# Regeneration Potential of the Young Permanent Tooth: What Does the Future Hold?

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**Abstract**

During the last 10–15 years, there has been a tremendous increase in our clinical "tools" (ie, materials, instruments, and medications) and knowledge from the trauma and tissue engineering fields that can be applied to regeneration of a functional pulp-dentin complex. In addition, recent case reports indicate that biologically based endodontic therapies can result in continued root development, increased dentinal wall thickness, and apical closure when treating cases of necrotic immature permanent teeth. The purpose of this review was to summarize these findings and illustrate a path forward for the development and evaluation of regenerative endodontic therapies. *(J Endod 2008;34: S51-S56)*

**Key Words**

Endodontics, pulp biology, regeneration, revascularization, tissue engineering

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Treatment of the young permanent tooth with a necrotic root canal system and an incompletely developed root is fraught with difficulty. Not only is the root canal system often difficult to fully debride, but the thin dentinal walls increase the risk of a subsequent fracture. Historically, acceptable endodontic results have been achieved through apexification procedures with use of long-term calcium hydroxide. Concerns have been raised, however, that long-term calcium hydroxide therapy might alter the mechanical properties of dentin. Recent treatment strategies include 1-step creation of an artificial apical barrier by using mineral trioxide aggregate (MTA) with or without an apical matrix followed by compaction of obturating material and placement of a coronal restoration. MTA has been shown to produce good sealing effects under these conditions (1, 2). In addition, bonded composite resins have been reported to increase fracture resistance under some (3, 4) but not all experimental conditions (5). Unfortunately, even after treatment, these teeth have an elevated risk for fracture (6).

An alternative approach is to provide treatment under conditions where continued dentin formation is promoted. Several reports document that under conditions where at least some pulp tissue appears vital, a pulp cap treatment permits continued dentin formation, described as either continued root development (maturogenesis) or apical closure (apexogenesis) (7). Although these findings and an emphasis for continued research on vital pulp therapy are important (8), in many clinical cases the dental pulp has already undergone tissue necrosis before specialist consultation. Moreover, conventional endodontic therapy is not expected to result in continued dentin formation in these circumstances. Thus, there is continued need to develop biologically based treatment regimens that offer the potential for continued hard tissue formation of the young permanent tooth with a necrotic root canal system and an incompletely developed root.

## Regenerative Endodontic Procedures

Several groups recently have published preclinical research or case reports that offer a biologically based alternative to conventional endodontic treatment of these complex clinical cases. In general, these studies have evolved from the trauma literature, where the following precepts have been established:

1. Revascularization occurs most predictably in teeth with open apices (9–12).
2. Instrumentation with NaOCl irrigation is not sufficient to reliably create the conditions necessary for revascularization of the infected necrotic tooth (13).
3. Placement of $Ca(OH)_2$ in root canal systems prevents revascularization coronal to the location of the $Ca(OH)_2$ (14).
4. The use of the "3 mix-MP" triple antibiotic paste, developed by Hoshino and colleagues and consisting of ciprofloxacin, metronidazole, and minocycline, is effective for disinfection of the infected necrotic tooth, setting the conditions for subsequent revascularization (15–19).

This triple antibiotic mixture has high efficacy. In a recent preclinical study on dogs, the intracanal delivery of a 20-mg/mL solution of these 3 antibiotics via a Lentulo spiral resulted in a greater than 99% reduction in mean colony-forming unit (CFU) levels, with approximately 75% of the root canal systems having no cultivable microorganisms present (19). Taken together, these studies provide a strong foundation level of knowledge from the trauma literature that permits subsequent research to focus on developing clinical methods for regeneration of a functional pulp-dentin complex.

Although the trauma literature has used the term *revascularization* to describe this treatment's outcome, the goal from an endodontic perspective is to regenerate a pulp-dentin complex that restores functional properties of this tissue, fosters continued root development for immature teeth, and prevents or resolves apical periodontitis. Thus, using the term revascularization for regenerative endodontic procedures has been questioned (20). Therefore, this review will focus on the concept of regenerating a functional pulp-dentin complex and will restrict the use of the term revascularization to trauma studies.

It should be appreciated that research on regeneration of a pulp-dentin complex has a long history. For example, during the last 30–50 years, Nygaard-Østby and others have reported a series of preclinical studies and case studies on patients attempting to regenerate pulp-like tissue in teeth with either vital or nonvital diagnoses (21–24). Connective tissue was demonstrated to grow as much as several millimeters into the apical portion of the root canal system in teeth with necrotic pulpal diagnoses (23). The results were variable, however, and histologic analysis failed to reveal regeneration of a functional pulp-dentin complex. This lack of outcome is not surprising, however, given the level of materials, instruments, and medications and the knowledge base available at the time. Instead, current research in regenerative endodontics uses greatly improved materials, instruments, and medications and applies many principles from the fields of trauma research and tissue engineering (25–28).

In part on the basis of this expanding base of tools and knowledge, several recent case reports have been published describing regenerative endodontic procedures applied to cases of necrotic immature permanent teeth. Key features of these published cases (20, 29–32) are summarized in Table 1.

There are several common factors observed in these cases. First, although structurally weak, it is important to realize that the immature permanent tooth in general has a very wide apical opening that likely is conducive to tissue ingrowth. Second, these patients are young (8–13 years old), and several (33–37), but not all (38), studies suggest that younger ages have greater healing capacity or stem cell regenerative potential. Third, none of the cases used instrumentation of the root canal walls, whereas all of the studies used NaOCl as an irrigant. Fourth, both $Ca(OH)_2$ paste and combinations of multiple antibiotics have been used in these patients. Outcome differences between these 2 medicaments might reveal an important aspect of regenerative methods, because many of the reported cases treated with $Ca(OH)_2$ display intracanal calcifications that appear to impede the continued thickening of the dentinal walls of these immature teeth (20). In addition, other investigators (30) have suggested that the use of $Ca(OH)_2$ might kill any remaining pulpal cells, including stem or progenitor cells known to be present in dental pulp tissue (39–41), or possibly disrupt the apical papilla (30) and its resident stem cells (42, 43), which is critical for continued root development. Fifth, the formation of a blood clot might serve as a protein scaffold, permitting 3-dimensional ingrowth of tissue. Sixth, nearly all of these studies report continued thickening of the dentinal walls and subsequent apical closure. It should be appreciated, however, that the radiographic finding of continued dentinal wall thickness does not address the cellular nature of this calcified material.

Largely on the basis of preclinical studies, it is possible that the radiographic presentation of increased dentinal wall thickness might be due to ingrowth of cementum, bone, or a dentin-like material (23, 24, 44–48). This diversity in cellular response is not surprising, given that human dental pulp cells can develop odontogenic/osteogenic, chondrogenic, or adipogenic phenotypes, depending on their exposure to different cocktails of growth factors and morphogens (49, 50). One advantage of case reports is that they are based on outcomes observed in actual patients and therefore might have great value in stimulating the development of subsequent treatment methods; indeed, the discovery of fluoride emerged from the keen observations of a practicing clinician. We now recognize, however, the critical importance of subjecting these initial findings to prospective randomized clinical trials to generate objective measures of treatment efficacy and the potential liability for adverse events.

Thus, these and other case reports (51) should be viewed as generating a strong impetus for developing future prospective clinical trials. Taken together, these recent case studies support the hypothesis that the immature necrotic permanent tooth might be particularly responsive to biologically based endodontic therapies. Not only do these treatments provide an important alternative in a clinical situation with an otherwise poor prognosis, but equally important, these cases might serve as an important clinical model to evaluate the application of tissue engineering concepts to the regeneration of a functional pulp-dentin complex.

## Application of Tissue Engineering Concepts to Regenerative Endodontics

The field of tissue engineering has literally exploded during the last decade, and extensive reviews on dental applications are available for the interested reader (25, 26, 52–57). Here we briefly review 3 major components of tissue engineering from the concept of developing regenerative endodontic treatment regimens. Although basic research has applied nearly all of the tools of molecular biology for engineering of dental tissues, including transfections and knockout animals, we will adopt a different perspective: What concepts of tissue engineering are most likely to be available to clinicians when treating their patients with regenerative endodontic techniques? We have used this rather practical perspective to shape our review of this field and to suggest a path forward for developing and evaluating regenerative endodontics.

The first component of tissue engineering is a cell source. Odontoblasts are of mesenchymal origin, and under appropriate conditions, cells from dental pulp, the apical papilla, and possibly other tissues can form odontoblast-like cells (49, 50, 56, 58–61). Controversies exist among several of these studies, because measuring only 1 or 2 characteristics of a cell might not be sufficient to conclusively determine whether the resulting cell is a true odontoblast. Indeed, even among odontoblasts, the phenotype varies in cells located in the apical versus coronal dentin. Recent molecular studies have identified many of the genes selectively expressed in odontoblasts (62, 63), however, and this is likely to aid future studies characterizing the conditions necessary for mesenchymal cells of multiple origins to differentiate into the odontoblast phenotype. To date, the precise cell source(s) supporting the continued root development of the cases described in Table 1 are unknown. It is possible that: residual pulp cells might have remained vital in some of the cases, cells from the apical papilla underwent proliferation, or bleeding-induced angiogenesis might have recruited stem/progenitor cells from apical tissues including the apical papilla. The clinical challenge will be to find a reliable cell source capable of differentiating into odontoblasts, convenient for harvesting, and autogenous to avoid tissue rejection or introduction of foreign pathogens (25). Moreover, a delivery method must be developed that permits controlled application of a known amount of cells into the apical region of the root canal system. Clearly, these are critical areas for future research.

The second component of tissue engineering is a physical scaffold. Tissues are 3-dimensional structures, and an appropriate scaffold is needed to promote cell growth and differentiation. It is known that extracellular matrix molecules control the differentiation of stem cells (64, 65), and an appropriate scaffold might selectively bind and localize cells (66), contain growth factors (67), and undergo biodegradation

**TABLE 1.** Key Features of Case Reports of Regenerative Endodontic Treatment Applied to Necrotic Immature Permanent Teeth

| Tooth no. | Patient age (y) | Patient sex | Preoperative pulpal diagnosis | Preoperative periradicular diagnosis | Treatment[1] | Outcome | Reference no. |
|---|---|---|---|---|---|---|---|
| 29 | 13 | Female | Necrosis | Chronic apical abscess | • 5 weekly visits, no instrumentation, irrigation with 5% NaOCl and 3% $H_2O_2$. Tooth left open between first and second appointments to permit drainage. Interappointment medicament: metronidazole and ciprofloxacin.<br>• 6 weeks later: Broach probed vital tissue in canal. Applied $Ca(OH)_2$ paste, glass ionomer cement, bonded composite resin. | • Sixth appointment: Vital tissue observed 5 mm apical to canal orifice.<br>• 15 months: Positive response to electrical pulp test.<br>• 30 months: Apical closure with thickening of dentinal walls. | 29 |
| 29 | 11 | Male | Necrosis | Chronic apical abscess | • First appointment: Rubber dam and access. No instrumentation. Deep irrigation with 10 mL 5.25% NaOCl and 0.12% chlorhexidine. Interappointment medicament: metronidazole, minocycline, and ciprofloxacin (Lentulo spiral). Cavit.<br>• 1 month: Irrigate 20 mL 5.35% NaOCl. Bleeding initiated with endo explorer. Stopped bleeding 3 mm from cementoenamel junction. MTA, wet pellet, Cavit.<br>• 2 weeks later: Composite restoration. | • 26 days: Vital tissue present 15 mm into canal system.<br>• 6–24 months: Gradual apical closure with thickening of dentinal walls.<br>• Positive response to pulpal cold test. | 30 |
| 20 | 10 | Female | (Partial) pulpal necrosis | Chronic periradicular abscess | • First appointment: Rubber dam and access. No instrumentation. Irrigate with 20 mL 2.5% NaOCl. Interappointment medicament: $Ca(OH)_2$ paste. Caviton/IRM.<br>• 2 weeks later: Repeat.<br>• 3 months: Replace $Ca(OH)_2$<br>• 11 months: Remove IRM and replace with amalgam. | • 3 months: Found hard tissue at $Ca(OH)_2$ site. Asymptomatic.<br>• 11 months: Thickening of dentinal walls.<br>• 35 months: Continued thickening of dentinal walls and apical closure. | 20 |
| 29 | 10 | Male | Necrosis | Acute periradicular abscess | • First appointment: Rubber dam and access. No instrumentation. Irrigate with 2.5% NaOCl. Interappointment medicament: $Ca(OH)_2$ paste.<br>• 1 month: Irrigate with 2.5% NaOCl. Interappointment medicament: $Ca(OH)_2$ paste.<br>• 2 months: Replace $Ca(OH)_2$.<br>• 7 months: Restore with Caiton/Ketac Silver. | • 2 months: Found hard tissue at $Ca(OH)_2$ site. Asymptomatic.<br>• 7 months: Apical closure, thickening dentinal walls, calcified coronal one third root canal system. | 20 |
| 20 | 10 | Female | (Partial) pulpal necrosis | Chronic periradicular periodontitis | • Formocresol pulpotomy.<br>• 9 days: Rubber dam and access. No instrumentation. Irrigate with 2.5% NaOCl. Interappointment medicament: $Ca(OH)_2$ paste.<br>• 1 month: Replace $Ca(OH)_2$.<br>• 2 months later and every 2–3 months for 11 months: Replace $Ca(OH)_2$.<br>• 18 months: Restore with amalgam. | • 1 month: Found hard tissue at mid-root.<br>• 11–54 months: Gradual apical closure, thickening dentinal walls in apical half of root canal system. | 20 |

**TABLE 1.** (Continued)

| Tooth no. | Patient age (y) | Patient sex | Preoperative pulpal diagnosis | Preoperative periradicular diagnosis | Treatment[1] | Outcome | Reference no. |
|---|---|---|---|---|---|---|---|
| 29 | 9 | Male | Necrosis | Chronic periradicular periodontitis | • Tooth left open for drainage at an emergency clinic.<br>• Rubber dam and access. No instrumentation. Irrigate 40 mL 2.5% NaOCl. Interappointment medicament: $Ca(OH)_2$ paste.<br>• 2 weeks and 5 weeks: Repeat.<br>• 5 months: Repeat.<br>• 36 months: Restore with amalgam to calcified bridge. | • 5 weeks: Hard tissue found at mid-root.<br>• 5–60 months: Gradual development of root length, thickening of dentinal walls, apical closure. | 20 |
| 8 | 8 | Male | Necrosis | Chronic periradicular abscess | • First appointment: Consultation.<br>• Second appointment: Rubber dam and access. No instrumentation. Deep irrigation with 10 mL 5.25% NaOCl and 0.12% chlorhexidine. Interappointment medicament: metronidazole, minocycline, and ciprofloxacin. Cavit.<br>• Third appointment: Irrigate with 5.25% NaOCl, induce bleeding with endo explorer. Cotton pellet placed 3 mm below cementoenamel junction for 15-minute control location of a blood clot. MTA, wet cotton pellet, Cavit.<br>• Fourth appointment: Remove Cavit and place bonded composite. | • 8 months: Asymptomatic. Apical closure with thickening dentinal walls. | 31 |
| 8 | 9 | Male | Necrotic | Acute apical abscess | • Trauma 2 years previously treated with Cvek pulpotomy procedure.<br>• Incision for drainage.<br>• Disinfect tooth surface with Betadine. Rubber dam and access. No instrumentation. Irrigate copiously with 1.25% NaOCl. Interappointment medicament: metronidazole, minocycline, and ciprofloxacin (Lentulo spiral). IRM.<br>• 11 weeks: Irrigate with 10 mL 1.25% NaOCl and 10 mL sterile water. Induce bleeding with endodontic file inserted beyond the apex. 15 minutes allowed for blood clot to reach cementoenamel junction. Place MTA over blood clot with moist cotton pellet. Remove cotton pellet 1 hour later and place bonded composite. | • 3 months: Asymptomatic. Diffuse radiopacities noted in root canal system. No response to pulp test ($CO_2$ ice).<br>• 6–12 months: Asymptomatic. Diffuse radiopacities noted in root canal system. No response to pulp test ($CO_2$ ice). Gradual apical development and closure. | 32 |

IRM, intermediate restorative material; MTA, mineral trioxide aggregate.

over time (68). Thus, a scaffold is far more than a simple lattice to contain cells. From our perspective of focusing on practical clinical applications, we believe that platelet-rich plasma (PRP) satisfies many of these criteria. PRP is autologous, fairly easy to prepare in a dental setting, rich in growth factors, degrades over time, and forms a 3-dimensional fibrin matrix (69–72). Interestingly, the case reports from Table 1 all include formation of a blood clot. The use of PRP as an alternative source for a fibrin clot might have several advantages, including increased concentration of growth factors and removal of erythrocytes that would be expected to undergo necrosis shortly after clot formation. To date, however, no publications have evaluated PRP for scaffold generation in regenerative endodontic applications. This and other potential scaffolds require future research.

The third component of tissue engineering to consider for regenerative endodontics is signaling molecules. Both growth factors and other compounds are capable of stimulating cellular proliferation and directing cellular differentiation. As aforementioned, the observed radiographic thickening of the dentinal walls might be due to production of cementum, bone, or dentin. It is likely that the cell source and the available signaling molecules play major roles in guiding the development of cells in the regenerating tissue. For example, the same cultures of human dental pulp cells can differentiate into cells resembling odontoblasts/osteoblasts, adipoyctes, or chondrocytes, depending on the combination of signaling molecules such as dexamethasone (49). Other investigators have shown that dentin or application of a dentin extract rich in growth factors will promote formation of an odontoblast phenotype (50, 62, 73). Extracts of dentin promote growth, because many growth factors are embedded into the dentin matrix during dentinogenesis. Interestingly, ethylenediaminetetraacetic acid (EDTA) very effectively releases growth factors from human dentin (74). It is not yet known, however, whether root canal irrigation with EDTA would promote the development of odontoblast proliferation in a regenerative endodontic procedure. It is likely that intracanal delivery of known signaling molecules or the solubilization of endogenous signaling molecules will promote the formation of dentin in regenerative endodontic methods.

## A Path to the Future

Collectively, there has been a tremendous increase in our clinical tools (ie, materials, instruments, and medications) and knowledge from the trauma and tissue engineering fields during the last decade. Moreover, recent case reports from multiple investigators support the feasibility of developing biologically based regenerative endodontic procedures designed to restore a functional pulp-dentin complex. Although these case reports primarily involve treating the immature permanent tooth, it is quite possible that knowledge gained from this clinical application will have value in developing regenerative endodontic procedures for the fully developed permanent tooth. In short, the question is no longer "can regenerative endodontic procedures be successful?" Instead, the important question facing us is "what are the issues that must be addressed to develop a safe, effective, and consistent method for regenerating a functional pulp-dentin complex in our patients?"

In our opinion, the path to the future should focus on translational research models that simulate likely clinical procedures. For example, although of clear scientific importance in understanding cellular mechanisms, we do not believe that gene transfection is likely to have major application in clinical endodontic procedures. Similarly, if natural tooth development takes several years to occur, then we are not convinced that the growth of artificial teeth with cells of allogenic or even xeongenic origin (42, 75) is likely to have major clinical application. Instead, we believe that research modeling clinical procedures designed to regenerate a functional pulp-dentin complex is likely to have the greatest impact. On the basis of current concepts, one approach would be to focus on methods permitting the delivery of known cells, signaling molecules, and a scaffold such as PRP into the apical 1–2 mm of a root canal system and then "backfilling" the root canal system with a solution of PRP and signaling molecules. Because most cells must be less than 1 mm away from a blood vessel to survive (76), research focusing on the initiation of pulpal regeneration at the apex is likely to have major impact in developing other clinically useful procedures. Cellular proliferation could then occur along the backfill scaffold.

One possible approach is to develop a model system resembling clinical application. For example, the revascularization of root canal systems has been evaluated in human tooth slices after implantation into nude mice (who are immunocompromised, thus avoiding tissue rejection) (77). Similarly, it might be possible to evaluate the initiation of pulpal regeneration after various treatments by implanting the apical 5–10 mm of sectioned human roots into nude mice. This approach has particular advantages, because it permits rapid evaluation of conditions necessary to initiate tissue regeneration and could be extended in future research by evaluating conditions necessary to optimally disinfect necrotic root canal systems before tissue regeneration.

A recent editorial has suggested that "little progress has been made" in the years since the Nygaard-Østby studies on the regrowth of dental pulp (8). From many perspectives, this statement is accurate. We believe, however, that the last decade has produced a critical mass of knowledge and methods that are likely to result in the generation of biologically based endodontic therapies that will answer the challenge issued decades ago.

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