Revascularization Outcomes: A Prospective Analysis of 16 Consecutive Cases

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Abstract

Introduction: Recent reviews lament the lack of evidence on the efficacy of regenerative procedures to induce further root maturation despite claims of a paradigm shift in the way infected, necrotic immature teeth with arrested root development can be endodontically treated. The majority of reports are either case series or successful case reports where nonstandardized images may make interpretation uncertain. Methods: This prospective clinical study reports on preliminary outcomes of regenerative endodontic procedures carried out on 16 teeth, 3 mandibular premolars and 13 traumatized central incisors, after 18-month reviews. Qualitative analysis of resolution of periapical radiolucencies and apical closure was undertaken. Quantitative analysis compared preoperative and recall radiographs by using a geometrical imaging program that calculated percentage changes in root length and dentin wall thickness. Results: Qualitative assessment showed 90.3% resolution of the periapical radiolucency. Apical closure was assessed as incomplete in 47.2% and complete apical closure in 19.4% of cases. Quantitative assessment showed change in root length varying from −2.7% to 25.3% and change for root dentin thickness of −1.9% to 72.6%. Conclusions: Patterns of continued root maturation were variable at 18-month review. Reviews at 36 months showed continued root maturation for 2 cases. Quantitative analysis can control for changes in angulation but may introduce other measurement errors. However, not all anterior teeth were suitable for TurboReg assessment because overlapping of the cementoenamel junctions and/or further eruption of teeth often precluded stable landmark positioning. Discoloration of the crown was a common consequence, with unaesthetic results in 10 of the 16 cases. (J Endod 2014;40:333–338)

Key Words

Regenerative endodontics, revascularization, traumatized teeth, tri-antibiotic paste, TurboReg

The promise and potential of regenerative endodontic therapies in necrotic teeth were first explored by Nygaard-Østby (1) in 1961 with limited success. During the last decade there has been renewed interest in regenerative endodontic procedures, which have been defined as "biologically based procedures designed to predictably replace damaged, diseased or missing structures, including dentine and root structures as well as cells of the pulp-dentine complex, with live viable tissues, preferably of the same origin, that restore the normal physiologic functions of the pulp dentine complex" (2, 3).

Numerous case reports have outlined successful outcomes when regenerative endodontic procedures have been used for the treatment of infected immature teeth (4−13). It has been reported that this treatment regime can result in apexogenesis, which is continued root maturation with closure of open apices, an increase in root length, and thickening of lateral dentin on root walls (14). Understandably, descriptions of these new techniques have created some excitement in the profession. A number of reviews have described this as a "paradigm shift for the treatment of immature permanent teeth" and as being the way of the future, going from traditional barrier formation approaches of calcium hydroxide and mineral trioxide aggregate (MTA) apexitication to biologically based treatment protocols (15, 16). Therefore, it is surprising that 2 recent reviews are more circumspect in their recommendations for regenerative endodontic procedures (3, 17). A recent invited review in Dental Traumatology stated that "because of the lack of long-term evidence to support the use of endodontic regenerative procedures in traumatised teeth with open apices, revascularization regeneration procedures should only be attempted if the tooth is not suitable for root canal obturation, and after apexogenesis, apexitication or partial pulpotomy treatments have already been attempted and have a poor prognosis" (3). Interestingly, the authors of that review have previously seemed to be avid proponents for regenerative techniques (2). The other recent review in the Journal of Endodontics states that "the outcome of revascularization procedures remains somewhat unpredictable and the clinical management of these teeth is challenging, when successful, they are an improvement to treatment protocols that leave the roots short and the walls of the root canal thin and prone to fracture" (17).

One identified drawback has been a lack of available evidence on outcomes (2). Although many case reports and some case series report favorable outcomes, these reports have to be considered a low level of evidence, particularly because one of the major problems with the interpretation of case reports is that often only successful individual outcomes are presented. Many of these reports also only describe outcomes limited to premolar teeth (4, 6, 8, 9, 11, 12). To date, there appears to be only one prospective pilot clinical study in the literature that examines outcomes for anterior
teeth (13). In that study, 14 infected nonvital immature incisor teeth were treated with regenerative procedures after trauma. However, the study used nonstandardized images, many of which appeared to be of poor quality and either foreshortened or elongated.

Unfortunately, even slight changes in angulation between preoperative and recall reviews have the capacity to produce inconsistent images and inaccurate interpretations. Thus the use of nonstandardized radiographs to measure outcomes in regenerative endodontic procedures, particularly to evaluate any increase in root length, has only allowed qualitative assessments to be made. Recently, Bose et al (14) reported on a geometrical imaging program aimed at minimizing the potential differences in angulations between preoperative and recall images that permitted quantitative determination of changes in root length and dentinal wall thickness to be made. This technique theoretically allows for calculations to capture the extent of any root maturation, which is an improvement of prior qualitative assessments on outcome. The validity of using this technique on anterior teeth has not been tested on traumatized teeth treated by regenerative endodontic procedures.

The first aim of this investigation was to present a preliminary prospective analysis of 16 teeth in 12 consecutive patients where a standardized protocol for regenerative endodontic procedure was undertaken for the treatment of immature infected teeth. Thirteen teeth were traumatized incisor teeth, and 3 teeth were premolar teeth. All teeth were followed up for a minimum of 18 months. Second, it aimed to assess the validity of using the mathematical modeling software described by Bose et al (14) to assess changes in root length and dentinal wall thickness after treatment. A comparison was made between the results obtained by qualitative assessment and those obtained where possible with the modeling software. This article reports on outcomes and describes some of the clinical difficulties encountered in treatment and alluded to in the literature.

**Methods**

In all cases the following protocol was followed. All parents of the patients signed a Consent Form advising them that data from this procedure were collected by Queensland Health and were to be used for research purposes, although patient anonymity was protected. Ethical approval for the research project had been granted by the Director of the Oral Health Centre. Clinical examination, pulp sensibility testing, and radiographic examination were carried out to confirm that the tooth was infected and had incomplete root maturation. The inclusion requirement was an open apex that was considered as greater than 1.1 mm in diameter. The exclusion requirements were a closed apex and any longitudinal fracture of the root. It was explained to all patients and/or their carer(s) that regenerative endodontic procedures were relatively new and that to date there were no guidelines for treatment protocols in the literature. A comprehensive discussion of the risks, complications, and alternative treatment options was undertaken, and parental consent was obtained.

This prospective clinical study was conducted on 16 teeth in 12 patients. The average age of the patients was 10 years and 5 months. There were 3 mandibular second premolar teeth and 13 traumatized maxillary central incisors in the sample. The mandibular premolar teeth had developmental anomaly dens evaginatus, which resulted in pulp necrosis. The incisor teeth had a history of a variety of traumatic injuries including crown fractures, luxations, and avulsion injuries, leading to pulpal necrosis and arrested root development.

Local anesthesia was administered, and the tooth was isolated with rubber dam. Access preparation was made, and working length was estimated with an apex locator (Sybron Endo, Orange, CA) and/or a periapical radiograph taken with a file inserted into the canal for length confirmation. The canal was then irrigated with 1% sodium hypochlorite 2 mm from the working length before drying the canal with large-size paper points. A tri-antibiotic paste was carefully introduced into the canal with a lentulo spiral root canal filler. The tri-antibiotic paste consisted of an equal mixture of metronidazole, ciprofloxacin, and amoxicillin mixed with 1 mL sterile water. The root canal was filled to a level just below the cementoenamel junction (CEJ). Care was taken to minimize placement in the coronal portion of the tooth. A double seal of approximately 4 mm Cavit (ESPE, Seefeld, Germany) and Fuji IX (GC America, Alsip, IL) was used to close the access cavity.

Teeth were usually reviewed 4 weeks later. Local anesthesia without a vasoconstrictor (Scandoc 3% Plain; Septodont, Saint-Maur-des-Fossés, France) was administered for this visit. The tooth was isolated with rubber dam. The canal was irrigated with 1% sodium hypochlorite. After the canal was dried with paper points, a D11T nickel-titanium hand spreader (Dentsply Tulsa Dental, Johnson City, TN) or a size 35 file (Sybron Endo) with a small bend at the tip to enable laceration of the periapical tissues was used to initiate bleeding into the root canal space. Where possible, blood was allowed to clot to a level 3 mm below the CEJ. Then 3 mm ProRoot white MTA (Dentsply Tulsa Dental) was placed onto the clot with Buchanan Pluggers (Sybron Endo). The access cavity was sealed with 3 mm glass ionomer cement.

The teeth were reviewed 6 months after access closure for at least 18 months. Assessments at review included an evaluation of clinical signs and symptoms, periapical status, the presence of further root maturation, and pulp sensitivity testing. Two teeth have been reviewed for 3 years. Because this is an ongoing study, further follow-up appointments are scheduled.

**Statistical Analysis**

The preoperative and postoperative images were saved in JPEG format, and preliminary qualitative assessments were undertaken by 3 endodontists and a pedodontist. The parameters assessed were as follows:

1. The presence or absence of a preoperative and/or postoperative periapical radiolucency
2. Whether apical closure was occurring
3. Whether apical closure was complete

The JPEG images were then transferred to the imaging program software Image J with TurboReg plug-in (Biomedical Imaging Group, Swiss Federal Institute of Technology, Lausanne, ND, Switzerland) as described by Bose et al (14). The change in root length was calculated by measuring along a straight line from the CEJ to the radiographic apex of the tooth. Adjacent teeth with open apices where continued root length growth had occurred during the 18-month review period were excluded from the quantitative analysis because a stable landmark position other than the CEJ was not able to be determined. The dentinal wall thickness for the preoperative and postoperative images was measured at two thirds of the root length from the CEJ. The root canal width and the pulp space were measured at this level, and the remaining dentin thickness was calculated by subtracting width of the pulp space from root canal width. All radiographic image transformations and measurements were repeated after 1 week, and the mean of the 2 sets was considered as the final value. Intraexaminer reliability was assessed by a kappa statistic. The data of dentin thickness and root length were calculated as the percentage change from the preoperative assessments. A strict radiographic criterion was used when assessing the presence of a periapical lesion. The data were statistically analyzed by using the Kruskal-Wallis nonparametric analysis of variance. A P value <.05 was considered significant.
Table 1 summarizes the age, gender, tooth type, type of traumatic injury sustained, and diagnosis at the time of first presentation of the patients for all 16 teeth. Response to electric pulp sensibility testing and presence of discoloration of the crown were noted at the time of the 18-month review.

Table 1 shows a mean score of 84.7% for the presence of periapical radiolucency in the preoperative films, consistent with a diagnosis of apical periodontitis in the majority of cases. The mean score for the presence of periapical radiolucency in the postoperative image was 9.7%. Apical closure after treatment was assessed as incomplete in 47.2%, and complete apical closure was recorded in 19.4% of cases.

Not all teeth were suitable for TurboReg assessments because of overlapping of CEJs precluding landmark positioning. Also, further root development occurred in some immature adjacent teeth during the 18-month review period, resulting in an unstable landmark position.

Figure 1 presents the data for cases 2–10 that were suitable for TurboReg assessment, with changes in root length varying in a range from 2.7% to 25.3% and for root dentin thickness from 1.9% to 72.6%. The kappa score for intraexaminer reliability was 0.46 for the quantitative analysis.

For cases 2 and 3 (right and left central incisors, respectively), the review period extended to 36 months, and that data with respect to root length and dentin thickness is presented in Figure 2. A sequential increase in the dentin thickness from 18 to 36 months is noted, which is consistent with further root maturation. The decrease in root length for case 2 suggests a margin of error in TurboReg assessment.

### Table 1. Summary of Cases at the 18-month Review

<table>
<thead>
<tr>
<th>Case</th>
<th>Age (y)</th>
<th>Gender</th>
<th>Tooth</th>
<th>Diagnosis</th>
<th>Type of injury</th>
<th>EPT response</th>
<th>Discoloration</th>
</tr>
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<tbody>
<tr>
<td>1</td>
<td>12</td>
<td>Female</td>
<td>Premolar</td>
<td>CPA</td>
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<td>No</td>
</tr>
<tr>
<td>2</td>
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<td>Incisor</td>
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<tr>
<td>3</td>
<td>8</td>
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<td>AAP</td>
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<td>8</td>
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<tr>
<td>9</td>
<td>10</td>
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<td>Incisor</td>
<td>Pulp necrosis</td>
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<td>Yes</td>
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<tr>
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<td>CPA</td>
<td>CCF</td>
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</tr>
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</table>

AAP, asymptomatic apical periodontitis; CCF, complicated crown fracture; CPA, chronic periapical abscess; EPT, electric pulp testing; evaginatus, lost occlusal tubercle; IRR, inflammatory root resorption; SAP, symptomatic apical periodontitis; UCCF, uncomplicated crown fracture.
of 5%. However, a 5% increase in root length is recorded for case 3, which may reflect further root maturogenesis. The periapical films taken preoperatively at the 18-month and 36-month review are presented (Fig. 3).

In case 5, 2 separate films taken at a different angulation appear to show differences in apical closure for 2 different periapical images that were both taken at the 18-month recall (Fig. 4). In Figure 4A, the periapical radiograph of the qualitative assessment suggests apical closure has occurred. In Figure 4B taken at a different angulation, apical closure has not yet occurred. Both 18-month images were analyzed with TurboReg to assess these differences. The margin of error in the TurboReg assessments was —0.5% for root length and 2.9% for dentin thickness. The qualitative assessments for case 1 suggested an increase in root dentin thickness and apical closure, but this case was excluded because TurboReg assessments showed implausibly large changes in the dimensions of untreated teeth during the treatment period, indicating a failure to accurately match the pretreatment and post-treatment images by the software.

In case 16, the incisor tooth experienced a horizontal root fracture from secondary trauma at 22 months. The tooth has been

Figure 3. (A) Preoperative film with GPP marker revealing periapical radiolucencies associated with both maxillary central incisors. (B) Periapical radiograph at 18-month review. (C) Periapical radiograph at 36-month review where apical closure appears more evident on the left central incisor.

Figure 4. (A) Periapical radiograph taken at 18-month recall where apical closure is evident. (B) Periapical radiograph also taken on same day at 18-month review but at a different angulation that suggests apical closure is incomplete. (C) Image J correction of the 2 radiographs for TurboReg assessment where analysis reveals differences in root length of -0.5% and 2.9% for dentin thickness.
successfully maintained in the arch with flexible splinting to the adjacent teeth.

**Discussion**

The results of this study indicate that similar and consistent outcomes in the management of endodontic outcomes are not always achieved. It is not surprising that the rate of root maturation is variable because of unique individual circumstances. It may be that teeth with longstanding necrosis are more likely not to have remaining viable pulp tissue and perhaps diminished regenerative capacity. Indeed, a number of case reports indicate cases of traumatized incisor teeth where the regenerative procedure failed to result in further root maturation (5, 9, 13, 18). Therefore, the many case series reporting 100% success for regenerative procedures as tabled in Torabinejad and Faras (19) may not reflect true outcomes for success because it is likely that unfavorable cases are not reported. Furthermore, there is a paucity of prospective studies (13) or case series where it appears consecutive cases are reported (9). This study reports on 16 consecutive cases where the 13 of the teeth treated with regenerative procedure were incisor teeth subjected to trauma, and 3 were premolar teeth. Quantitative assessment by using software imaging programs that control for angulation of preoperative and recall radiographs for changes in root length and dentin thickness appears to add validity to the biological changes that may occur after regenerative endodontic procedures. Furthermore, the importance that longer periods of review are required is illustrated because continued root maturation was observed in 2 cases followed for 36 months (Fig. 2).

In this prospective study, 90.3% of the infected immature teeth were assessed to have periapical radiolucency at the 18-month review. The absence of periapical radiolucency is a well-established measure of a favorable healing outcome (20). If this is considered a success in the endodontic literature, then are regenerative procedures with or without root maturation more or less successful than other endodontic outcomes provided by MTA apicification or root canal obturation? The current authors suggest that teeth where there is evidence of resolution of periapical pathology do not necessarily require further endodontic treatment, even if no further root maturation has occurred. Interestingly, Nosrat et al (18) reported a case where no further root maturation had occurred after 6 years, despite the resolution of periapical pathology, and the teeth were re-treated with MTA apicification. In a prospective study on 14 immature teeth treated with a revascularization protocol, Shah et al (13) reported resolution of periapical radiolucencies in only 78% of cases. Fornocresol was used as an intracanal medicament instead of triple antibiotic paste, which may account for the lower value.

This current study reported quantitative results in changes in root length and root dentin thickness after images had been transformed to eliminate errors of angulation by utilization of ImageJ software first used by Bose et al (14). Changes in root length and dentin thickness are recorded in Figure 1. Of note is that of the 16 cases, only 8 were considered suitable for assessment because of difficulty in assigning a landmark position of the CEJ in crowded dentition because it was sometimes not possible to identify clear landmarks widely spaced in 2 dimensions across the image, as is optimal for accurate transformation. Bose et al also excluded 6 teeth because of difficulties in selecting consistent landmarks, despite the fact that their study focused predominantly on premolar teeth where the first mandibular molar may have achieved complete root maturation in regard to length. In the current study, TurboReg gave an inconsistent result in one mandibular premolar case where the angulation between pretreatment and post-treatment radiographs was obviously too large for TurboReg analysis to effectively match the 2-dimensional images through a 3-dimensional affine transformation. In this case there were difficulties in placement of the film because of behavioral issues with the child. In some cases in the current study, despite careful selection of landmarks, the results of TurboReg image transformation were determined by visual inspection to be implausible. This factor alone questions the reliability of the ImageJ software in assessing root changes in anterior teeth, particularly because trauma and subsequent loss of vitality and endodontic infections to maxillary incisor teeth are often associated with Class II malocclusions where the incidence of crowding and overlapping of teeth is higher (21).

Repeated image transformation introduces more potential for inconsistency between trials because the analyst must re-select landmarks before image transformation, and multiple factors can affect this selection. In the current study the kappa reliability was 0.46, which is considered moderate reliability (22). Bose et al (14) did not report a kappa reliability score for repeated analysis, whereas Jeeruphan et al (23) in a study of 7 incisor and 13 premolar teeth reported an intraexaminer agreement of 0.985, showing excellent reliability between the 2 measurements taken a week apart. It is important to note that here, repeated image comparison included both image transformation by using TurboReg and measurements of the resulting images. Jeeruphan et al (23) did not specify whether in their study both of these steps were repeated, or only the measurements, by using the images resulting from the initial TurboReg transformation.

In case 1, 2 postoperative images taken on the same day appeared to show different interpretations of apical closure (Fig. 4). It could be argued that qualitative assessment of Figure 4A suggests apical closure, whereas Figure 4B reveals incomplete apical closure, resulting in a different assessment of outcome success. However, when assessed by TurboReg, the differences in root length between the 2 images were less than 0.5%, and root thickness was 2.9%, suggesting an error margin of less than 3%. Also in case 2, differences between the TurboReg assessments at the 18-month and 36-month reviews for case 2 actually showed a reduction in root length of 5% at 36 months, suggesting a margin of error in this assessment technique (Fig. 2).

Another reported factor critical to the outcome of regenerative procedures has been the initiation of bleeding into the canal to provide the stem cells and scaffold for ingrowth of new tissue (2, 15). Bleeding was often difficult to initiate in some teeth, despite using an anesthetic without a vasoconstrictor. Other studies have reported that bleeding occurred at the apex but not into the canal (18). Ding et al (9) reported failure to induce bleeding in 4 of 12 teeth treated with a regenerative technique where a vasoconstrictor, epinephrine, was used. In the current study, if bleeding did not occur, a file was bent and passed into the apical tissue to lacerate the tissues and promote bleeding. Despite this, substantial bleeding did not always occur. The inability to promote bleeding may be considered a negative factor in case selection when case selection guidelines for revascularization procedures are written in the future. The problems with initiating bleeding in canals were recently reviewed (18). Research on other types of stem cell therapies and scaffolds are under development (3, 16). The current recommended protocol recommends the induction of bleeding to provide undifferentiated mesenchymal stem cells (24) and the scaffold. Torabinejad and Turman (25) have recently reported further root development and apical closure where a canal was filled with platelet-rich plasma, and bleeding was not induced into the canal. García-Godoy and Murray (5) have suggested that the standard revascularization procedure can be improved by placing other scaffolds that may have been tissue engineered and incorporated growth factors into the root canal.

Discoloration of the crown was a common consequence of the regenerative procedure, with unaesthetic results in 10 of the 16 cases. Kim et al (26) reported that discoloration occurred when minocycline used in the tri-antibiotic paste contacted the coronal dentin. The current study had attempted to eliminate that problem by substituting amoxicillin
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for minocycline. An earlier report on a mandibular premolar had demonstrated no discoloration by using the same modified tri-antibiotic paste (11). Although minocycline has been attributed as the main cause of discoloration (18), several studies have reported that both gray MTA (10) and white MTA (27) can cause discoloration after treatment. Placement of MTA just below the CEJ also proved to be difficult in some teeth, and in some cases the material may not have developed a complete seal. Some of these difficulties in the placement of MTA may have been avoided if a collagen matrix had been used as advocated by Petroj et al. (27). It has been suggested that one possible way to avoid discoloration is to seal the dentinal walls of the access cavity with a dentin bonding agent (10). It has been suggested, without supporting evidence, that avulsion is a contraindication for regenerative treat-

The recent review in Dental Traumatology (3) does seem timely because there does appear to be a lack of evidence and conflicting results as well as a paucity of prospective studies. However, it is perhaps too strong in its recommendation in advising that regeneration should only be attempted as a last resort in failing treatment with a poor prognosis where apexitogenesis, nonsurgical root canal apexitogenesis, or obturation has already been attempted. The aforementioned procedures are generally associated with good outcomes, so if these recommendations were followed, there would be fewer instances when regenerative procedures would be used. Furthermore, undertak-

The current authors suggest that if regenerative protocols are to be tried, they should be considered as the primary care for treatment of traumatized infected immature teeth. Cvek (28) reported on the higher incidence of fracture in incisor teeth treated by calcium hydroxide apexitogenesis where root maturation was arrested. This may be due to the fragility of the remaining root structure, which may be compounded by the detrimental effects of calcium hydroxide on root dentin strength (29). Even if regenerative procedures are used in incisor teeth, there is often little change in the thickness of the dentin in the cervical and mid-root regions of the tooth, which remain thin. In one case in this study the tooth was subjected to further trauma, resulting in a cervical horizontal root fracture.

Conclusion

The results of this study have showed that the pattern for further root maturation of roots after regenerative procedures was variable when assessed at 18 months, although in almost all cases, periapical pathology appeared to resolve completely. That root maturation in one case was only evident at 36-month review suggests the need for longer follow-up assessment before assessment of further maturogen-

Acknowledgments

The authors deny any conflicts of interest related to this study.

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