Injuries to Permanent Dentition Symposium

Treatment Options: Biological Basis of Regenerative Endodontic Procedures

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Abstract

Dental trauma occurs frequently in children and often can lead to pulpal necrosis. The incidence of pulpal necrosis in the permanent but immature tooth represents a challenging clinical situation because the thin and often short roots increase the risk of subsequent fracture. Current approaches for treating the traumatized immature tooth with pulpal necrosis do not reliably achieve the desired clinical outcomes, consisting of healing of apical periodontitis, promotion of continued root development, and restoration of the functional competence of pulpal tissue. An optimal approach for treating the immature permanent tooth with a necrotic pulp would be to regenerate functional pulpal tissue. This review summarizes the current literature supporting a biological rationale for considering regenerative endodontic treatment procedures in treating the immature permanent tooth with pulpal necrosis. (J Endod 2013;39:530–543)

Key Words

Children, pulpal revascularization, regenerative endodontics, stem cells, trauma

This Symposium includes a number of papers focused on diagnosis, prognosis, and treatment of the traumatized tooth. Here we provide a biological rationale for considering regenerative endodontic treatment procedures. A companion paper in this Symposium discusses considerations for the clinical protocol of a regenerative endodontic procedure.

Dental trauma occurs frequently in children and often can lead to pulpal necrosis. Population-based studies from around the world indicate that the prevalence of dental trauma injuries is about 4%–59%, with the majority of cases occurring in incisors. The broad range in estimated prevalence rates may be due in part to differences in sampling methods or study populations. In one study of 262 Swiss children aged 6–18 years, the prevalence of dental trauma was nearly 11%, and about 12% of enamel-dentin fractures led to pulpal necrosis. In another study of 889 permanent teeth with traumatic injuries, pulpal necrosis occurred in about 27% of the sampled population. The risk of developing pulpal necrosis is well recognized to be dependent on the type of dental trauma. In an analysis of 10,673 permanent teeth seen at a tertiary care center, pulpal necrosis was estimated to range from 0% (infraction), to 5% (conclusion), to 26% (extrusion), to 58% (lateral luxation), to 92% (avulsion), to 94% (inclusion). The occurrence of pulpal necrosis in the permanent but immature tooth often represents a challenging clinical situation because the thin and often short roots increase the risk of subsequent fracture; indeed, overall survival of the replanted permanent teeth has been reported to range from 39%–89%. In treating the immature tooth with pulpal necrosis, the ideal clinical outcomes would be to prevent or heal the occurrence of apical periodontitis, promote continued root development, and restore the functional competence of pulpal tissue, particularly from both immunologic and sensory perspectives. These outcomes would very likely increase the long-term probability of retaining the natural dentition. Unfortunately, alternative procedures (eg, implants) are often contraindicated because of the still growing craniofacial skeleton in these young patients.

Treating the Immature Necrotic Tooth by Revascularization or Apexification

Current approaches (eg, replantation) for treating the traumatized immature tooth with pulpal necrosis do not reliably achieve healing of apical periodontitis, continued root development, and reestablishment of pulpal immunologic and sensorial competence. In one study, only 34% of replanted immature permanent teeth (32 of 94) exhibited pulpal healing. Another study reported an 8% revascularization rate (13 of 154) in replanted teeth, with this outcome defined as continued root development and an absence of radiographic signs of apical periodontitis or root resorption. These values are similar to a reported range of pulpal healing of about 4%–15% in a series of 470 replanted teeth reported by various authors. Importantly, the diameter of the apical opening (≥1 mm), extraoral time (<45 minutes), and the arch (mandibular) were all significant predictors for improved revascularization of replanted avulsed teeth. Thus, the classic revascularization procedure of simply replanting an avulsed permanent tooth does not reliably achieve the goals of preventing apical periodontitis, triggering continued root development, and restoring functional competence of the pulp tissue.

An alternative approach for treating the immature permanent tooth is apexification procedures. The classic apexification method involves...
Ca(OH)$_2$, which may weaken teeth (9, 10) and is associated with increased risk of cervical fractures (11, 12). A more recent method of apexification involves the use of mineral trioxide aggregate (MTA) as an apical barrier, followed by placing either a root filling or obturating material (13). MTA apexification appears to offer superior advantages over the traditional Ca(OH)$_2$ method (14), reducing the number of treatment appointments, increasing patient compliance, improving rate of healing (15), and reducing the risk of subsequent fracture (12), although other outcomes such as apical barrier formation may be similar between the 2 methods (16). However, it is important to note that apexification by either Ca(OH)$_2$ or MTA completely prevents any further root development in terms of increased radiographic measures of either root length or width (12, 17). Thus, the immature tooth treated by apexification procedures shows healing of apical periodontitis but does not achieve the goals of continued root development or restoration of functional pulp tissue.

**Regeneration of Functional Pulpal Tissue**

An optimal approach for treating the immature permanent tooth with a necrotic pulp would be to regenerative functional pulp tissue. Regenerative endodontics has been defined as “biologically based procedures designed to replace damaged structures, including dentin and root structures, as well as cells of the pulp-dentin complex” (18). As recently observed, the goal of tissue regeneration (eg, formation of new tissue reproducing both the anatomy and function of the original tissue) is distinct from tissue repair (eg, development of a replacement tissue, such as scar tissue, without restoration of function) (19, 20). The concept of regenerating pulpal tissue was promulgated by the classic studies of Nygaard-Ostby, who evaluated the effects of evoked bleeding by overinstrumentation of human or dog root canal systems. Unfortunately, histologic analysis revealed tissue repair (eg, fibroblasts, collagen, and sparse vascularity) without histologic evidence of regeneration of the pulp-dentin complex (21–23). Together with findings from the trauma literature regarding the negative impact of infection on revascularization, the studies of Nygaard-Ostby and others (24–26) contributed to a fundamental shift in the focus of endodontic research from the 1970s onward, which was away from a biological regenerative objective and toward a restorative dental philosophy for endodontic treatment, including disinfection and placement of root fillings.

During the period of 1993–2007, several key publications prompted a reemergence of a biological or regenerative approach for endodontic treatment. During this period, several case reports were published in which immature permanent teeth with pulp necrosis were disinfectected, followed by laceration of the apical tissue and placing a coronal restoration. The resulting clinical outcome was a resolution of sinus tracts, pain, and swelling and a dramatic increase in radiographic root length and width, which often occurred 0.5–2 years after treatment (27–31). Since these original case reports, numerous studies have been published reporting varying clinical outcomes after regenerative procedures (12, 30, 32–50). Successful outcomes have been reported after treating immature permanent teeth with pulp necrosis caused by trauma, development defects, or caries. A retrospective analysis by Bose et al (17) (Fig. 1) on 48 regenerative cases reported a significant increase in radiographic root development for both root length and root width as compared with MTA apexification procedures. These findings were recently replicated in an independent patient population (12), which extended the original findings of Bose et al by demonstrating significantly greater tooth survival after regenerative treatment (100%) compared with teeth treated with Ca(OH)$_2$ apexification (77%). Although caution must be applied to these clinical findings because case reports may be biased for reporting positive outcomes (51), the preponderance of publications to date suggest that regenerative endodontic treatment of the immature permanent tooth can lead to healing of apical periodontitis, continued radiographic root development, and improved tooth survival.

There is clearly a need for randomized controlled studies comparing various procedures for treating the immature tooth with pulp necrosis. However, because these studies have not yet been completed, clinicians are forced to use a lower level of clinical evidence that is based on available reports. Alternatively, some investigators have suggested that the lack of randomized controlled studies or long-term

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**Figure 1.** (A) Percentage change in root length from preoperative image to postoperative image, measured from the cementoenamel junction 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. (From Bose R, Numnikoski P, Hargreaves K. A retrospective evaluation of radiographic outcomes in immature teeth with necrotic root canal systems treated with regenerative endodontic procedures. J Endod 2009;35:1343.)

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**Table 1**

<table>
<thead>
<tr>
<th>Treatment</th>
<th>% Change in Root Length</th>
<th>% Change in Dentin Thickness</th>
</tr>
</thead>
<tbody>
<tr>
<td>MTA Control</td>
<td>100%</td>
<td>15%</td>
</tr>
<tr>
<td>NSRCT Control</td>
<td>80%</td>
<td>10%</td>
</tr>
<tr>
<td>Triple Antibiotics</td>
<td>70%</td>
<td>8%</td>
</tr>
<tr>
<td>Ca(OH)$_2$</td>
<td>60%</td>
<td>5%</td>
</tr>
<tr>
<td>Formocresol</td>
<td>50%</td>
<td>3%</td>
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</table>
follow-up should restrict the use of regenerative procedures to only those cases when all other treatments are either not suitable or have failed (44). However, this is not a practical solution. Practicing endodontists must treat patients on the basis of the best available evidence, which in many situations is not a randomized clinical trial with years of follow-up. As examples of this general issue, both traditional surgical and nonsurgical endodontic treatments provide strong benefit in treating apical periodontitis, even though comparatively few randomized controlled trials have been conducted (52, 53). Although we agree with the need for continued clinical studies on regenerative endodontics, the available level of evidence is sufficient to provide patients with this treatment option. Finally, as active clinicians who must make real-life decisions in treating our patients, we respectfully observe that many clinical decision points will never be evaluated by randomized controlled trials (eg, randomizing patients to intravenous bisphosphonates or placebo before apical surgery or randomizing patients to a retained instrument within a root canal system before completion of nonsurgical endodontic treatment), and yet, clinicians are still expected to use their best judgment of available evidence in caring for their patients (54, 55). Indeed, the entire concept of “level of evidence” is to use the best available evidence and not to withhold treatment simply because the level of evidence is not ideal.

**Biological Basis for Regeneration**

A second major contribution during the period of 1993–2007 was the development of the field of tissue engineering (56). Simply put, tissue engineering integrates the fields of biology and engineering into a discipline that is focused on tissue regeneration instead of tissue repair. Figure 2 illustrates the core principles of tissue engineering, namely that tissue regeneration requires an appropriate source of stem/progenitor cells, growth factors, and scaffolds to control the development of the targeted tissue.

The first element of tissue engineering is a source of cells capable of differentiating into the desired tissue component. Figure 3 is based on an elegant review by Egusa et al (57) and summarizes several stem cell types available in the oral and craniofacial region. Detailed reviews are available that summarize the properties of these orofacial stem cells (57–62). Interestingly, stem cells are found in dental pulp (63, 64), the apical papilla (59, 60), and even the inflamed periapical tissue collected during endodontic surgical procedures (inflamed periapical progenitor cells) (65). These findings suggest an opportunity for harvesting stem cells during clinical procedures. Indeed, the evoked bleeding during endodontic regenerative procedures conducted on immature teeth with pulp necrosis reveals a massive influx of mesenchymal stem cells into the root canal space (66). As shown in Figure 4, laceration of the apical papilla in patients triggers an inflow of blood into the root canal space that has a 400-fold to 600-fold greater concentration of mesenchymal stem cell markers (CD73 and CD105) as compared with concentrations of these cells circulating in the patient’s systemic blood. Thus, several local sources of stem cells are available for clinical dental procedures, and stem cells can be delivered into the root canal system of patients.

The second element of tissue engineering focuses on growth factors or other tissue-inducing mediators. Stem cells have the capacity to differentiate into a number of cell phenotypes depending on their lineage and exposure to environmental stimuli such as growth factors, extracellular matrix, hypoxia, or other conditions (65, 67–73). Thus, the environment is a critical factor in regulating tissue differentiation. For example, Figure 5 is taken from the study of Wei et al (67) and shows that exposure of the same population of dental pulp cells to 3 different combinations of growth factors results in cells that express a mineralizing phenotype (Fig. 5D), or a cartilage-like phenotype (Fig. 5E and F). These general findings have been repeated in numerous studies on orofacial stem cells and represent a distinctive property of stemness. Thus, the mere step of lacerating the apical papilla and delivering a high local concentration of stem cells into the root canal space may not be sufficient to guide their differentiation into cells of the pulp-dentin complex. Instead, growth factors should be considered as important adjuncts. This is an important concept to remember when interpreting histologic studies after regenerative procedures.

The third element of tissue engineering is a scaffold. A scaffold is much more important than simply forming a three-dimensional tissue structure. In addition, scaffolds play a key role in regulating stem cell differentiation by local release of growth factors or by the signaling cascade triggered when stem cells bind to the extracellular matrix and to each other in a three-dimensional environment (72, 74–76). Scaffolds may be endogenous (eg, collagen, dentin) or synthetic.
substances (eg, hydrogels, MTA, or other compounds) (77, 78). This principle may play a very important role in interpreting clinical regenerative studies. For example, instrumentation of dentin cylinders that was followed by irrigation with 5.25% NaOCl and extensive washing led to a dentin surface that promoted differentiation of cells into calcified-like cells capable of resorbing dentin (71). In contrast, irrigation of dentin cylinders with 17% EDTA either alone or after NaOCl treatment produced a dentin surface that promoted cell differentiation into cells expressing an appropriate marker for a mineralizing phenotype (eg, dentin sialoprotein) (71). Accordingly, the selection of irrigants and their sequence (EDTA last) may play critical roles in conditioning dentin into a surface capable of supporting differentiation of a desired cell phenotype.

Some controversy exists over the terms regeneration versus revascularization (79, 80). The term revascularization emerged from the trauma literature and the observation that pulp in teeth with transient or permanent ischemia, in certain cases, could have reestablishment of its blood supply. These studies laid the foundational knowledge of factors important for revascularization to occur, notably the evidence that teeth with immature roots and open apices had increased rates of revascularization and continued root development. Although these important findings have a significant influence in contemporary regenerative endodontic procedures, they do not include the intentional use of bioengineering principles in achieving repair and regeneration of a missing dental pulp. Instead, contemporary regenerative endodontic procedures consider the presence of an enriched source of stem cells within the apical papilla, their delivery into root canal systems, and the intentional release and use of local growth factors embedded into the dentin. Thus, contemporary regenerative endodontics departs from its origins that are based on the trauma literature and embarks in the field of bioengineering.

From our perspective, regeneration indicates an overall goal of reproducing the original tissue histology and function. To date, the approach that appears to offer the greatest opportunity for regeneration is tissue engineering. Because high concentrations of stem cells are delivered into the root canal space when lacerating the apical papilla in the immature permanent tooth (66), this clinical procedure accomplishes one key component of the triad of tissue engineering (Fig. 2). Ongoing research, much of it preclinical, has evaluated combinations of stem cells, growth factors, and scaffolds that lead to histologic regeneration of pulp tissues that fulfill many of the criteria for a pulp-dentin complex (81–86). In contrast, the concept of revascularization focuses only on the delivery of blood into the root canal space as a means of prompting wound healing, similar to healing after extraction of a tooth (21). In addition, revascularization is a term better used for the reestablishment of the vascularity of an ischemic tissue, such as the dental pulp of an avulsed tooth. From this perspective, a focus on revascularization would ignore the potential importance of growth factors and scaffolds that are required for histologic recapitulation of the pulp-dentin complex. Although we appreciate that angiogenesis and the establishment of a functional blood supply are a key feature in the maintenance and maturation of a regenerating tissue, it is noteworthy that some of the published cases report positive responses to pulp sensitivity tests such as cold or electric pulp test. This is evidence that a space that was previously vacant (debrided root canal) may become populated with an innervated tissue supported by vascularity. Taken together, the core concepts of tissue engineering (Fig. 2) distinguish a regenerative treatment philosophy from a revascularization philosophy derived from certain trauma cases (which only occur in a low percentage of replanted teeth).

**Overview of Current Literature from a Tissue Engineering Perspective**

From the perspective of the triad of tissue engineering, current clinical regenerative protocols may not achieve histologic regeneration. Current clinical protocols partially meet the triad criteria, because stem cells are delivered into the root canal space (66), dentin and the fibrin clot may serve as scaffolds (42, 69, 71, 87), and certain growth factors are both embedded in dentin (88, 89) and released from platelets during the clotting cascade (90, 91). However, the composition of cells, growth factors, and scaffolds is not controlled and likely to vary with differences in clinical protocol or tooth condition. In addition, an emerging body of evidence indicates that both irritants (71, 92) and medicaments such as the triple antibiotic paste (93) may adversely affect stem cell differentiation or survival. Thus, current clinical protocols are largely tailored toward the disinfection of root canal systems but fail to incorporate many of the required procedures needed for more complete and organized regeneration of the pulp-dentin complex.

These issues are perhaps best exemplified by preclinical and clinical histology of tissues found in the root canal space after the current clinical protocols. In general, the use of current protocols results in the production of many histologic elements of pulp tissue (eg, fibroblasts, blood vessels, collagen), but other cell types are missing (eg, odontoblasts), and nontargeted cell types or tissues may be present (eg, osteoblasts, cementum) (42, 94–100). In contrast, preclinical studies that deliver specific growth factors, scaffolds, and stem cells into the root canal space have demonstrated the histologic regeneration of pulp tissues that fulfill nearly all the criteria for a pulp-dentin complex,

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**Figure 4.** Evoked-bleeding step in endodontic regenerative procedures in immature teeth with open apices leads to significant increase in expression of undifferentiated mesenchymal stem cell markers in the root canal space. Systemic blood, saline irrigation, and intracanal blood samples were collected during second visit of regenerative procedures. Real-time reverse transcriptase–polymerase chain reaction was performed by using RNA isolated from each sample as template, with validated specific primers for target genes and 18S ribosomal RNA endogenous control. Expression of mesenchymal stem cell markers CD73 and CD105 was up-regulated after the evoked-bleeding step in regenerative procedures. (From Lovelace TW, Henry MA, Hargreaves KM, Diogenes A. Evaluation of the delivery of mesenchymal stem cells into the root canal space of necrotic immature teeth after clinical regenerative endodontic procedure. J Endod 2011;37:133.)
including production of cells with an odontoblast-like phenotype (81–86). Thus, continued translational research is needed to evaluate the effects of delivery of specific growth factors and scaffolds to determine whether these elements impact histologic regeneration of the pulp-dentin complex in patients.

The second outcome from a regeneration perspective is a functional or clinical success. The available clinical reports are consistent with the notion that current clinical regenerative protocols achieve many aspects of successful clinical outcomes. For example, the vast majority of published cases report closure of sinus tracts (if present), resolution of pain, healing of apical periodontitis, increased radiographic development of root length and width, and overall tooth survival (12, 27–50). Although this clinical evidence is at the level of case reports/case series, they are based on actual outcomes reported by clinicians in treating real-life patients. Thus, there is good evidence for functional outcomes that are clinically meaningful.

Understanding the distinction between clinically meaningful functional outcomes and histologic outcomes is important. This general issue applies to all aspects of clinical endodontics. For example, several histologic studies have been conducted with human material after nonsurgical endodontic treatment (NSRCT). In general, studies have reported the presence of histologic signs of periapical inflammation even in teeth with radiographically normal periapical tissues. Although the presence of inflammatory tissue has been reported in about 94% of cases by using a historical treatment method (101), more contemporary studies report histologic signs of periapical inflammation in 26%–32% of cases without radiographic signs of apical periodontitis (102, 103). These histologic studies are consistent with a dichotomy between clinical measures of success and actual tissue histology. Because treatment success is primarily based on clinical, and not histologic, outcomes for evaluating contemporary NSRCT treatments, it seems reasonable that the more important factors in evaluating treatment success in regenerative endodontics are functional measures of clinical outcome.

From this perspective, we have conducted a retrospective analysis of published cases on regenerative treatment of immature teeth with pulp necrosis. A PubMed search was conducted in September 2012 by using the search terms (revascularization or regeneration) AND (endodontic or "root canal"). The resulting 1044 hits were then analyzed by title and abstract, and the results of the 29 accepted studies

**Figure 5.** Multilineage differentiation capacity of dental pulp cells (DPCs). DPCs cultured in alpha-modified Eagle medium with 15% fetal bovine serum (control medium) are shown in (A) (original magnification, ×40). Odontogenic differentiation was shown by deposition of a mineralized matrix indicated by von Kossa stain shown in (B) (original magnification, ×100) and by positive immunostaining of dental sialoprotein shown in (C) (original magnification, ×200). Adipogenic differentiation was shown by accumulation of neutral lipid vacuoles stainable with oil red O shown in (D) (original magnification, ×100). Chondrogenic differentiation was shown by the secretion of cartilage-specific proteoglycans stainable with alcian blue shown in (E) (original magnification, ×400) and by positive immunostaining of collagen type II shown in (F) (original magnification, ×400). These results were representative of 3–4 independent experiments. (From Wei X, Ling J, Wu L, Liu L, Xiao Y. Expression of mineralization markers in dental pulp cells. J Endod 2007;33:703.)
<table>
<thead>
<tr>
<th>Authors</th>
<th>Reference no.</th>
<th>Sample size</th>
<th>Etiology</th>
<th>Diagnosis</th>
<th>Clinical outcomes</th>
<th>Post-treatment vitality (sensitivity) responses</th>
</tr>
</thead>
<tbody>
<tr>
<td>Nygaard-Ostby, 1961</td>
<td>(21)</td>
<td>N = 17</td>
<td>N/A</td>
<td>Vital pulp (n = ?); necrotic pulp with apical periodontitis (n = ?)</td>
<td>Histologic outcome: healing of the periodontal tissue damaged by overinstrumentation occurred very quickly in as little as 35 days. In addition, all cases showed ingrowth of connective tissue into the canal space, with fiber bundles running parallel to the canal wall or inserting into the newly formed intracanal mineralized tissue (identified as cementum). The tissue resembles fibrous connective tissue with numerous capillaries and &quot;undifferentiated mesenchymal elements&quot; near the capillaries.</td>
<td>N/A</td>
</tr>
<tr>
<td>Rule and Winter, 1966</td>
<td>(26)</td>
<td>n = 5</td>
<td>Trauma (n = 3); dens invaginatus (n = 1); not disclosed (n = 1)</td>
<td>Necrotic pulp (n = 5) with acute apical abscess (n = 2); chronic apical abscess (n = 2) or symptomatic apical periodontitis (n = 1)</td>
<td>Resolution of signs and symptoms of pathosis, including periapical radiolucencies. In addition, all cases showed continued root development and/or apical closure (6 months–1 year)</td>
<td>N/A</td>
</tr>
<tr>
<td>Nygaard-Ostby and Hjortdal, 1971</td>
<td>(22)</td>
<td>n = 47</td>
<td>N/A</td>
<td>Vital pulp (n = 35); necrotic pulp (n = 12)</td>
<td>Histologic outcome: there was evidence of a vascularized fibrous connective, with often deposition of cellular cementum along the root canal walls in teeth previously diagnosed as having a vital pulp, whereas no repair was seen in teeth previously diagnosed with a necrotic pulp (9 days–3 years follow-up).</td>
<td>N/A</td>
</tr>
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<td>Iwaya et al, 2001</td>
<td>(27)</td>
<td>n = 1</td>
<td>Dens evaginatus (tooth #29)</td>
<td>Necrotic pulp and chronic apical abscess</td>
<td>Complete resolution of signs and symptoms of pathosis. Initial apical closure observed at the 5-month recall visit, with complete closure and evidence of root development at 30-month recall visit.</td>
<td>Yes</td>
</tr>
<tr>
<td>Banchs and Trope, 2004</td>
<td>(28)</td>
<td>n = 1</td>
<td>Dens evaginatus (tooth #29)</td>
<td>Necrotic pulp and chronic apical abscess</td>
<td>Periradicular lesion healed (6-month recall); continued root development (12-month recall); tooth development, apical closure, and cold response (2-year follow-up)</td>
<td>Yes</td>
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<tbody>
<tr>
<td>Thibodeau and Trope, 2007</td>
<td>(93)</td>
<td>n = 1</td>
<td>Trauma (complicated crown fracture, tooth #9)</td>
<td>Necrotic pulp and acute apical abscess</td>
<td>Patient was asymptomatic with evidence of continued root development and apical closure and partial canal obliteration (12.5-month follow-up)</td>
<td>No</td>
</tr>
<tr>
<td>Shah et al, 2008</td>
<td>(38)</td>
<td>n = 14</td>
<td>Trauma, n = 10 (complicated coronal fractures, n = 9, and lateral luxation, n = 1); not disclosed (n = 4)</td>
<td>Pulp necrosis (n = 14) with chronic apical abscess (n = 4), acute apical abscess (n = 5), or apical periodontitis (n = 6)</td>
<td>Periapical resolution of radiolucencies (93% or 13/14). Root development (21.4% or 3/14) with evident thickening if lateral dentinal walls (57% or 8/14) and resolution of clinical signs and symptoms (78% or 11/14).</td>
<td>N/A</td>
</tr>
<tr>
<td>Cotti et al, 2008</td>
<td>(33)</td>
<td>n = 1</td>
<td>Trauma (complicated crown fracture)</td>
<td>Pulp necrosis with chronic apical abscess</td>
<td>Resolution of signs and symptoms of pathosis, including sinus tract and associated radiolucency; continued root development (3- to 30-month follow-up).</td>
<td>No</td>
</tr>
<tr>
<td>Jung et al, 2008</td>
<td>(45)</td>
<td>n = 8</td>
<td>Dens evaginatus (tooth #29, n = 2); not disclosed (n = 5); caries (n = 1)</td>
<td>Necrotic pulp (n = 5) or previously initiated therapy (n = 3) with chronic apical abscess (n = 4), acute apical abscess (n = 1), symptomatic apical periodontitis (n = 2), or asymptomatic apical periodontitis (n = 1)</td>
<td>All patients became asymptomatic and demonstrated healing of apical periodontitis (100% or 8/8 at 1- to 3-month recall). In addition, root development was evident in 75% of the cases (6/8 at 10-month to 5-year recall).</td>
<td>N/A</td>
</tr>
<tr>
<td>Reynolds et al, 2009</td>
<td>(46)</td>
<td>n = 2</td>
<td>Dens evaginatus (teeth #20 and #29)</td>
<td>Necrotic pulp and chronic apical abscess</td>
<td>Both teeth remained asymptomatic, with demonstration of periradicular healing and continued root development with apical closure. Both teeth responded to EPT. In addition, tooth #29 did not show discoloration often seen with TAP treatment caused by the sealing of dentinal tubules with a bonding material.</td>
<td>Yes</td>
</tr>
<tr>
<td>Chueh et al, 2009</td>
<td>(32)</td>
<td>n = 23</td>
<td>Dens evaginatus (n = 22); trauma (n = 1, complicated crown fracture); caries (n = 1)</td>
<td>Pulp necrosis (n = 18), previously initiated therapy (n = 5) with acute apical abscess (n = 8), chronic apical abscess (n = 8), symptomatic apical periodontitis (n = 3) or asymptomatic apical periodontitis (n = 4)</td>
<td>There was evidence of resolution of apical periodontitis with resolution of radiolucencies and continued root development (100% of cases, n = 23, 3- to 29-month follow-up)</td>
<td>N/A</td>
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<tbody>
<tr>
<td>Shin et al, 2009</td>
<td>(40)</td>
<td>n = 1</td>
<td>Caries (tooth #29)</td>
<td>Pulp necrosis and chronic apical abscess</td>
<td>There was evidence of periradicular lesion healing (7-month recall). Also, there was thickening of the dentinal walls and continued root development (13- to 19-month follow-up).</td>
<td>No</td>
</tr>
<tr>
<td>Ding et al, 2009</td>
<td>(47)</td>
<td>n = 3</td>
<td>Dens invaginatus (tooth #29); caries (tooth #29); trauma (complicated crown fracture, tooth #9)</td>
<td>Pulp necrosis (n = 3) with acute apical abscess (n = 2) and undisclosed periradicular status (n = 1)</td>
<td>There was evidence of thickening of the dentinal walls and closure of apex (15- to 18-month follow-up).</td>
<td>Yes</td>
</tr>
<tr>
<td>Thomson and Kahler, 2010</td>
<td>(41)</td>
<td>n = 1</td>
<td>Dens evaginatus (tooth #20)</td>
<td>Pulp necrosis with chronic apical abscess</td>
<td>There was resolution of signs and symptoms of pathosis, including closure of sinus tract; evidence of root development and positive response to EPT testing (18-month follow-up)</td>
<td>Yes</td>
</tr>
<tr>
<td>Petrino et al, 2010</td>
<td>(37)</td>
<td>n = 6</td>
<td>Trauma (complicated crown fracture, n = 2, avulsion, n = 2); caries (n = 2)</td>
<td>Pulp necrosis (n = 6), with asymptomatic apical periodontitis (n = 4) or chronic apical abscess (n = 2)</td>
<td>There was evidence of periradicular healing with resolution of signs and symptoms of pathosis (100% of cases, n = 6). In addition, continued root development was seen in 3 cases (50% of cases), whereas response to pulpal sensitivity testing was seen in 2 cases (33.3% of cases).</td>
<td>Yes</td>
</tr>
<tr>
<td>Kim et al, 2010</td>
<td>(108)</td>
<td>n = 1</td>
<td>Trauma (uncomplicated crown fracture)</td>
<td>Pulp necrosis and symptomatic apical periodontitis</td>
<td>Tooth was asymptomatic with resolution of periradicular radiolucency. There was evidence of continued root development with apical closure (8-month recall).</td>
<td>N/A</td>
</tr>
<tr>
<td>Nosrat et al, 2011</td>
<td>(36)</td>
<td>n = 2</td>
<td>Trauma (n = 2) (complicated crown fracture, tooth #8, and uncomplicated coronal fracture, tooth #9)</td>
<td>Pulp necrosis and symptomatic apical periodontitis</td>
<td>Tooth was asymptomatic and apices had closed, but root development was not evident (6-year follow-up). In addition, teeth were not responsive to sensitivity testing.</td>
<td>No</td>
</tr>
<tr>
<td>Nosrat et al, 2011</td>
<td>(36)</td>
<td>n = 2</td>
<td>Caries</td>
<td>Necrotic pulp with symptomatic apical periodontitis</td>
<td>Teeth were asymptomatic, had healed periradicular lesions, and showed thickening of the dentinal walls (3- to 18-month follow-up).</td>
<td>N/A</td>
</tr>
<tr>
<td>Jung et al, 2011</td>
<td>(107)</td>
<td>n = 1</td>
<td>Unknown</td>
<td>Previous initiated therapy with chronic apical abscess</td>
<td>Tooth was asymptomatic with resolution of periradicular lesion and sinus tract. There was evident development of the apical 1/3 that was found to be detached from the main root.</td>
<td>N/A</td>
</tr>
</tbody>
</table>
### TABLE 1. (Continued)

<table>
<thead>
<tr>
<th>Authors</th>
<th>Reference no.</th>
<th>Sample size</th>
<th>Etiology</th>
<th>Diagnosis</th>
<th>Clinical outcomes</th>
<th>Post-treatment vitality (sensitivity) responses</th>
</tr>
</thead>
<tbody>
<tr>
<td>Cehreli et al, 2011</td>
<td>(49)</td>
<td>n = 6</td>
<td>Caries (n = 6)</td>
<td>Pulp necrosis with symptomatic apical periodontitis</td>
<td>There was average of 7.7% increase in root length and 26.5% root width increase. In addition, 33.3% (n = 2) responded positively to sensitivity testing (12-month follow-up).</td>
<td>Yes</td>
</tr>
<tr>
<td>Torabinejad and Turman, 2011</td>
<td>(43)</td>
<td>n = 1</td>
<td>Accidental extraction</td>
<td>N/A</td>
<td>There was evidence of resolution of apical lesion and symptoms, further root development, and apical closure (5½-month recall).</td>
<td>No</td>
</tr>
<tr>
<td>Kootor and Velmurugan, 2012</td>
<td>(103)</td>
<td>n = 1</td>
<td>Trauma (complicated crown fracture, tooth #7)</td>
<td>Pulp necrosis and symptomatic apical periodontitis</td>
<td>Tooth was asymptomatic (3-month recall); evidence of continued root development and apical closure (1-year follow-up); further development was evident, but no positive responses to sensitivity tests were observed (3- and 5-year follow-up).</td>
<td>No</td>
</tr>
<tr>
<td>Jeeruphan et al, 2012</td>
<td>(12)</td>
<td>n = 20</td>
<td>Trauma (n = 7); dens evaginatus (n = 12); caries (n = 1)</td>
<td>Pulp necrosis (n = 20) with symptomatic apical periodontitis (n = 17) and asymptomatic apical periodontitis (n = 3)</td>
<td>There was resolution healing of apical periodontitis and detection of 28.2% increase in root width, 14.9% increase in root length, and 100% survival rate. On the other hand, teeth treated with Ca(OH)2 or MTA apexification procedures showed no root development and a survival of 77.2% and 95%, respectively.</td>
<td>N/A</td>
</tr>
<tr>
<td>Shimizu et al, 2012</td>
<td>(39)</td>
<td>n = 1</td>
<td>Trauma, tooth #9 (complicated crown fracture).</td>
<td>Symptomatic irreversible pulpitis with normal periradicular tissues</td>
<td>Histologic evaluation of the extracted tooth revealed a connective tissue that resembled pulp, with flattened cells surrounding the dentinal wall resembling odontoblasts. Also, cells of the Hertwig’s epithelial root sheath could be seen surrounding the apical tissues.</td>
<td>N/A</td>
</tr>
<tr>
<td>Narayana et al, 2012</td>
<td>(104)</td>
<td>n = 1</td>
<td>Dens invaginatus (tooth #7)</td>
<td>Pulp necrosis with symptomatic apical periodontitis</td>
<td>Healing of periradicular lesion was detected (5-month follow-up), as well as formation of a dentinal bridge (12-month follow-up). No evident change in length or thickness was seen (12-month follow-up).</td>
<td>No</td>
</tr>
</tbody>
</table>

(Continued)
## Table 1. (Continued)

<table>
<thead>
<tr>
<th>Authors</th>
<th>Reference no.</th>
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<th>Clinical outcomes</th>
<th>Post-treatment vitality (sensitivity) responses</th>
</tr>
</thead>
<tbody>
<tr>
<td>Aggarwal et al, 2012</td>
<td>(105)</td>
<td>n = 1</td>
<td>Trauma (teeth #8 and #9)</td>
<td>Pulp necrosis and chronic apical abscess (teeth #8 and #9)</td>
<td>Periapical healing, appreciable dentinal wall thickening, and apical closure were detected when comparing tooth #9 (regenerative treatment) with tooth #8 (apexification treatment) (2-year follow-up).</td>
<td>No</td>
</tr>
<tr>
<td>Miller et al, 2012</td>
<td>(106)</td>
<td>n = 1</td>
<td>Trauma (avulsion); avulsed tooth was kept in cold milk for 4.5 h before replantation</td>
<td>Asymptomatic irreversible pulpitis and asymptomatic apical periodontitis</td>
<td>There was arrestment of the resorption process, periradicular healing, and positive response to pulpal sensitivity tests (1- to 3-month follow-up). There was evident root development and apical closure, while remaining asymptomatic without any other signs or symptoms of pathosis (18-month follow-up).</td>
<td>Yes</td>
</tr>
<tr>
<td>Cehreli et al, 2012</td>
<td>(107)</td>
<td>n = 1</td>
<td>Trauma (extrusive luxation injury)</td>
<td>N/A</td>
<td>There was periapical healing and evidence of continued root development (3 months). The tooth responded positively to cold tests (12-month follow-up) and EPT (18-month follow-up).</td>
<td>Yes</td>
</tr>
<tr>
<td>Chen et al, 2012</td>
<td>(50)</td>
<td>n = 20</td>
<td>Caries (n = 3); dens evaginatus (n = 7); trauma (n = 10)</td>
<td>Pulp necrosis (n = 20) with chronic apical abscess (n = 11); acute apical abscess (n = 5) and asymptomatic apical periodontitis (n = 4)</td>
<td>There was evidence of periradicular healing with resolution of signs and symptoms of pathosis (100% of cases, n = 20). There was evidence of continued root development at the follow-up visits in 15 cases (75%), whereas in 5 cases there was no further root development, but apical closure was evident (25%).</td>
<td>N/A</td>
</tr>
</tbody>
</table>

N/A, information not available; TAP, triple antibiotic paste.

Sample sizes listed in the table denote only the number of teeth treated with revascularization/regeneration protocols.
describing regenerative treatment are summarized in Table 1. The etiology of the pulp and periapical disease of these published cases is composed of dens evaginatus (39.7%), trauma (35.5%), caries (14%), dens invaginatus (1.6%), and undisclosed-unknown factors (9.2%). Thus, about one-third of the published studies on regenerative endodontic treatment involve treatment of trauma. A common feature of the published case reports is the rapid resolution of apical periodontitis and other signs and symptoms of pathosis such as sinus tracts and swelling (104–109). In addition, clinically evident root development and/or apical closure were reported in most cases. Thus, regenerative endodontic protocols have been used successfully in clinical cases of varied etiology, including a significant number of trauma cases.

In San Antonio, our residents routinely provide regenerative treatment to immature permanent teeth with pulp necrosis. The following describes a case with pulp necrosis caused by trauma. A 9-year-old male patient came to our graduate clinic for evaluation and treatment of tooth #8. The child had a traumatic injury to his maxilla, which caused an avulsion of tooth #6 and lateral luxation of teeth #7, #8, and #9. Tooth #8 had uncomplicated crown fracture with loss of the incisal edge. According to the patient’s mother, the general dentist repositioned and splinted the permanent teeth. However, they never persevered further treatment. Several months after treatment by the general dentist, the patient was taken by his mother to an emergency department at a local hospital because he woke up with severe facial swelling on the right side extending up around his eye. Emergency treatment was then provided with incision and drainage above tooth #8, and the patient was placed on oral clindamycin. The patient presented to our clinic 2 days after the acute infection, with moderate to severe pain still present and significant periorbital swelling and swelling of the upper right lip with loss of labial fold. Purulence drainage was clinically observed from the sulcus between teeth #8 and #9. It was not possible to perform pulpal responsivity tests because of the severe pain and inflammation in the area. A decision was made to treat tooth #8 because it was the most obvious etiology from both clinical and radiographic examination. The patient’s mother was informed that we would thoroughly examine teeth #7 and #9 once the acute infection subsided. Radiographic findings (Fig. 6A–D) indicated that teeth #7, #8, and #9 had open apices, and tooth #8 had apical periodontitis. Clinically, all teeth were only partially erupted. Tooth #8 had a diagnosis of pulp necrosis with acute apical abscess. Pulpal regeneration of tooth #8 was recommended. After reviewing the risks, benefits, and treatment options with the patient and his parent, an informed consent and consent were obtained for performing the recommended procedure on tooth #8. A topical anesthetic was applied to the mucosa before anesthetizing the tooth with 2% lidocaine with 1:100,000 epinephrine, and the tooth was anesthetized with 2% lidocaine with 1:100,000 epinephrine, and a rubber dam was placed. The temporary was removed, and the canal was irrigated with 17% EDTA under microscopic view. The canal was then dried with paper points, and intentional bleeding was induced. A Colla-tape (Zimmer Dental, Carlsbad, CA) matrix was placed 4–5 mm below the cementoenamel junction level, and an MTA (Pro-Root MTA; Dentsply Tulsa Dental Specialties, Tulsa, OK) barrier was placed. The access was then sealed with glass ionomer (Fuji II; GC America, Alsip, IL) and Built-It light cure—core material (Pentron, Orange, CA). The patient’s parent was informed that follow-up visits would be necessary at 1, 3, 6, and 12 months. Patient was then referred to the postgraduate pediatric clinic for restoration of incisal edge fracture. Tooth #8 was restored with Z100 (3M Espe Dental Products) composite restoration; the occlusion was checked, and postoperative instructions were provided (Fig. 6E).

At the 1-month follow-up visit, tooth #8 and an evaluation of pulpal and periradicular status of teeth #7, #9, and #10 were conducted (Fig. 6F). The patient’s mother reported that her son had not had any discomfort or history of pain since his last visit. Tooth #8 did not respond to Endo Ice or an electric pulp tester (EPT). No discoloration was observed on tooth #8. All the other teeth did not respond to cold, but they were positive to EPT. No mobility was observed, and probing measurements were less than 3 mm on all teeth. A 6-month follow-up visit was recommended.

At the 6-month follow-up visit, the patient’s mother reported that the patient had been asymptomatic since the last evaluation. No changes on medical history were recorded. All teeth tested (#7 through #10) did not respond to cold testing but were positive to EPT. No mobility was observed, and probing depths were within normal limits. Tooth #8 was tested several times with cold and EPT, with variations to test the patient’s reliability, and the patient correctly reported positive response to EPT all times. Tooth #8 showed continued root development with a substantial gain in length and wall thickness (Fig. 6G). It was noted that tooth #8 is continuing to develop at a similar rate compared with tooth #9. The parents were informed of the need for a 1-year follow-up examination.

**Are We There Yet? Considerations of Current Clinical Protocols**

Although this review documents good clinical outcomes in the majority of studies treating the immature permanent tooth, there are several caveats. First, no randomized controlled trials have been conducted, and therefore, clinicians must rely on lower levels of clinical evidence. This is similar to numerous other examples of clinical decision points (eg, selection of instrumentation methods, irrigation methods, obturation methods, antibiotics) where clinicians must rely on the best available evidence, which in some cases ranges to the clinician’s expert opinion that is based on preclinical studies. Second, the strongest evidence for success after regenerative treatment is based...
on clinical outcomes (eg, healing of apical periodontitis, continued radiographic root development). Third, current clinical protocols do not fully enact the triad of tissue engineering, and preclinical studies indicate that the addition of specific growth factors and scaffolds is necessary for anatomic regeneration of pulp tissue. Fourth, the etiology of pulp necrosis may be an important factor in dictating clinical outcomes after regenerative procedures. For example, a traumatic injury that disrupts Hertwig’s epithelial root or the apical papilla may result in aberrant root development (19, 110). Fifth, continued translational research is needed to continue to improve this method. This last caveat is the same as that across the entire field of dentistry; new methods must be constantly introduced and refined to allow clinicians to deliver better treatment for our patients.

**Acknowledgments**

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**References**

Injuries to Permanent Dentition Symposium


