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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 occurrence of pulpal necrosis in the permanent but immature tooth represents a challenging clinical situation since 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 pulp necrosis.

Keywords

regenerative endodontics; pulpal revascularization; stem cells; trauma; children

Introduction

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 (1).

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 (2). 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, the prevalence of dental trauma was nearly 11% and about 12% of enamel-dentin fractures led to pulpal necrosis (3). In another study of 889 permanent teeth with traumatic injuries, pulpal necrosis occurred in about 27% of the sampled population (4). The risk of developing pulpal necrosis

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is well recognized to be dependent upon 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% (infracture), to 3% (concussion), to 26% (extrusion), to 58% (lateral luxation), to 92% (avulsion), to 94% (intrusion), (5).

The occurrence of pulpal necrosis in the permanent but immature tooth often represents a challenging clinical situation since 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% (6). 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 immunological and sensory perspectives (7). These outcomes would very likely increase the long term probability of retaining the natural dentition. Unfortunately, alternative procedures (eg., implants) are often contraindicated due to 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 immunological and sensorial competency. In one study, only 34% (32 of 94) of replanted immature permanent teeth exhibited pulpal healing (6). 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 (8). 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 (6). Importantly, the diameter of the apical opening (≤ 1 mm), extraoral time (<45 min) and the arch (mandibular) were all significant predictors for improved revascularization of replanted avulsed teeth (8). 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 long term application of 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 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 two 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 demonstrates 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 regenerate functional pulpal 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 (e.g., formation of new tissue reproducing both the anatomy and function of the original tissue) is distinct from tissue repair (e.g., 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 over-instrumentation of human or dog root canal systems. Unfortunately, histological analysis revealed tissue repair (e.g., fibroblasts, collagen, sparse vascularity) without histological 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, away from a biological regenerative objective and towards a restorative dental philosophy for endodontic treatment, including disinfection and placement of root fillings.

Over the period of 1993–2007, several key publications prompted a re-emergence of a biological, or regenerative approach for endodontic treatment. During this period, several case reports were published where immature permanent teeth with pulp necrosis were disinfected followed by laceration of the apical tissue and placing a coronal restoration. The resulting clinical outcome was a resolution of sinus tracts, pain, 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 following regenerative procedures (12, 30, 32–50). Successful outcomes have been reported after treating immature permanent teeth with pulpal necrosis due to trauma, development defects, or caries. A retrospective analysis by Bose et al., (Fig 1) on 48 regenerative cases reported a significant increase in radiographic root development for both root length and root width as compared to MTA apexification procedures (17). These findings were recently replicated in an independent patient population (12), which extended the original findings of Bose and colleagues by demonstrating significantly greater tooth survival after regenerative treatment (100%) compared to teeth treated with Ca(OH)₂ apexification (77%). Although caution must be applied to these clinical findings since 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 pulpal necrosis. However, given that these studies have not yet been completed, clinicians are forced to use a lower level of clinical evidence, based upon available reports. Alternatively, some investigators have suggested that the lack of randomized controlled studies or long term 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 based upon 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 non-surgical 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 i.v. bisphosphonates or placebo prior to apical surgery; or randomizing patients to a retained instrument within a root canal system prior to completion of non-surgical 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 over 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. Fig 2 illustrates the core principles of tissue engineering, namely that tissue regeneration requires an appropriate source of stem/progenitor cells, growth factors and scaffolds in order 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. Fig 3 is based upon an elegant review by Egusa and colleagues (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 (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 pulpal necrosis reveals a massive influx of mesenchymal stem cells into the root canal space (66). As shown in Fig 4, laceration of the apical papilla in patients triggers an inflow of blood into the root canal space that has a 400- to 600-fold greater concentration of mesenchymal stem cell markers (CD73 and CD105) as compared to 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, Fig 5 is taken from the study of Wei and colleagues and demonstrates that exposure of the same population of dental pulp cells to three different combinations of growth factors results in cells that express a mineralizing phenotype (Fig 5B, C), an adipocyte (fat cell) phenotype (Fig 5D) or a cartilage-like phenotype (Fig 5E, 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 histological 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 3-dimensional environment (72, 74–76). Scaffolds may be endogenous (eg., collagen, dentin, etc) or synthetic substances (e.g., 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 followed by irrigation with 5.25%

NaOCl and extensive washing led to a dentin surface that promoted differentiation of cells into clastic-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 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. Since 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 histological 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 employed 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 histological recapitulation of the pulp-dentin complex. Although we appreciate that angiogenesis and the establishment of a functional blood supply is 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 EPT. 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 histological regeneration. Current clinical protocols partially meet the triad criteria, since 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 irrigants (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 towards 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 histological elements of pulp tissue (eg., fibroblasts, blood vessels, collagen), but other cell types are missing (e.g., odontoblasts) and non-targeted 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 histological regeneration of pulp tissues that fulfill nearly all the criteria for a pulp-dentin complex, 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 if these elements impact histological 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 histological outcomes is important. This general issue applies to all aspects of clinical endodontics. For example, several histological studies have been conducted with human material after non-surgical endodontic treatment (NSCRT). 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 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. Since treatment success is primarily based on clinical, and not histological, 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 pulpal necrosis. A PubMed search was conducted in September 2012 using the search terms (revascularization or regeneration) AND (endodontic or “root canal”). The resulting 1,044 hits were then analyzed by title and abstract and the results of the 29 accepted studies describing regenerative treatment are summarized in Table 1. The etiology of the pulpal and periradicular disease of these published cases are comprised of dens evaginatus (39.7%), trauma (35.5%), caries (14%), dens invaginatus (1.6%) or undisclosed-unknown factors (9.2%). Thus, about 1/3 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 was reported in most cases. Thus, regenerative endodontic protocols have been used successfully in clinical cases of varied etiology, including a significant number trauma cases.

In San Antonio, our residents routinely provide regenerative treatment to immature permanent teeth with pulpal necrosis. The following describes a case with pulpal necrosis due to trauma. A 9-year-old male patient was 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 #G 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 persuaded further treatment. Several months after treatment by the general dentist, the patient was taken by his mother to an emergency room 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 placed on oral clindamycin. The patient presented to our clinic two days after the acute infection, with moderate to severe pain still present and significant peri-orbital swelling and swelling of the upper right lip with loss of labial fold. Purulence drainage was clinically observed from the sulcus between #8 and #9. It was not possible to perform pulpal responsiveness tests due to the severe pain and inflammation in the area. A decision was made to treat #8 since it is most obvious etiology from both clinical and radiographic examination. The patient's mother informed that we would thoroughly exam teeth #7 and #9 once the acute infection subsides. 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 pulpal 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 assent 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 via labial and palatal infiltrations. After isolation with a rubber dam, a lingual access cavity was performed by using a Zeiss surgical microscope (Carl Zeiss Meditac Inc, Dublin, CA). Copious purulent and hemorrhagic drainage through the canal was observed upon access. The root canal system was mechanically debrided and copiously irrigated with 1.5% NaOCl followed by 17% EDTA. Several minutes were allowed for canal drainage between subsequent irrigations. The canal was then dried with paper points and calcium hydroxide was placed. The tooth was sealed with Cavit (ESPE, Chergy Pontoise, France).

The patient returned 2 weeks later for continuation of treatment. The patient's parent reported that he had no pain since last visit. The swelling was no longer noted. After applying 20% benzocaine topical anesthetic to the mucosa, 36.0 mg of mepivacaine was used for labial and palatal infiltrations. After isolation with rubber dam, the temporary restorative material was carefully removed. Copious irrigation with 20ml of 1.5% NaOCl followed by 10ml of 17% EDTA was performed. The canal was the dried with paper points. The pulp chamber space was then etched and coated with prime and bond (Optibond FL Prime™, Kerr Corporatino, Orange CA and Adhesive™ Kerr) to diminish possible staining. A thin watery mixture (92) of double antibiotic paste (DAP, Champs Pharmacy) containing metronidazole and ciprofloxacinin in a 1:1 ratio was then placed to below the CEJ level, a sterile sponge was packet in the canal and access cavity sealed with Cavit. A double antibiotic paste without minocycline was selected to reduce the risk of staining (109). The patient was schedule to return between 28 to 30 days to complete treatment.

At the final visit, the patient reported no pain or swelling. He was anesthetized with 2% lidocaine with 1:100,000 epinephrine and a rubber dam was placed. The temporary removed and canal was irrigated with 17% EDTA under microscopic view. The canal was then dried with paper points and intentional bleeding induced. A Colla-tape (Zimmer Dental, Carlsbad, CA, USA) matrix placed 4 to 5 mm below the CEJ 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™) and Built-It™ light cure – core material (Pentron, Orange, CA, USA). The patient's parent was informed that follow-ups visits would necessary at 1, 3, 6 and 12 months. Patient was then referred to Postgraduate Pediatric Clinic for restoration of incisal edge fracture. Tooth #8 was restored with Z100™ (3M Espe Dental Products, USA) composite restoration; the occlusion was checked and post-op instructions 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 his son neither has had any discomfort nor history of pain since 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 3mm on all teeth. The 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 demonstrated 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 to tooth #9. The parents were informed of the need for a one year follow up examination.

Are we there yet? Considerations of current clinical protocols

Although the above 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 (e.g., selection of instrumentation methods, irrigation methods, obturation methods, antibiotics, etc), where clinicians must rely on the best available evidence, which in some cases ranges to the clinician's expert opinion based upon preclinical studies. Second, the strongest evidence for success after regenerative treatment is based upon clinical outcomes (e.g., 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 are necessary for anatomical 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.

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Fig 1.

(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. Reprinted from Bose et al., J Endod 35:1343, 2009 with permission.

Fig 2.
Three main components of tissue engineering

Fig 3.

Schematic drawing illustrating potential sources of post-natal stem cells in the oral environment. Cell types include tooth germ progenitor cells (TGPCs), dental follicle stem cells (DFSCs), salivary gland stem cells (SGSCs), stem cells of the apical papilla (SCAP), dental pulp stem cells (DPSCs), stem cells from human exfoliated deciduous teeth (SHED), periodontal ligament stem cells (PDLSCs), bone marrow stem cells (BMSCs) and, as illustrated in the insert, oral epithelial stem cells (OESCs), and gingival-derived mesenchymal stem cells (GMSCs)

Fig 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 RT-PCR was performed by using RNA isolated from each sample as template, with validated specific primers for target genes and 18S ribosomal RNA endogenous control. (A) Expression of mesenchymal stem cell markers CD73 and CD105 was up-regulated after the evoked-bleeding step in regenerative procedures. Reprinted from Lovelace, et al., *J Endod* 37:133, 2011 with permission.

Fig 5.

Multilineage differentiation capacity of DPCs. DPCs cultured in alpha-MEM with 15% FBS (control medium) was shown in A (original magnification X 40). Odontogenic differentiation was shown by the deposition of a mineralized matrix indicated by von Kossa stain shown in B (original magnification X 100) and by the positive immunostaining of DSP shown in C (original magnification X 200). Adipogenic differentiation was shown by the accumulation of neutral lipid vacuoles stainable with Oil Red O shown in D (original magnification X100). Chondrogenic differentiation was shown by the secretion of cartilage-specific proteoglycans stainable with Alcian blue shown in E (original magnification X400) and by the positive immunostaining of collagen type II shown in F (original magnification

X400). These results were representative of three to four independent experiments.
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Fig 6.

Example of a regenerative protocol applied to an immature permanent tooth with pulpal necrosis due to trauma. A: pre-operative image, B: Pre-operative cone-beam computed tomography (CBCT) 3D view, C: pre-operative CBCT coronal view, D: pre-operative CBCT sagittal view, E: immediate post-operative radiograph, F: one month follow-up radiograph, G: six month follow-up radiograph

Table 1

Current published case reports or case series of revascularization/ regenerative procedures in endodontics. Sample size listed on the table denote only to the number of teeth treated with revascularization/regeneration protocols. N/A= information not available.

Authors	Reference #	Sample Size	Etiology	Diagnosis	Clinical Outcomes	Post-treatment Vitality (Sensitivity) Responses
Nygaard-Ostby, 1961	21	n=17	N/A	Vital pulp (n=7) Necrotic Pulp with Apical Periodontitis (n=7)	Histologic outcome: Healing of the periodontal tissue damaged by over instrumentation occurred very quickly in as little as 35 day. In addition, all cases demonstrated in-growth 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 resembled fibrous connective tissue with numerous capillaries and "undifferentiated mesenchymal elements" near the capillaries.	N/A
Rule and Winter, 1966	26	n=5	Trauma (n=3); Dens invaginatus (n=1); not disclosed (n=1)	Necrotic pulp (n=5) with Acute Apical Abscess (n=2); Chronic Apical Abscess (n=2) or Symptomatic Apical Periodontitis (n=1)	Resolution of signs and symptoms of pathosis, including periapical radiolucencies. In addition, all cases demonstrated demonstrated continued root development and/or apical closure (6 months to 1 year)	N/A
Nygaard-O and Hjortdal, 1971	22	n=47	N/A	Vital pulp (n=35); Necrotic pulp (n=12)	Histological 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 to 3 years follow-up).	N/A
Iwaya et al., 2001	27	n=1	Dens evaginatus (tooth #29)	Necrotic pulp and Chronic Apical Abscess	Complete resolution of signs and symptoms of pathosis. Initial apical closure observed at the 5-months recall visit with complete closure and evidence of root development at 30-months recall visit	Yes
Banchs and Trope, 2004	28	n=1	Dens evaginatus (Tooth #29)	Necrotic pulp and chronic apical abscess	Periapical lesion healed (6 months recall); continued root development (12-month recall); Root development, apical closure and cold response (2 year follow-up)	Yes
Thibodeau and Trope, 2007	93	n=1	Trauma (complicated crown fracture; tooth #9)	Necrotic pulp and acute apical abscess	Pt was asymptomatic with evidence of continued root development and apical closure and partial canal obliteration (12.5 months follow-up)	No
Shah et al., 2008	38	n=14	Trauma [n=10 (complicated coronal fractures, n=9 and lateral luxation, n=1)]; not disclosed (n=4)	Pulp Necrosis (n=14) with Apical Chronic Apical Abscess (n=4) or Acute Apical Abscess (n=5) or Apical Periodontitis (n=6)	Periapical resolution of radiolucencies (93% or 13/14). Root development (21.4% or 3/14) with evident thickening of lateral dentinal walls (57% or 8/14) and resolution of clinical signs and symptoms (78% or 11/14).	N/A

Authors	Reference #	Sample Size	Etiology	Diagnosis	Clinical Outcomes	Post-treatment Vitality (Sensitivity) Responses
Cotti et al., 2008	33	n=1	Trauma (complicated crown fracture)	Pulp Necrosis with Chronic Apical Abscess	Resolution of signs and symptoms of pathosis, including sinus tract and associated radiolucency; continued root development (3–30 months follow-up).	No
Jung et al., 2008	45	n=8	Dens Evaginatus (tooth #29; n=2); Not disclosed (n=5); Caries (n=1)	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)	All patients became asymptomatic and demonstrated healing of apical periodontitis (100% or 8/8 at 1 to 3 months recall). In addition, root development was evident in 75% of the cases (6/8 at 10 months –5 years recall).	N/A
Reynolds et al., 2009	46	n=2	Dens evaginatus (tooth #20 and #29)	Necrotic pulp and Chronic Apical Abscess	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 demonstrate discoloration often seen with TAP treatment due the sealing of dentinal tubules with a bonding material.	Yes
Chueh et al., 2009	32	n=23	Dens evaginatus (n=22); Trauma (n=1, complicated crown fracture) and Caries (n=1)	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).	There was evidence of resolution of apical periodontitis with resolution of radiolucencies and continued root development (100% of cases; n=23; 3–29 months follow-up)	N/A
Shin et al., 2009	40	n=1	Caries (tooth #29)	Pulp Necrosis and Chronic Apical Abscess	There was evidence of periradicular lesion healing (7 months recall). Also, there was thickening of the dentinal walls and continued root development (13–19 months follow-up).	No
Ding et al., 2009	47	n=3	Dens invaginatus (tooth #29); Caries (Tooth #29); Trauma (complicated crown fracture; tooth #9)	Pulp Necrosis (n=3) with acute apical abscess (n=2) and undischarged periradicular status (n=1)	There was evidence of thickening of the dentinal walls, closure of apex (15–18 months follow-up).	Yes
Thomson and Kahler., 2010	41	n=1	Dens evaginatus (tooth #20)	Pulp Necrosis with Chronic Apical Abscess	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 months follow-up)	Yes
Petrino et al., 2010	37	n=6	Trauma (complicated crown fracture n=2, avulsion n=2); Caries (n=2)	Pulp Necrosis (n=6), with Asymptomatic Apical Periodontitis (n=4) or Chronic Apical Abscess (n=2).	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),	Yes

Authors	Reference #	Sample Size	Etiology	Diagnosis	Clinical Outcomes	Post-treatment Vitality (Sensitivity) Responses
					whereas response to pulpal sensitivity testing was seen in 2 cases (33.3% of cases).	
Kim et al., 2010	108	n=1	Trauma (uncomplicated crown fracture)	Pulp Necrosis and Symptomatic Apical Periodontitis	Tooth was asymptomatic with resolution of periradicular radiolucency. There was evidence of continued root development with apical closure (8 months recall)	N/A
Nosrat et al., 2011	36	n=2	Trauma (n=2) (complicated crown fracture tooth #8, and uncomplicated coronal fracture tooth #9)	Pulp Necrosis and Symptomatic Apical Periodontitis	Tooth was asymptomatic and apices had closed, but root development was not evident (6 years follow-up). In addition, teeth were not responsive to sensitivity testing.	No
Nosrat et al., 2011	36	n=2	Caries	Necrotic pulp with Symptomatic Apical Periodontitis	Teeth were asymptomatic, had healed periradicular lesions and demonstrated thickening of the dentinal walls (3–18 months follow-up).	N/A
Jung et al., 2011	107	n=1	Unknown	Previous Initiated Therapy with Chronic Apical Abscess	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.	N/A
Cehreli et al., 2011	49	n=6	Caries (n=6)	Pulp Necrosis with Symptomatic Apical Periodontitis	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 months follow-up).	Yes
Torabinejad and Turman, 2011	43	n=1	Accidental extraction	N/A	There was evidence of resolution of apical lesion and symptoms, further root development and apical closure (5 1/2 month recall).	No
Kootor and Velmurugan, 2012	103	n=1	Trauma (complicated crown Fracture; tooth #7)	Pulp Necrosis and Symptomatic Apical Periodontitis	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 response to sensitivity tests were observed (3 and 5 years follow-up).	No
Jeeruphan et al., 2012	12	n=20	Trauma (n=7); Dens evaginatus (n=12); Caries (n=1)	Pulp Necrosis (n=20) with Symptomatic Apical Periodontitis (n=17) and Asymptomatic apical periodontitis (n=3)	There was resolution healing of apical periodontitis and detection of a 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 demonstrated no root development and a survival of 77.2% and 95%, respectively.	N/A
Shimizu et al., 2012	39	n=1	Trauma #9 (complicated crown fracture).	Symptomatic Irreversible Pulpitis with Normal Periradicular Tissues	Histological 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.	N/A

Authors	Reference #	Sample Size	Etiology	Diagnosis	Clinical Outcomes	Post-treatment Vitality (Sensitivity) Responses
Narayana et al., 2012	104	n=1	Dens invaginatus (tooth #7)	Pulp necrosis with Symptomatic Apical Periodontitis	Healing of periradicular lesion was detected (5 months follow-up), as well as formation of a dentinal bridge (12 months follow-up). No evident change in length or thickness was seen (12 months follow-up).	No
Aggarwal et al., 2012	105	n=1	Trauma (tooth #8 and #9)	Pulp necrosis and Chronic Apical Abscess (Tooth #8 and #9)	Periapical healing, appreciable dentinal wall thickening and apical closure was detected when comparing tooth #9 (regenerative treatment) to tooth #8 (apexification treated) (2 year follow-up)	No
Miller et al., 2012	106	n=1	Trauma (avulsion). Avulsed tooth was kept in cold milk for 4.5 hrs prior to replantation.	Asymptomatic irreversible pulpitis and asymptomatic apical periodontitis	There was arrestment of the resorption process, periradicular healing and positive response to pulpal sensitivity tests (1-3 month follow-up). There was evident root development and apical closure while remaining asymptomatic without any other signs or symptoms of pathosis (18 months follow-up).	YES
Cehreli et al., 2012	107	n=1	Trauma (extrusive luxation injury)	N/A	There was periapical healing and evidence of continued root development (3 months). The tooth responded positively to cold tests (12 months follow-up) and EPT (18 months follow-up).	YES
Chen et al., 2012	50	n=20	Caries (n=3); Dens evaginatus (n=7); Trauma (n=10)	Pulp Necrosis (n=20) with Chronic Apical Abscess (n=11); Acute Apical Abscess (n=5) and Asymptomatic Apical Periodontitis (n=4).	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%).	N/A