image J + Turboreg plug-in - Straightline measurement Dropped from X-ray analysis: None Kappa: Not reported	▪ Apical closure ▪ Increased root length (reported as %) ▪ Increased root thickness (reported as %) ▪ PA pathology resolution	Not reported
Chen et al 2012 (n=20)	6-26	Tooth type: ▪ Incisors = 10 ▪ Premolars =10	▪ Trauma=10 ▪ Caries=3 ▪ Anomaly=7	▪ Tooth survival	Visual evaluation Kappa: Not reported	▪ Root development ▪ Increased root thickness ▪ PA pathology resolution	▪ PCO ▪ Hard tissue barrier formation (not at apex) ▪ Discoloration
Mctigue et al 2013 (n=32)	12	Tooth type: ▪ Incisors = 29 ▪ Premolars = 3	▪ Anomaly=4 ▪ Trauma=28 ❖ Luxation = 3 ❖ Intrusion =3 ❖ Extrusion=4 ❖ Avulsion=4 ❖ Cx =4 ❖ UnCx=10	▪ Tooth survival ▪ Sinus track resolution	Visual evaluation Kappa: 0.985	▪ Apical closure ▪ Increased root length (reported as %) ▪ Increased root thickness (reported as %) ▪ PA pathology resolution	▪ Discoloration
Dabbagh et al 2012 (n=16)	24	Tooth type: ▪ Incisors = 13 ▪ Premolars = 1 ▪ Molar =2	▪ Trauma=12 ▪ Caries=2 ▪ Anomaly=2	▪ Tooth survival ▪ Other details not reported	Visual evaluation Kappa: Not reported	▪ Increased root length ▪ PA pathology resolution	▪ Hard tissue barrier ▪ Discoloration ▪ MTA material collapsing into canal
Kahler et al 2014 (n=16)	18	Tooth type: ▪ Incisors = 13 ▪ Premolars = 3	▪ Anomaly=3 ▪ Trauma=13 ❖ Luxation = 2 ❖ Avulsion=2 ❖ Cx =2 ❖ UnCx=5 ❖ Unknown=2	▪ Tooth survival ▪ EPT	Image J + Turboreg plug-in -Straightline measurement Kappa score: 0.46 Dropped from X-ray analysis: 7/16	▪ Apical closure (reported as %) ▪ Increased root length (reported as % increase) ▪ Increased root thickness (reported as % increase) ▪ PA pathology resolution (reported as %)	▪ Discoloration

Saoud et al 2014 (n=20)	12	Tooth type: ▪ Incisors = 20	▪ Trauma=20 ❖ Cx, n=16 ❖ UnCx, n=2 NoCx, n=2	▪ Tooth survival ▪ EPT and cold test ▪ Resolution of clinical signs and symptoms	Image J + Turboreg plug-in Straightline measurement Kappa: Not reported Dropped from X-ray analysis: None	▪ Apical closure ▪ Increased root length (reported as mean % change) ▪ Increased root thickness (reported as mean % change) ▪ PA pathology resolution	▪ Hard tissue bridge formation (not at apex)
Bose et al 2009 (n=88)	0 - >36	Tooth type: ▪ Incisors = 33 ▪ Canine = 1 ▪ Premolars = 51 ▪ Molar =1 ▪ Unknown = 2	Mixed (Trauma/ caries/anomaly) Breakdown not specified RET = 48	▪ Tooth survival	Image J + Turboreg plug-in Straightline measurement Kappa: Not reported Dropped from X-ray analysis: ▪ 6/54 initially included	▪ Increased root length (reported as clinically significant change) ▪ Increased root thickness (reported as clinically significant change)	Not reported
Jeeruphan at al 2012 (n=61)	▪ RET =21 ± 12 ▪ MTA =14 ± 8 ▪ CaOH =27 ± 30	Tooth type: ▪ Incisors = 36 ▪ Premolars = 25	▪ Trauma=36 (RET =7) ▪ Caries=5 (RET=1) ▪ Anomaly=20 (RET =12)	▪ Tooth survival ▪ Resolution of clinical signs and symptoms	Sopro software Kappa: 0.985 Dropped from X-ray analysis: None	▪ Increased root length (reported as % increase) ▪ Increased root thickness (reported as % increase) ▪ PA pathology resolution	Not reported
Alobaid et al 2014 (n=31)	▪ RET= 14.5 ± 8.5 ▪ Apex= 21.8 ± 12.0 ▪ Xray= 15.5 ± 10.4	Tooth type: ▪ Incisors = 28 ▪ Others = 3	▪ Trauma=24 (RET =15; 6/11 severe trauma) ▪ Caries=4 (RET=1) ▪ Anomaly=3 (RET =3)	▪ Tooth survival ▪ Resolution of clinical signs and symptoms	Image J + Turboreg plug-in RRA measurement Kappa: Not reported Dropped from X-ray analysis: ▪ T =2/18 ▪ C = 3/12	▪ Apical closure ▪ Clinically sig. change (>20%) in either RRA, width or length: PA pathology resolution	▪ PCO ▪ Hard tissue barrier formation (not at apex) ▪ Discolouration: ▪ Other adverse events
Jadhav et al 2012 (n=20)	12	Tooth type: ▪ Incisors = 20	Not specified	▪ Tooth survival ▪ Resolution of clinical signs and symptoms	Visual evaluation Kappa: Not reported	▪ Apical closure ▪ Increased root length ▪ Increased root thickness ▪ PA pathology resolution	Not reported

Nagata et al 2014 (n=23)	1-19	Tooth type: ▪ Incisors = 23	▪ Trauma=23 (RET =12) ❖ Lat. lux = 11 ❖ Intrusion =1 ❖ Extrusion=9 ❖ Avulsion=2	▪ Tooth survival ▪ EPT and cold test ▪ Resolution of clinical signs and symptoms	Visual evaluation Kappa: Not reported	▪ Apical closure ▪ Increased root length ▪ Increased root thickness ▪ PA pathology resolution	▪ Discolouration
Nagy et al 2014 (n=36) ▪ Drop out: T_1=2 T_2=2 C=3	3-18	Tooth type: ▪ Incisors = 36	Not specified	▪ Tooth survival ▪ Resolution of clinical signs and symptoms	Image J + Turboreg plug-in - Straightline measurement Diagora – PA bone density measurement Kappa: Not reported Dropped from X-ray analysis: None	▪ Apical closure ▪ Increased root length ▪ Increased root thickness ▪ PA pathology resolution	▪ Not reported
Bezgin et al 2015 (n=20)	18	Tooth type: ▪ Incisors = 14 (BC =8/10;PRP=6/10) ▪ Premolars =6 (BC =2/10;PRP=4/10)	▪ Trauma=14 (BC=8/10;PRP=6/10) ▪ Caries=6 (BC=2/10;PRP=4/10)	▪ Tooth survival ▪ EPT ▪ Resolution of clinical signs and symptoms	Image J + Turboreg plug-in RRA measurement Kappa: Not reported Dropped from X-ray analysis: None	▪ Apical closure ▪ Relative radiographic Area (RRA) ▪ PA pathology resolution	▪ Discoloration
Narang et al 2015 (n=20)	6 & 12	Tooth type: ▪ Not specified	Not specified	▪ Tooth survival ▪ Resolution of clinical signs and symptoms	Visual evaluation Kappa: Not reported	▪ Apical closure ▪ Increased root length ▪ Increased root thickness ▪ PA pathology resolution	▪ Not reported

Table 3: Summary of Outcome Measures for included studies

Study	Primary outcomes						Secondary outcomes	
	Clinical evaluation		Radiographic evaluation				Late stage effects	Side effects/ Drawbacks
	Tooth survival (n)	Healing or findings	Continue root formation/maturation			PA pathology resolution*		
			Apical closure	Increase Root length	Increase Root thickness			
Cehrelli et al 2011	6/6	▪ Cold: 2/6 +ve ▪ EPT: 6/6 no response	6/6	6/6 - in 9-10 months (2.23-18.09%)	6/6 - in 9-10 months (14.83-38.47%)	6/6 (within 3 mths)	Not reported	Not reported
Chen et al 2012	20/20	Details not specified	Root development: 15/20 in 20 months		20/20 (Marked in 4/20)	20/20	▪ PCO: 4/20 ▪ Hard tissue barrier :2/20	Discoloration: 2/20
Mctigue et al 2013	32/32	Sinus track resolution: 10/10	23/32	21/32	22/32	31/32	Not reported	Discoloration: 14/32 ▪ Mino+ gray MTA = 7 ▪ MTA or Mino NOT used = 7
			9 teeth failed to achieve 2 out of 3 root maturation parameters: ▪ Trauma: 5 (3 avulsion;2 intrusion;1 Cx;1 UnCx) ▪ Anomaly:2 (dens evaginatus)					

Dabbagh et al 2012	16/16	Not reported	Not reported	12/16	Not reported	9/10 (1 lost to f/u)	Hard tissue barrier : 2/16	▪ Discoloration: 2/16 -before minocycline changed to cefaclor ▪ MTA material collapsing into canal
Kahler et al 2014	16/16	EPT: 5/16 +ve	▪ Incomplete: 47.2% ▪ Complete: 19.4%	% increase: 2.7 to 25.3% (9 cases)	% increase: 1.9 to 72.6% (9 cases)	90.37%	Not reported	Discoloration: 13/ 16
Saoud et al 2014	20/20	▪ EPT: 20/20NR ▪ Cold: 20/20NR ▪ Swelling/sinus track resolution: 10/10 ▪ Pain resolution: 12/12	▪ Complete: 11/20 - by12 months ▪ >20% decrease in apical diameter: 20/20	Mean % change at 12 months: from <1% 5%	Mean %change at 12 months: from <3% to 21%	18/20	Hard tissue bridge formation (not at apex): 5/20	Not reported
Bose et al 2009	Not reported (Assumed to be 100% as all cases collected from other sources)	Not reported	Not reported	†Clinically sig. change ($\geq$20%) ▪ T1= 1/14 ▪ T2=10/24 ▪ T3= 0/10 ▪ C= 0/40	†Clinically sig. change ($\geq$20%) ▪ T1=12/14 ▪ T2= 9/24 ▪ T3= 0/10 ▪ C= 0/40	Not reported	Not reported	Not reported
Jeeruphan at al 2012	T=20/20 C_1=18/19 C_2=17/22	Resolution of clinical signs & symptoms: T=20/20 C_1=18/19 C_2=17/22	Not reported	Increased by: *T=15% C_1=6% C_2=0.5%	Increased by: *T=28% C_1=0% C_2=2%	T=16/20 C_1=13/19 C_2=17/22	Not reported	Not reported
Alobaid et al 2014	T= 18/19; (1 tooth extracted; re-traumatized)	Resolution of clinical signs & symptoms:	▪ T = 6/18 ▪ C= 1/12	Clinically sig. change (>20%) in either RRA, width or length: ▪ T=4/15 ▪ C=1/15		RET=7/8; C=8/8	PCO: T=2/18; C=0	Adverse Events: T=8/18; C=1/12

	C=12/12	T=15/19; C=12/12					IC: T=3/18; C=0	• Pain:1/15 • Discolouration:2/15 • Re-traumatized: ❖ T =1/15; ❖ C=1/8 • Reinfection: T =3/15 • Fracture:1/15 • Ext. root resorption: ❖ T=4/15; C=0/8
Jadhav et al 2012	T_1= 10/10 T_2=10/10	Resolution of clinical signs & symptoms: T_1= 10/10 T_2=10/10	*T_1= 10/10 T_2=10/10	*T_1= 10/10 T_2=10/10	*T_1= 10/10 T_2=10/10	T_1= 10/10 T_2=10/10	Not reported	T_2 = Venous blood drawn from young patients
			Stat. sig. difference in PA healing, apical closure, and dentine thickening, but not root lengthening between T_1 & T_2 groups					
Nagata et al 2014	T_1=12/12 T_2=11/11	Cold test and EPT: All no response Resolution of clinical signs & symptoms: T_1=*11/12 T_2=*11/11	*T_1=8/12 *T_2=0/11	T_1=5/12 T_2=3/11	T_1=5/12 T_2=5/11	*T_1=6/6 T_2= 4/5	Not reported	Discoloration *T_1=10/12 T_2=3/11
Nagy et al 2014	T_1=10/10 T_2=10/10 C= 12/12	Resolution of clinical signs & symptoms: T_1= 9/10 T_2= 8/10 C= 12/12	† T_1= 9/10 T_2= 8/10 C= 0/12	†T_1= 9/10 T_2= 7/10 C= 0/9	†T_1= 9/10 T_2= 7/10 C= 0/9	†T_1= 9/10 T_2= 8/10 C= 12/12	Not reported	Not reported
Bezgin et al 2015	T1= 10/10 T2= 10/10	▪ EPT +ve: T1=2/10 T2=5/10 ▪ Pain/swelling/sinus track resolution: T1=10/10 T2=10/10	T1= 6/10 T2= 7/10	Relative Radiographic Area (RRA): T1= 9/10 T2= 9/10		T1= 8/9 T2= 7/7	▪ PCO-8/20 : T1= 4/10 T2= 4/10	▪ Discoloration: 12/20 (related to MTA use) ▪ T_2 = Venous blood drawn from young patients

Narang et al 2015	T1=5/5 T2=5/5 T3=5/5 C=5/5	Resolution of clinical signs & symptoms: T1=5/5 T2=5/5 T3=5/5 C=5/5	T1=5/5 *T2=5/5 T3=5/5 C=0/5	T1=5/5 T2=5/5 *T3=5/5 C=5/5	T1=5/5 T2=5/5 T3=5/5 C=5/5	T1=5/5 T2=5/5 T3=5/5 C=5/5	Not reported	T_2 & T_3 = Venous blood drawn from young patients

*Dependant on number of teeth reported with symptoms or lesions at baseline
†Calculated based on raw data obtained or available

Table 4: I^2 values for each subgroup

I^2 (p value)	PA healing	Apical closure	Increased root length	Increased dentine thickness
Control	0.00 (p=0.863)	67.49 (p=0.046)*	48.083 (p=0.165)	48.083 (p=0.165)
BC	0.00 (p=0.946)	36.7 (p=0.176)	70.366 (p=0.034)*	70.366 (p=0.034)*
PRP	20.163 (p=0.286)	0.00 (p=0.387)	0.00 (p=0.755)	0.00 (p=0.755)

Significant, indicative of high heterogeneity

Fig 1. PRISMA flowchart summarising the systematic review process in identification of the included studies

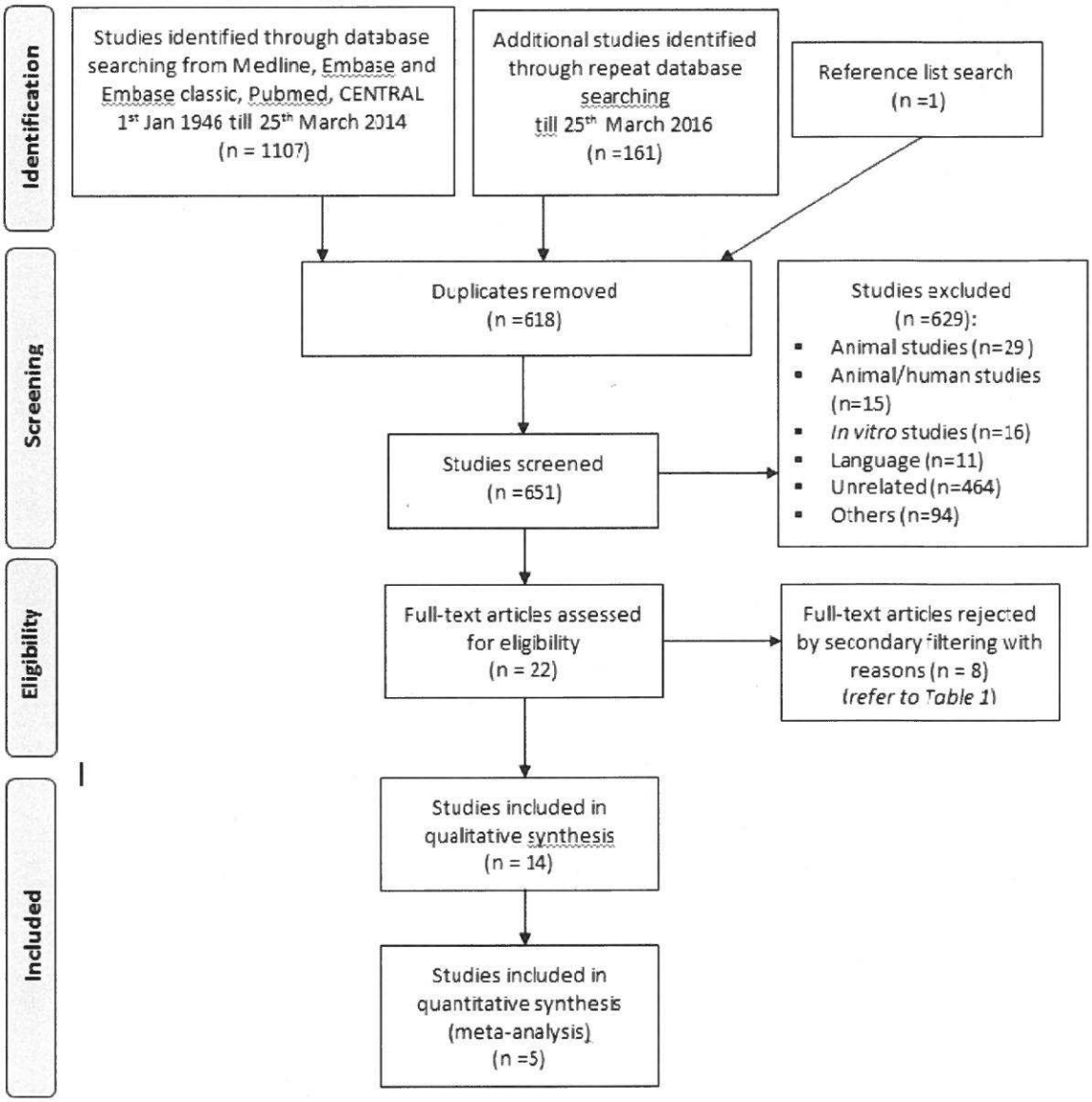


Fig 2: Risk of Bias summary and classification of LOE

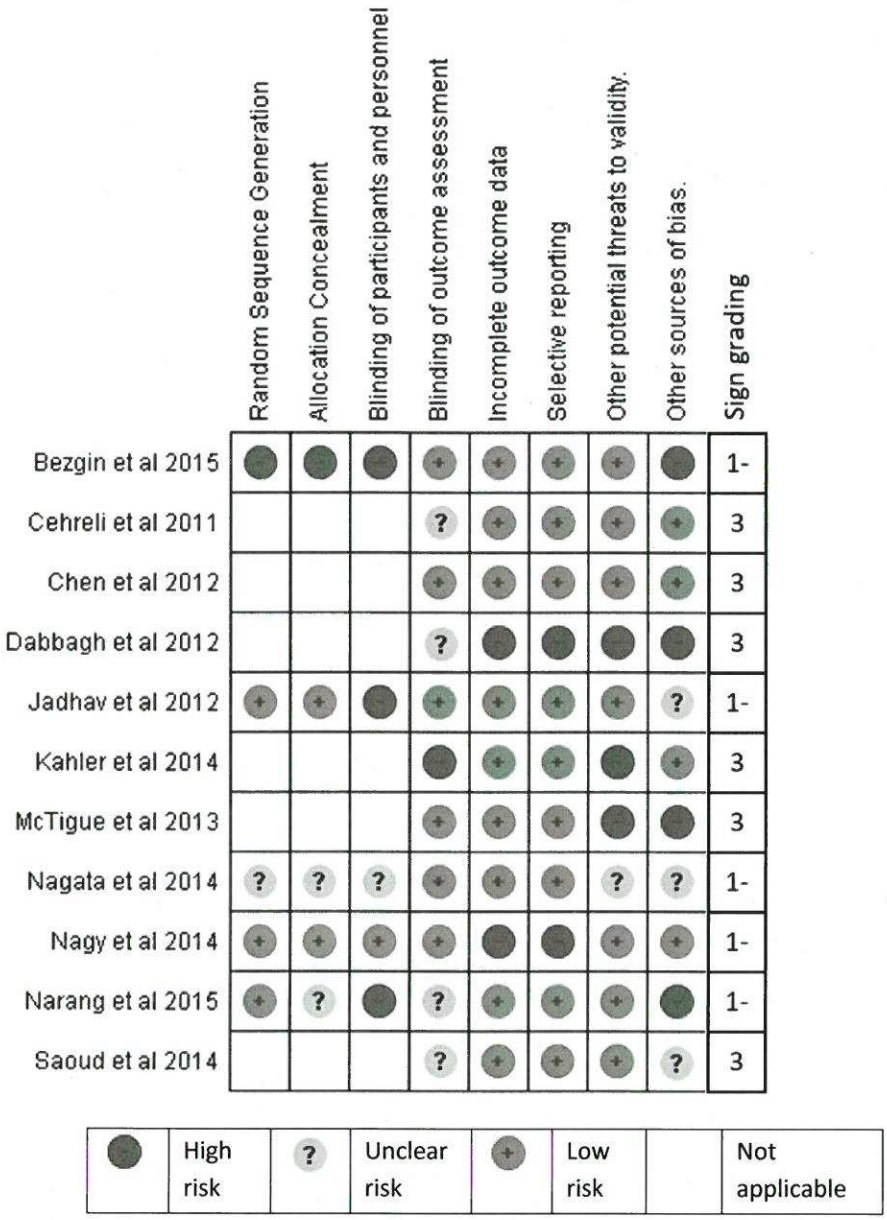
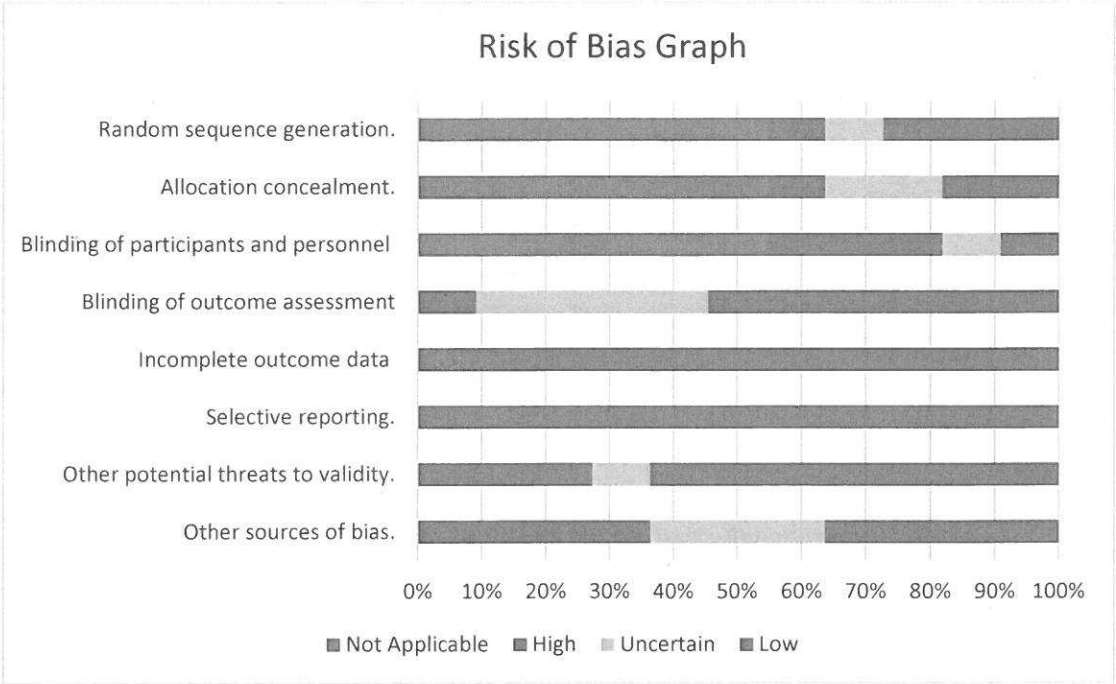
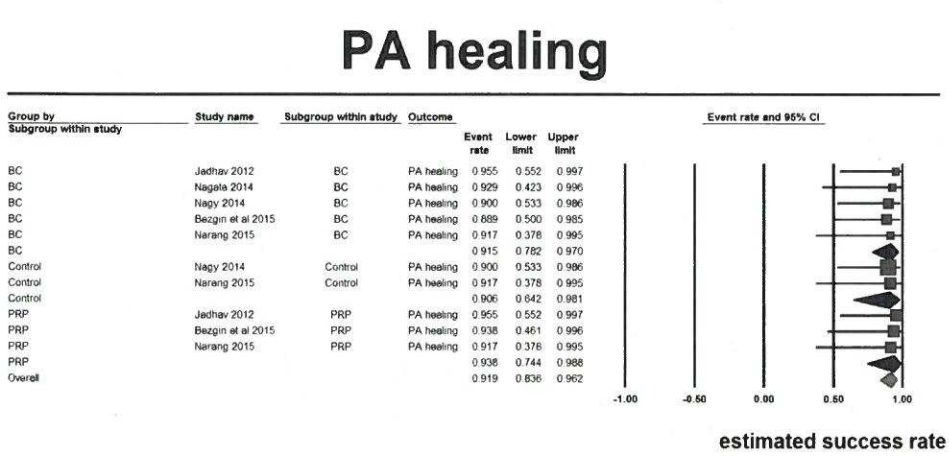


Figure 3: Risk of bias graph

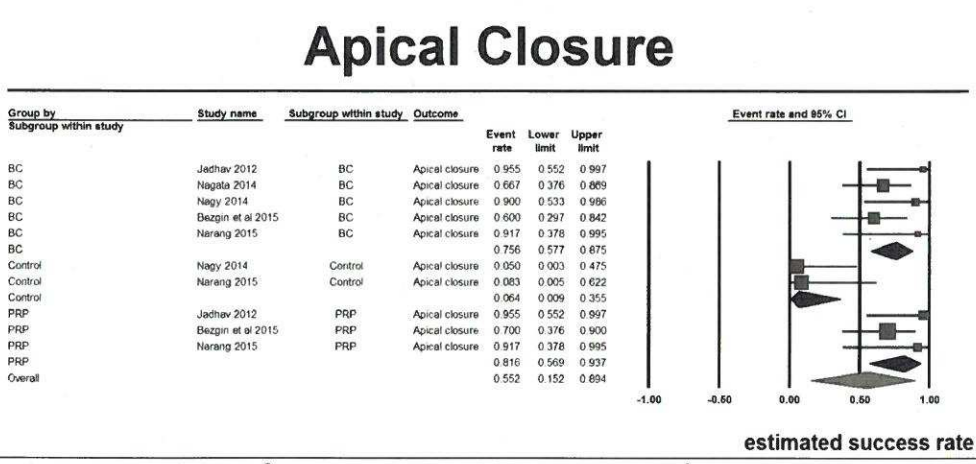


Figures 4a-d: Forrest Plot of success rates for periapical healing, apical closure, root lengthening and root dentin formation

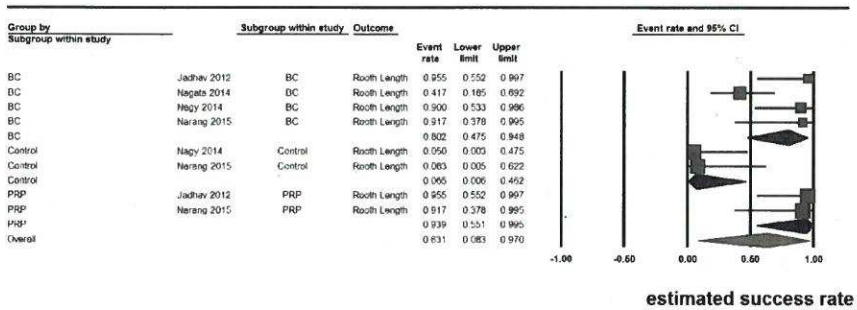
a. Heterogeneity: tau^2= 1.28, Q = 12.58, df = 2, p = 0.002



b. Heterogeneity: tau^2= 1.28, Q = 12.58, df = 2, p = 0.002



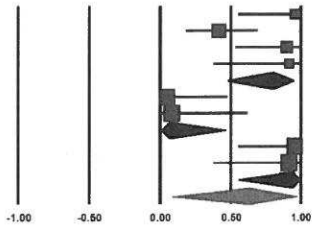
c. Heterogeneity: tau^2= 3.3, Q = 10.14, df = 2, p = 0.006, I^2= 70.32



d. Heterogeneity: tau^2= 3.3, Q = 10.14, df = 2, p = 0.006, I^2= 70.32

Root Dentine Formation

Group by Subgroup within study	Subgroup within study		Outcome	Event rate	Lower limit	Upper limit
BC	Jadhav 2012	BC	Root dentine formation	0.955	0.552	0.997
BC	Nagata 2014	BC	Root dentine formation	0.417	0.185	0.692
BC	Nagy 2014	BC	Root dentine formation	0.900	0.533	0.986
BC	Narang 2015	BC	Root dentine formation	0.917	0.378	0.995
BC				0.802	0.475	0.948
Control	Nagy 2014	Control	Root dentine formation	0.050	0.003	0.475
Control	Narang 2015	Control	Root dentine formation	0.083	0.005	0.622
Control				0.065	0.006	0.462
PRP	Jadhav 2012	PRP	Root dentine formation	0.955	0.552	0.997
PRP	Narang 2015	PRP	Root dentine formation	0.917	0.378	0.995
PRP				0.939	0.551	0.995
Overall				0.631	0.083	0.970



estimated success rate