Healing after Regenerative Procedures with and without Pulpal Infection

Ashraf F. Fouad, DDS, MS, and Prashant Verma, DDS

Abstract

Regenerative endodontic procedures for immature teeth with pulp necrosis have gained a lot of interest. Basic scientific research has documented the potential for dental pulp regeneration in preclinical studies. A number of case reports and case series have shown the control of clinical infection and increases in thickness and length of the roots. Preoperative infection is an important factor that predicts outcome in nonsurgical endodontic treatment in mature teeth and seems to also be an important determinant of outcome in treating immature teeth. However, antimicrobial strategies for the immature tooth in which pulp regeneration is contemplated are different from those used in mature cases. This is because of the interest in disinfecting the root canal to a higher level to promote tissue growth without disrupting the bioactive potential of root dentin and the vialility of stem cells from which the regenerated tissue would develop. This review addresses the factors involved in making clinical decisions in this area in light of the information available on the microbiology of endodontic infections, the efficacy to antimicrobial strategies, and the outcomes of regenerative and alternative procedures. (J Endod 2014;40:S58–S64)

Key Words

Endodontics, irrigants, molecular microbiology, pulp regeneration

Dental pulp regeneration has received considerable interest in recent years. Clearly, the main impetus for this interest is the clinical problem of pulp necrosis with established infection before complete maturation of the tooth root. Although this is a formidable problem, it is generally accepted that cases with this presentation constitute a small proportion of all cases with pulpal pathosis. However, it is also generally recognized that if predictable treatment procedures can be achieved to control the infection and promote pulp regeneration in cases with immature teeth, these procedures could potentially be extended to the treatment of the vast majority of endodontic infections that occur in mature teeth, as was recently proposed (1, 2).

Pulp regeneration in an immature tooth that was rendered pulpless by trauma, caries, or a congenital abnormality is advantageous because of the potential for strengthening of the tooth to the same extent as mature teeth. In addition, the presence of vital pulp in any tooth is preferable biologically compared with a filling material because it would restore the host immune response to the pulp and could also potentially regenerate any missing coronal dentin. In this article, the factors that determine success in promoting tooth maturation, the central role that pulp infection and its elimination play, and the different antimicrobial strategies for controlling infection are reviewed.

Pulp Revascularization without an Established Infection

Pulp revascularization procedures have been advocated in immature teeth for decades. It has generally been recognized that for teeth with immature apex after traumatic avulsion with short extraoral time, intrusive or extrusive luxations, replantation/repositioning of teeth, or any transplantation of teeth with immature apex, the teeth are monitored for spontaneous revascularization (3–6). In these clinical conditions, the prognosis for revascularization and continued root maturation ranges from 33%–100%. Moreover, cases have been described in which revascularization is attempted by the clinician in the absence of any evidence for infection and is also shown to yield clinical success (7, 8) and histologic revascularization (9). The contemporary recommendations for treating traumatic injuries of immature teeth in the absence of active infection are to promote revascularization with minimal clinical intervention, if possible (Fig. 1A–D), but monitor the case carefully in case the pulp becomes necrotic. The general understanding in these cases is that if there is no infection or minimal contamination without an established bacterial biofilm, host responses will allow sufficient connective tissue to revascularize the pulp space through the relatively large apical foramen and continue mineralization, leading to increases in the width and length of the root. Pulp revascularization through the immature apex or a mature apex that had been resected was shown in principle in older animal studies (10–13). In fact, the success of revascularization after traumatic injuries of immature teeth has been shown to increase with the size of the apical foramen (14).

Pulp Revascularization with an Established Infection

The situation is very different for a case with necrotic pulp and an established infection manifesting clinically as pulp necrosis with an acute or chronic abscess or a radiographically visible apical lesion. In these cases, the infection has been established for a sufficient duration to allow the development of bacterial biofilms inside the root canal. In addition, cases in which spontaneous revascularization fails present
with some form of an infection. This may include the typical signs mentioned before and/or a lack of continued root development or external infection-related (inflammatory) root resorption. These cases have historically been thought to not respond to the revascularization procedures because of the inability to disinfect the root canal to a degree that would permit regeneration of vital pulp (15). Thus, the important innovation in the last decade has been the demonstration through case reports and case series that cases with established clinical infections could be disinfected sufficiently to allow the revascularization process to yield what appears to be clinical success. This disinfection has been achieved through a variety of methods but mainly with the use of various antibiotics, antibiotic combinations, or calcium hydroxide.

The apparent success in these cases that have been described is primarily related to the control of the established infection and the radiographic healing of the periapical lesion. Although this is an important outcome, it is not the original outcome for which regenerative procedures are contemplated. Apexification procedures with calcium hydroxide or more recently with a mineral trioxide aggregate (MTA) plug have actually been shown to yield the same outcome. Furthermore, there are many cohort studies for MTA apexification now showing clinical success, primarily in controlling periapical infections (16–20).

As stated previously, the interest in regenerative procedures seeks another more important outcome related to strengthening of the teeth involved. This outcome is difficult to evaluate. Clinicians can show control of the endodontic infection within 1–2 years; however, the strengthening of teeth requires decades of monitoring. Calcium hydroxide apexification has been shown to fail in many cases within 5 years, particularly in cases with very thin dentin (21). It is speculated that this may be related to weakening of dentin exposed to long-term calcium hydroxide (22, 23). MTA does not appear to weaken dentin like calcium hydroxide does (24); however, the tooth remains structurally weak and may fracture in the long-term (Fig. 2A–H).

**Transformation of the Oral Microbiome from the Oral to the Endodontic Environment**

Because the development of an endodontic infection plays such a critical role in treatment considerations and the success of regenerative procedures, it is essential to examine the type and characteristics of infection that results after pulp necrosis. This allows the clinician to understand the location of bacterial biofilm, the microbial virulence and adhesion characteristics, and the antibiotic sensitivity of the organisms involved. This knowledge would assist in identifying the best antibacterial strategies.

Naturally, endodontic microbiology has gone through different phases that parallel advances in the science of microbiology. For most of the research performed in the 20th century, culturing of root canal microflora was the state of the art, and clinical decisions were frequently based on cultivation results. Culturing is still the gold standard when it comes to the identification of antibiotic resistance or the study of virulence traits. It is also an important determinant that persistent microorganisms after treatment modalities are alive. However, it has recently been determined that only about 10% of the human microbiome (the bacteria that occupy different sites in the human body) is cultivable (25). In addition, many of the cultivable bacteria may be rendered temporarily uncultivable if they are exposed to reagents or techniques that may exert metabolic stress but resume their cultivability once these stressors are eliminated (26, 27). Therefore, culturing information is at best incomplete in defining the microbial content of infected root canals.

Several generations of molecular technologies have led to a dramatic improvement in our knowledge of endodontic microbiology. Older molecular studies merely investigated the presence of organisms...
that had been identified by culturing or used the inefficient and expensive cloning and sequencing methodologies (28). More recently, next-generation sequencing studies have revealed previously unrecognized diversity and richness in endodontic infections (29, 30). There are also remarkable differences observed in specimens from different patients (29, 31) and the expression of different virulence traits in different strains of the same microbial species (32, 33). These facts make it very difficult to identify specific microorganisms that are responsible for clinical symptoms, resistance to antimicrobial agents, and markers of disease persistence. Newer strategies have sought to link the vastly diverse endodontic infections to the normal oral microbiota of the same individuals from which they originate (34). The basic principle here is to identify the members of the endodontic microbiome that become over-represented relative to the original source in acute or symptomatic conditions and would therefore be the true putative pathogens. This study showed remarkable diversity of the endodontic microbiome in 3 locations: normal oral cavity, necrotic root canal space, and apical abscess (Fig. 3). It can be seen from the differential abundance of microbiota how the proportion of streptococci and Veillonella spp., which are very abundant in the oral cavity, decrease in endodontic infections and the abundance of gram-negative anaerobes such as Fusobacterium spp., Prevotella spp., and Porphyromonas spp. and the gram-positive Parvimonas spp. increase. Lesser known genera like Afipia spp., Phocaeicola spp., Gemella spp., and 1 unclassified genus also increase to different extents in necrotic root canal spaces and apical abscesses. These could play an important role in the overall pathogenicity of the infection. This remarkable overview of the dynamics of bacterial changes in endodontic infections provides a novel insight into the pathogenesis of these infections.

The Role of Infection in Treatment Outcomes

Preoperative endodontic infection is generally determined by the lack of response to vitality tests and the presence of a radiolucent periapical lesion. Numerous outcome studies have identified preoperative infection as the single most important factor in the success of endodontic treatment of teeth with a mature apex. The presence of infection reduces the chances for complete clinical and radiographic healing by about 15%–20% compared with cases with vital pulp (35–38). These findings appear to be consistent in many studies, regardless of the proficiency of the operator and recent technological advances in the field.

Furthermore, one prospective study (37) showed that the probability of long-term healing was not only significantly lower in the presence of a preoperative periapical lesion but also that larger periapical lesions, the presence of a sinus tract, and preoperative symptoms or perforations during treatment in these cases were associated with a worse prognosis. These findings seem to suggest a dose-dependent and duration-dependent effect of preoperative infection on endodontic treatment outcomes.

At the same time, it has been shown that effective debridement in the apical third of root canals significantly improved healing. A recent
randomized clinical trial showed that mechanically enlarging the canals to a minimum of 3 sizes larger than the initial binding apical file led to significantly higher success 1 year postoperatively (39). In this study, to a minimum of 3 sizes larger than the initial binding apical file led to

Several case reports and case series have shown successful results of regenerative endodontic treatment in cases of preoperative infection, including control of the infectious process, radiographic thickening of canal walls, and continued root development (41–46). However, there are currently no clinical trials or outcome studies that have specifically looked at the effect of preoperative infection on the prognosis of regenerative endodontic treatment. More research is needed in this area.

The Selection of Optimal Antimicrobials for Pulp Canal Disinfection

It follows from the previous discussion that there should be some selectivity in the choice of antimicrobial agents, given what is known about the nature of endodontic infections. There are 2 additional factors that further support this notion that specific antimicrobials need to be identified for the treatment of teeth with immature apex. Given that these teeth have a very thin dentinal wall, minimal mechanical instrumentation is advocated so as not to further weaken the tooth structure. However, it is important to note that without the frictional force applied by a file to dentinal wall, bacterial biofilms remain intact and are much more resistant to antimicrobial agents than if they were rendered planktonic by this mechanical disruption. Therefore, a small amount of filing is performed, the intent of which is not to shape the root canal (such in mature teeth) but rather to create inroads through the biofilm to allow maximum permeation by the antimicrobials.

The second important factor is that although maximum antimicrobial efficacy is needed to prevent bacterial irritation of the revascularized/regenerated tissue, minimal toxicity of these antimicrobials on the soft and hard tissues surrounding this newly formed tissue is critical. For example, it is known that 2.5%–5.25% sodium hypochlorite and 2% chlorhexidine are among the most effective antimicrobials in nonsurgical endodontic treatment of teeth with mature apex. However, in vitro and animal model studies have shown that these materials at these concentrations may be toxic to stem cells of the apical papilla (47), may prevent adhesion of stem cells to dentin (48), and may abrogate the bioactivity of growth factors sequestered in dentin (49). Therefore, of these agents, current clinical guidelines advocate only the use of 1.25% sodium hypochlorite at the first clinical appointment.

The use of antibiotics becomes the obvious next choice because of their selectivity, their relatively reduced toxicity, and their potential residual effect while the tissue is growing. Several different antibiotics and antibiotic combinations have been proposed. The most widely used is triple antibiotic paste (ciprofloxacin, metronidazole, and minocycline), which was historically introduced after trials on root canal cultivable microflora (50–52). Triple antibiotic paste has been
found in 1 animal in situ study to disinfect 70% of root canals compared with only 10% disinfected by 1% sodium hypochlorite (53). However, because of the staining effect of minocycline, it was replaced with cefaclor (54) or eliminated altogether (55). Other strategies that prevent discoloration, such as dentin bonding (56), may be cumbersome or ineffective. Augmentin (GlaxoSmithKline, Philadelphia, PA) was used in a recent report (57) because it has been shown to be most effective against root canal flora (58, 59), has the clavulanic acid that inactivates beta-lactamases that are prevalent in endodontic infections (58), and does not discolor teeth. In the case treated with Augmentin, despite continued normal apical maturation of the tooth, access to the root canal to bleach the crown from discoloration because of MTA revealed no tissues in the root canal space (57). Therefore, it is not clear to what extent repair tissue needs to be present in the pulp space to ensure normal apical maturation in these cases.

A creamy mix of antibiotics in a powder form with water or another sterile fluid, as is commonly advocated, results in high concentrations of the antibiotics. These high concentrations have been recently found to be toxic to the stem cells of the apical papilla (60). Therefore, lower concentrations of the antibiotics need to be used, and work is currently underway to determine the concentrations that would achieve effective disinfection with the least toxicity to the apical regenerative tissue. Interestingly, calcium hydroxide was not found to be toxic in the same study (60). This medicament has been found to provide clinically acceptable results in many case reports and case series (61, 62), and so it provides an important alternative to be considered.

Outcomes of Alternative Therapies to Pulp Regenerative Procedures

Endodontics has traditionally approached the problem of pulpal necrosis by attempting to eliminate the bacteria causing the infection and preventing recurrence by sealing the tooth against future ingress of bacteria. Although this is highly successful in many circumstances, teeth with immature apices present some specific challenges. Root canal instrumentation, disinfection, and sealing are more technically difficult to perform in such circumstances because of the tubular or reverse tapering of the canal, together with the open apex. Furthermore, depending on the degree of root maturation, thin root canal walls can render the tooth weak and susceptible to fracture (21).

In the initial phases of pulp inflammation, vital pulp therapy may be effective in maintaining the ability of the radicular pulp to continue root formation in immature teeth (63–65). However, if there is a preoperative infection, then the current treatment options are long-term apexification with calcium hydroxide, the MTA plug technique, or regenerative endodontics. The long-term use of calcium hydroxide for apexification has several disadvantages including multiple treatment appointments, probable recontamination of the root canal system, and weakening of root dentin, which might increase the risk of cervical root fractures (22, 23). The long-term success of apexification with calcium hydroxide ranged from 28%–77% depending on the degree of apical maturation in 1 classic study (21).

The MTA plug technique has been shown to be successful in terms of healing of periapical disease (16, 17, 19, 20); however, it does not allow for continued root development in length or width (61) and so is not likely to strengthen the root. One large recent retrospective cohort study examined the outcomes of MTA plug at 2–4 years postoperatively (18). In this study, 252 cases were examined 1–10 years postoperatively; 90% of them healed, and 96% were fully functional. Using multivariate analysis, the study showed that the presence of a preoperative lesion and the experience of the operator were significant predictors of outcome. Although this is an admirable outcome for this alternative approach to regenerative procedures, the outcome is still a relatively small period in the lives of these children, who have a life expectancy of 60–70 years after treatment is rendered. The value of further strengthening of the roots through regenerative procedures remains to be determined.

The treatment approach of dental pulp regeneration has the potential for restoring the vitality of the tooth. In immature teeth, this can allow for continued normal physiologic development. The pulp has the ability to promote dentin formation, which would increase root thickness and length to prevent fracture and would potentially also develop coronal dentin. Vital pulp has the immune response that allows it to resist future bacterial irritation because of caries or microleakage of restorations. Finally, a normally developed root results in a morphology that is more appropriate for conventional endodontic therapy if future treatment becomes necessary. Other benefits of the pulp include the ability to repair defects because of caries and fractures through tertiary dentin, to provide normal vascular and sensory responses, and to promote normal function. Therefore, pulp regeneration is an ideal treatment outcome for immature teeth with or without preoperative infection. A retrospective review of radiographic outcomes (61) and a recent prospective study (62) showed that the regenerative endodontic treatment produced better outcomes in some parameters compared with calcium hydroxide or MTA apexification. Higher levels of evidence from randomized clinical trials that examine multiple treatment variables are yet to be reported. As noted previously, long postoperative periods are needed in order to ascertain that the original goals of treatment are achieved.

Dentin Regeneration versus Repair with Other Types of Mineralized Tissue

It is generally recognized that tissue regeneration requires an interaction of stem cells and growth factors in a bioactive resorbable scaffold, referred to as the tissue engineering triad (66). Current clinical procedures appear to provide components of these elements sufficient to produce clinically acceptable results. The published case reports and case series have shown clinical success (41–46); however, because these are clinical case reports, it has rarely been possible to determine the histologic nature of the regenerated tissue.

Results from in vitro animal studies using similar protocols with an induced blood clot in the canal suggest that the regenerated tissue is not pulp tissue but, in fact, repair tissue consisting of bone, cementum, and inflammatory tissue (67–70). There are several reports in the literature on the histologic outcome of pulp regeneration in human teeth (71–73). In 1 of them (73), vital pulplike connective tissue was found in the root canal of a tooth treated 14 months previously with a regenerative endodontic procedure using platelet-rich plasma. In another report (72), 3.5 weeks after a regenerative endodontic procedure, more than half the canal was filled with loose connective tissue similar to pulp tissue, and a layer of flattened odontoblast-like cells lined the predentin. A third report (71) found that in a molars tooth extracted 2 years 1 month after a regenerative procedure, the tissues formed in the canals were mineralized tissue similar to cementoid/osteoid tissue and uninflamed fibrous connective tissue. No pulplike tissue characterized by the presence of odontoblastlike cells lining the mineralized tissue was observed. Finally, 1 other report (74) revealed that a tooth treated using the currently acceptable methods failed mechanically 26 months after treatment. Histologic analysis revealed the presence of cementum, bone, and fibrous tissue in the pulp space.

The clinical techniques advocated for regenerative procedures today call for MTA to be placed in the cervical area of the tooth and
extend into the coronal third of the root canal. This prevents mineralized tissues from forming in this area, which clearly is critical for the mechanical strength of the tooth, and could discolor the crown (57). Horizontal fracture in the cervical area occurred in the recent report in which MTA was placed in this area (74). This outcome calls into question 1 of the important goals of the regenerative procedures and underscores the importance of undertaking randomized clinical trials to study both healing and survival of these teeth after treatment with alternative techniques.

The Need for an In Situ Animal Model
To predictably regenerate the dentin-pulp complex, sound tissue engineering principles must be used. Tissue engineering models used include the tooth slice/scaffold (75), the 6- to 7-mm long human tooth root fragment (76), and in vitro whole pulp regeneration animal studies (77), each of which represents a step closer to the clinical situation, both in terms of simulating the biological response and simulating the root canal anatomy. The findings from these studies provide proof of concept for whole pulp regeneration, but they really do not present an in situ model for human regenerative procedures; they would be used on patients, particularly in cases with established infections. The reason for this is that rodent model studies use immunodeficient animals, which would not reject the transplanted tissues and stem cells and are too small for in situ endodontic procedures. Larger mammals are reasonable alternatives, but their stem cells have not been characterized because of the absence of specific antibodies to stem cells or dentin-specific proteins in these species. Furthermore, they may reject xenografts of human stem cells or allografts from noninbred members of their own species. Therefore, a good in situ model for regenerative endodontics still needs to be identified. The ideal model would precisely simulate the clinical situation and allow for testing of treatment protocols and materials that could be directly translated into a clinical trial on human patients.

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

References
Pulp Regeneration—Translational Opportunities


