A Retrospective Evaluation of Radiographic Outcomes in Immature Teeth With Necrotic Root Canal Systems Treated With Regenerative Endodontic Procedures

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Abstract

Introduction: Several case reports on endodontic regeneration involving immature permanent teeth have recently been published. These case series have used varying treatments to achieve endodontic regeneration including triple antibiotic paste, $Ca(OH)_2$, and formocresol. However, no study has analyzed the overall results. **Methods:** In this retrospective study, we collected radiographs from 54 published and unpublished endodontic regenerative cases and 40 control cases (20 apexification and 20 nonsurgical root canal treatments) and used a geometrical imaging program, NIH ImageJ with TurboReg plug-in, to minimize potential differences in angulations between the preoperative and recall images and to calculate continued development of root length and dentin wall thickness. **Results:** The comparison to the 2 control groups provided a validation test for this method. Forty-eight of the 54 regenerative cases (89%) had radiographs of sufficiently similar orientation to permit analysis. The results showed regenerative endodontic treatment with triple antibiotic paste ($P < .001$) and $Ca(OH)_2$ ($P < .001$) produced significantly greater increases in root length than either the MTA apexification or NSRCT control groups. The triple antibiotic paste produced significantly greater differences in root wall thickness than either the $Ca(OH)_2$ or formocresol groups ($P < .05$ for both). The position of $Ca(OH)_2$ also influenced the outcome. When $Ca(OH)_2$ was radiographically restricted to the coronal half of the root canal system, it produced better results than when it was placed beyond the coronal half. **Conclusions:** $Ca(OH)_2$ and triple antibiotic paste when used as an intracanal medicament in immature necrotic teeth can help promote further development of the pulp-dentin complex. *(J Endod 2009;35:1343–1349)*

Key Words

Immature teeth, regenerative endodontics, retrospective studies, revascularization

Bacterial infection of dental pulp and dentin in immature permanent teeth with open apices poses numerous challenges to the dentist. Traditionally, the treatment of immature permanent teeth with necrotic pulps involves long-term application of calcium hydroxide to induce apexification at the root apex (1). However, the remaining thin fragile dentinal walls predispose these teeth to fracture. Moreover, some studies have shown that long-term use of calcium hydroxide can weaken dentin (2). Recently, mineral trioxide aggregate (MTA) has been used in 1-step apexification procedures to create an artificial apical barrier on which the obturation material can be compacted (3). Although clinically successful for treatment of apical periodontitis, these techniques do not help strengthen the root, and in the absence of continued development of the root, the roots remain thin and fragile.

Several case reports on endodontic regeneration involving immature permanent teeth with pulpal necrosis have been recently published (4–11). Although there are no established standardized treatment protocols for endodontic regeneration, many of these cases have shown favorable results, with continued radiographic evidence of development of the dentin pulp complex and an absence of clinical symptoms. A triple antibiotic paste consisting of metronidazole, ciprofloxacin, and minocycline has been shown to be very effective against the pathogens commonly found inside the root canal system (12–14). Calcium hydroxide has also been shown to be very effective as an intracanal medicament (1).

Although histologic studies are still lacking, the clinical outcome of these endodontic regeneration studies is promising. The most frequent pretreatment diagnosis of these cases was of pulpal necrosis with or without apical periodontitis. Postoperative recalls demonstrated regression of clinical symptoms in addition to radiographic evidence of continued root development and increased dentinal wall thickness (15). However, the application of nonstandardized radiographs to evaluate an increase in the root length and dentin thickness should be interpreted with caution because a slight change in the angulation at the preoperative or recall appointment might produce inconsistent images and, accordingly, inaccurate interpretations. Ideally, prospective studies should fabricate customized jigs for each patient to consistently reposition the x-ray tube during radiographic exposure. However, most of the available retrospective case series on regenerative procedures did not use this method. Accordingly, the quantitative determination of changes in root length and dentinal wall thickness requires a mathematical modeling of the preoperative and postoperative images that permits calculation of changes in root development. In this retrospective study, we collected radiographs from 54 published and unpublished endodontic regenerative case series as well as 40 control cases (20 apexification cases and 20 nonsurgical root canal treatments [NSRCTs]) and used an image transformation and analysis

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program to estimate quantitative differences in the development of root length and dentinal wall thickness. The preoperative versus postoperative comparison to the 2 control groups provided 2 independent validation tests for this mathematical method. This method was then used to interpret differences in the outcome of root development for various intracanal treatments and during reported follow-up times.

Materials and Methods

Acquisition of Data

Radiographs of 54 clinical cases of immature permanent teeth that underwent endodontic regeneration procedures were obtained from practicing dentists around the globe (5–11, and unpublished cases by Drs Frederic Barnett and Milton Davenport). The initial diagnosis of these regeneration cases was pulp necrosis with or without periradicular pathology. On the basis of the intracanal medication that was placed subsequent to access opening and irrigation with NaOCl, the cases were subdivided into 3 groups: triple antibiotic paste, calcium hydroxide, and formocresol. The control group consisted of 20 case series that used MTA for apexification procedures from a recently published case series (16) and an additional 20 conventional NSRCTs generated from our residency program at the University of Texas Health Science Center at San Antonio, with follow-up times similar to the regenerative procedure cases.

Mathematical Image Correction

The preoperative and postoperative images were saved in the JPEG format and transferred to the Image J software (version 1.41; National Institutes of Health, Bethesda, MD) for measurement and recording of results. The plug-in application TurboReg (Biomedical Imaging Group, Swiss Federal Institute of Technology, Lausanne, VD, Switzerland) was used to mathematically minimize any dimensional changes that might have been incorporated into the preoperative or postoperative radiographs as a result of angulation differences to the x-ray central beam at the time of image acquisition. The TurboReg Algorithm performs an affine geometric image transformation to match the projection in 2 sequential radiographs. By using an approach previously described (17, 18), the preoperative or postoperative image with the least visible distortion was selected to be the source image, and the other image, which needed to be corrected, was termed the target image. The same 3 landmarks were selected on both the source and target image. The landmarks most commonly selected were well-defined, easily identifiable structures such as cementoenamel junction (CEJ), restoration margins, and apices of adjacent nonerupting mature teeth; in >90% of cases the CEJ and root apices were selected as radiographic landmarks. Care was taken to ensure that the landmarks were as widely spaced as possible on the image and that no positional changes had taken place to the reference points during the time interval the preoperative and postoperative images were taken (ie, landmarks were not selected on erupting teeth or immature root apices). Of the 94 total number of cases, a total of 6 regenerative endodontic cases were excluded because of problems with selecting consistent landmarks (4 formocresol and 2 $Ca(OH)_2$ cases); thus, the final analysis consisted of 48 regenerative endodontic cases (89% of submitted cases) and 40 control cases. The TurboReg "automatic mode" was selected for image correction for all cases (both regenerative and the 2 control groups), and this feature eliminated a potential for investigator bias. With the landmark positions of the source image, the target image was adjusted by TurboReg. A representative set of unadjusted and postadjusted radiographic images is presented in Fig. 1 as illustrations of this process.

Calibration

The images were then calibrated according to the type of the images received. For example, if the image was that of a size #2 intraoral conventional photographic film, the "set scale" option in Image J was used to set the horizontal dimension to be 31 mm and the vertical dimension to be 41 mm. Similarly, the scale was set for the digital films on the basis of the pixel size used in the particular digital system. If the edges of the warped images were cut during the process of calibration, the distance between any 2 stable reference points was selected from the source image and was used to set the scale of the adjusted target image. The calibration process permitted measurement of changes in root size to be based on a millimeter scale.

Measurements

The "straight-line" tool of TurboReg was used to measure the root length and dentin thickness. The root length was measured as a straight line from the CEJ to the radiographic apex of the tooth. The dentinal wall thickness for both the preoperative and recall images was measured at the level of the apical one third of the preoperative root canal length measured 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 the pulp space from the root canal width (Fig. 2). All the calibrations and measurements were repeated after a 1-week period to confirm the reproducibility of the procedures.

The first part of the study evaluated the validity of the NIH software Image J algorithm to mathematically model any dimensional changes between the preoperative and postoperative radiographs. This was done by evaluating 2 series of control cases in which we would predict that the treatment resulted in no clinical change in root length. The first control group consisted of 20 previously published cases (16) of necrotic immature permanent teeth with MTA apexification. The second group of 20 control cases involved conventional NSRCT in fully developed premolars. We predicted that neither treatment would result in a measurable increase in root dimensions and therefore would serve as control groups to validate the directional accuracy of the TurboReg analysis of nonstandardized radiographs.

The 48 analyzed endodontic regenerative cases were divided into 3 groups on the basis of the intracanal medication (ie, triple antibiotic paste, calcium hydroxide, and formocresol).

Statistical Analysis

After image standardization by using TurboReg, the primary outcome measures, root length and dentinal wall thickness at the apical third, were measured by using NIH Image J. All measurements were repeated after 1 week, and the mean of the 2 replicates was considered as the final value. Although the results were generated in millimeter units, we present the data as percentage change from preoperative values rather than the calculated millimeter change. We believe that this presentation is a conservative analysis because each case is normalized to its own preoperative measurement, minimizing one potential source of systematic errors in the overall analysis of treatment outcome. In addition, the units of percentage change provide a clinically meaningful outcome when considering the impact of regenerative endodontic procedures. However, for the interested reader, we include all calculations, including the actual millimeter data, in an online supplement to this article (Supplemental Appendix 1). The 2 data sets were then analyzed by the Bartlett test for homogeneity of variance. Both outcome measures had significantly different variances among the treatment groups ($P < .001$ for both root length and dentinal wall thickness); therefore, a parametric analysis could not be conducted. Accordingly, the data were analyzed by the Kruskal-Wallis nonparametric analysis of

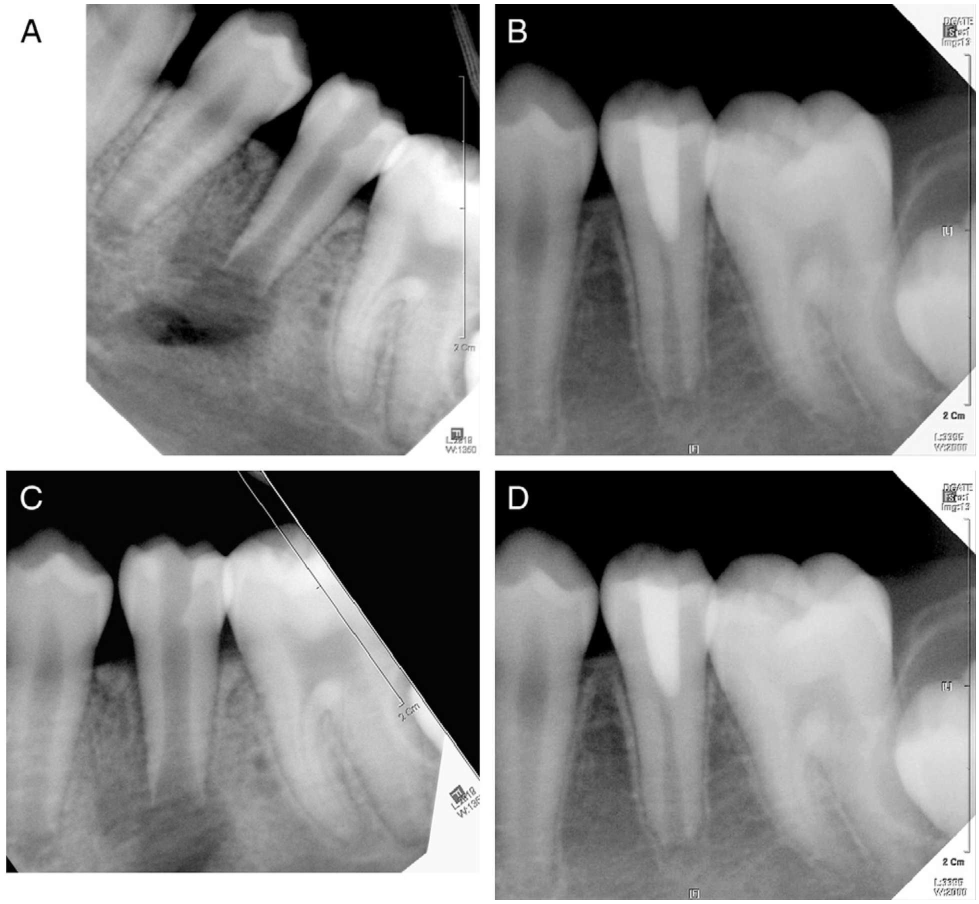

Figure 1. (*A*) Preoperative radiograph of tooth #20 with an open apex and a diagnosis of pulpal necrosis with apical periodontitis (6). (*B*) Recall radiograph at 10 months. Notice change in the angulations between (*A*) and (*B*). (*C*) Preoperative radiograph after image correction with the TurboReg plug-in application of NIH Image J. (*D*) Recall radiograph used as the source image to define the modeling function used on the preoperative (target) image. Note the final degree of parallelism of tooth #20 between the modified preoperative and recall radiographs.

variance, with Dunn multiple comparison test to identify differences between treatment groups. A *P* value <.05 was considered for significance, and median values are reported.

Results

The treatment groups differed significantly in the development of root length (Kruskal-Wallis statistic, 63.61; $P < .0001$). The 2 control groups, MTA apexification and NSRCT, displayed essentially no change in overall root length, indicating that the mathematical transformation by TurboReg appropriately detected these controls (Fig. 3*A*). Regenerative endodontic treatment with either the triple antibiotic paste ($P < .001$) or $Ca(OH)_2$ ($P < .001$) produced significantly greater increases in root length compared with either the MTA or NSRCT control groups. The formocresol group differed only compared with the MTA apexification control group ($P < .05$). There was no significant difference among the 3 medication groups.

The treatment groups also differed significantly in the development of dentinal wall thickness at the apical third of the root (Kruskal-Wallis statistic, 49.25; $P < .0001$). The calculated percentage change in dentinal wall thickness for the 2 control groups, MTA apexification and NSRCT, differed significantly from each other ($P < .05$), probably as a result of differences in the magnitude of instrumentation between the 2 groups (Fig. 1*B*). Regenerative endodontic treatment with the triple antibiotic paste produced significantly greater ($P < .001$) increases in dentin wall thickness compared with the MTA and NSRCT control groups. Treatment with $Ca(OH)_2$ ($P < .05$) or formocresol ($P < .05$) resulted in significantly greater change in dentinal wall thickness compared with the NSRCT group, but no differences were observed between these medicaments and the MTA apexification group. Finally, the triple antibiotic paste produced significantly greater differences in dentinal wall thickness compared with either the $Ca(OH)_2$ or formocresol groups ($P < .05$ for both).

Two secondary analyses were next conducted. First, we evaluated whether any time-related changes in outcome measures could be detected (Fig. 4). In general, the percentage increase in root length and root wall thickness increased with time, although the sample size became so small in this subset analysis that the reliability of the findings could be questioned. Second, we evaluated whether radiographic location of $Ca(OH)_2$ placement influenced the outcome. When $Ca(OH)_2$ was radiographically restricted to the coronal half of the root canal system, the median percentage increase in dentinal wall thickness was 53.8%, as compared with a 3.3% increase when it was placed beyond coronal half (ie, into the apical half of the root canal system). Interestingly, the percentage increase in root length was similar in both of these subgroups.

Discussion

All regenerative endodontic cases included in this retrospective study were considered by their authors to be clinically successful on the basis of the regression of signs and symptoms associated with

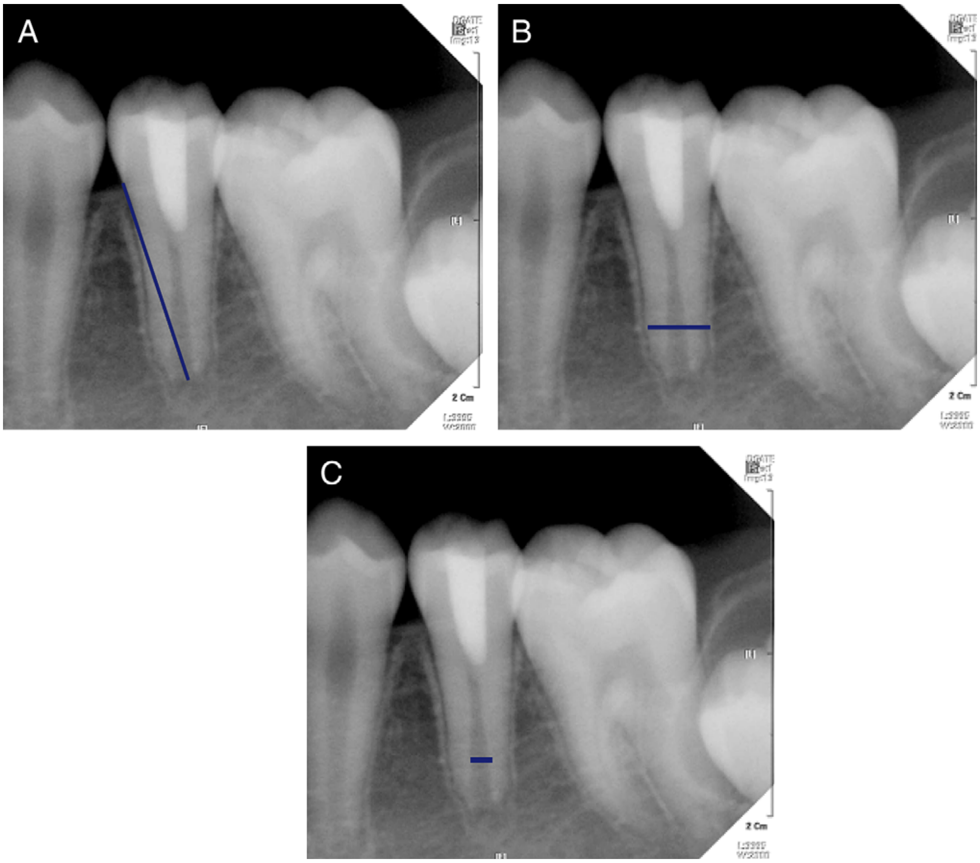

Figure 2. (*A*) Root length is measured from the CEJ to the radiographic apex. (*B*) Root canal width was measured at the level of two thirds the preoperative root canal length. (*C*) Pulp space measured at the same level and the remaining dentin thickness were calculated by subtracting the pulp space from the root canal width.

infected necrotic teeth as well as radiographic evidence of continued root development. However, to our knowledge, no previous study has attempted to standardize preoperative and postoperative radiographs for measuring the magnitude of these changes. Although the inclusion of a customized jig is ideal for collecting standardized radiographic images, this was not available in this retrospective analysis. As an alternative, we applied mathematical modeling of the preoperative and postoperative images by using NIH Image J with TurboReg plug-in to obtain morphologically standardized images. As seen in Fig. 3*A* and *B*, the percentage changes in root length and dentin wall thickness of both the control groups are nearly zero. The slight decrease in the dentin wall thickness of the NSRCT control group is most likely due to a detected decrease in dentin wall thickness after instrumentation. Thus, we believe that this method is sufficiently sensitive to permit estimation of treatment outcomes in regenerative endodontic cases. We believe that the use of mathematical image transformation to estimate these values has potential relevance for comparing the outcomes between groups and across times and for generating potential sample size estimates needed to conduct prospective clinical trials on regenerative endodontic treatments. The results indicated that regenerative endodontic treatments with various intracanal antimicrobial methods result in a significant increase in root length and dentinal wall thickness, as compared with similar cases treated with MTA apexification (16) or conventional NSRCT.

The most critical analytical step in this study was image correction with TurboReg. Any error incorporated during the image correction phase might substantially affect the results. Extra care was taken to ensure that no positional changes occurred to the landmarks used for image correction. For example, if the experimental tooth was surrounded on both sides by erupting teeth, then image correction with TurboReg would be of little value as a result of the lack of stable reference points. TurboReg also would not be able to correct images recorded with extreme deviations in the horizontal angulation. Most roots are oval-shaped in the buccolingual direction; hence, extreme deviations in the horizontal angulations might produce images that are distorted beyond the scope of TurboReg. NIH Image J with TurboReg also does not attempt to correct the 4%–8% magnification errors that are inherent in all periapical radiographs. The comparison of the 2 control groups supports the validity of this method because NSRCT resulted in no change in root length, and a predictable slight reduction in dentinal wall thickness, presumably as a result of instrumentation, was observed for changes in dentinal wall thickness. In addition, MTA apexification also resulted in negligible changes in root dimensions.

The results indicated that there is radiographic evidence of continued root development in all the experimental groups during the observation time. There were no significant differences observed among the 3 groups for changes in root length. Conversely, the triple antibiotic group showed the highest percentage increase in the dentin wall thickness compared with the other 2 groups. However, it is worth noting that we measured dentin wall thickness at a level of two thirds the preoperative root canal length measured from the CEJ. In a majority of the $Ca(OH)_2$ cases, the medicament appeared to be placed at the level of the apical third, which was beyond the level at which we took our measurements. Hence, we conducted secondary analyses in which the $Ca(OH)_2$ group was subdivided into 2 subgroups on the basis of the radiographic position of the $Ca(OH)_2$. When $Ca(OH)_2$ was placed

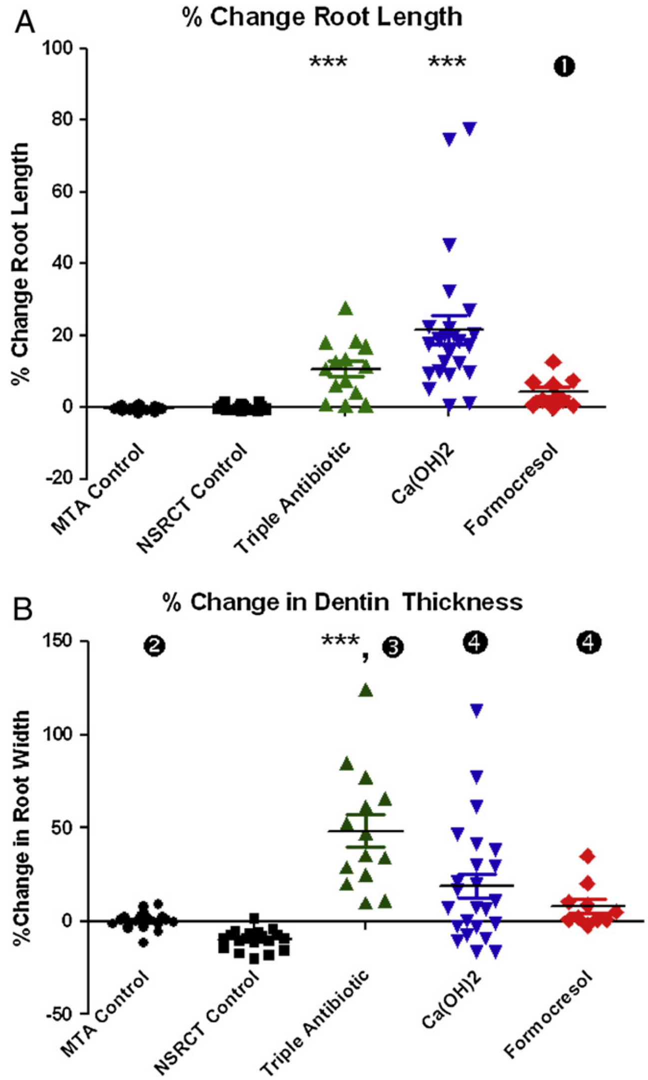

Figure 3. (*A*) Percentage change in root length from preoperative image to postoperative image, measured from the CEJ to the root apex. $^{***}P < .001$ versus MTA apexification control group (n = 20) and NSRCT control group (n = 20). (1) $P < .05$ versus MTA control group only. Median values for each group are depicted by horizontal line, and individual cases are indicated by the corresponding symbol. (*B*) Percentage change in dentinal wall thickness from preoperative image to postoperative image, measured at the apical third of the root (position of apical third defined in the preoperative image). $^{***}P < .001$ versus MTA apexification control group and NSRCT control group. (2) $P < .05$ versus NSRCT control group only. (3) $P < .05$ versus $Ca(OH)_2$ and formocresol groups. (4) $P < .05$ versus NSRCT control group only.

in the coronal half of the root canal, the percentage increase in dentinal wall was 53.8%, as compared with a mere 3.3% increase when it was placed beyond coronal half. Interestingly, the percentage increase in root length was similar in both the subgroups. The formocresol group showed the lowest increase in root length and dentin wall thickness. These results are comparable to previous animal studies with formocresol (19).

Although there are differences in the treatment protocols, all groups demonstrated several common factors that could have contributed toward the continued development of the root. All cases involved immature permanent teeth with open apices that might offer higher regenerative potential (15). In addition, the rich blood supply through the wide open apex could have helped preserve or promote vital pulp cells (8). In contrast to conventional endodontic treatment, all cases were treated as conservatively as possible, with little if any instrumentation of the root canal walls.

Pioneering studies by Kakehashi et al (20) have demonstrated healing of exposed pulps in germ-free rats. Hence, complete disinfection of the root canal is pivotal to the success of any attempt at endodontic regeneration of the pulp-dentin complex. Irrigation with NaOCl alone cannot render the canal free of bacteria, and thus, various intracanal medicaments including triple antibiotic paste, $Ca(OH)_2$, and formocresol have been used. Another requirement is developing a bacterial-tight coronal seal by using various combinations of materials including bonded composites, MTA, or glass ionomer to prevent any recontamination of the root canal.

Although radiographic evidence of hard tissue deposition was noticed, it has been theorized that this hard tissue could be due to the ingrowth of dentin, cementum, or bone (8, 21). Our study did not attempt to differentiate between these possibilities, and definitive human histologic studies are required to verify the exact nature of the hard tissue. The source of the cells that produce the biologic root development is also not clear. One question for future studies is to determine whether the continued increase in the root length and root wall thickness was due to the proliferation of multipotent mesenchymal

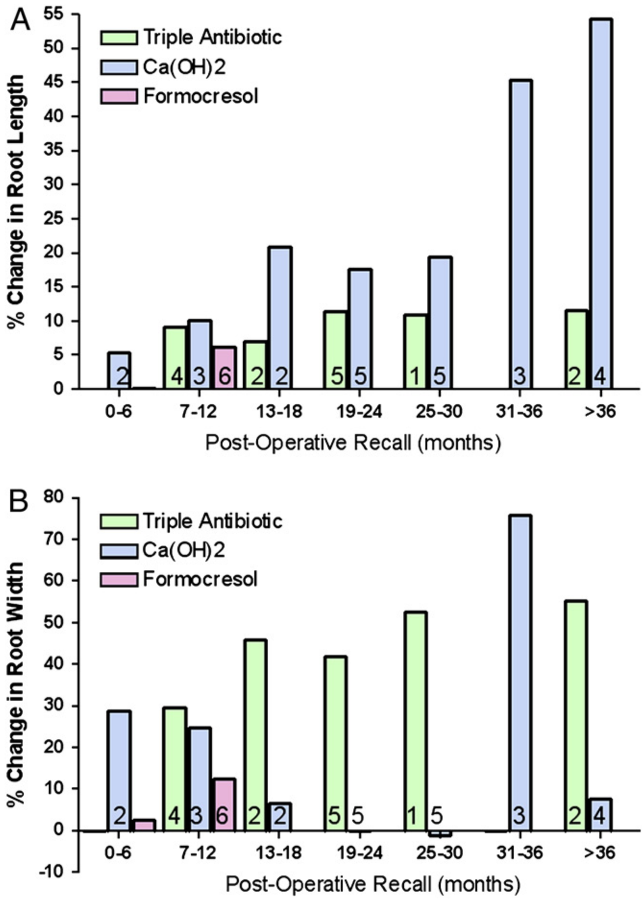

Figure 4. (*A*) Effect of postoperative recall time on percentage change in root length from preoperative image to postoperative image, measured from the CEJ to the root apex. Numbers at base of each bar are the sample size (n) for each group at that recall time. (*B*) Effect of postoperative recall time on percentage change in root width from preoperative image to postoperative image, measured at the level of two thirds the preoperative root canal length.

stem cells from the apical papilla or from the dental pulp stem cells remaining in the pulp tissue (22).

This retrospective study aimed to introduce a new radiographic analysis technique to mathematically transform nonstandardized radiographs and use the output to calculate estimates of radiographic success of clinical regenerative endodontic procedures. The results of the study indicate that $Ca(OH)_2$ and triple antibiotic paste, when used as an intracanal medicament in immature necrotic teeth, can help promote functional development of the pulp-dentin complex as measured by changes in root morphology. Although these data do not provide a definitive answer for outcomes of regenerative endodontic procedures, the approach does provide quantitative estimates of the magnitude of the treatment outcomes, and these values can be used to calculate sample sizes for future prospective randomized controlled clinical trials.

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