Microbial Factors and Antimicrobial Strategies in Dental Pulp Regeneration

Ashraf F. Fouad, DDS, MS

Abstract

Dental pulp regeneration after pulp necrosis in immature teeth represents a major departure from traditional endodontic therapy of these conditions. Preliminary clinical attempts have shown the feasibility of developing mineralized repair tissue, which may provide a clinically acceptable outcome. However, this outcome may not provide sufficient host response and root strength to ensure the longevity of the involved teeth. It is not clear if these preliminary suboptimal results are caused by the inability to fully disinfect the pulp space or the absence of a suitable progenitor cell/scaffold template together with adequate vascularity. Moreover, it is not known to what degree the root canal system needs to be disinfected in order for clinical success to be evident. This article describes the current clinical strategies and protocols for the optimal disinfection and preparation of the pulp space environment to promote periapical healing as well as soft and hard tissue development after an infectious process. Current and future strategies for disinfecting the pulp space with minimal disruption of the necessary biological factors from dentin, the progenitor cells in periapical vital tissues, and the vascularity are discussed. The potential for success of pulp regeneration after necrosis and infection would transform the practice of endodontics, even for mature teeth. This is a goal worth pursuing because it would achieve the restoration of normal host responses in the pulp space and the regeneration of destroyed dental tissues. *(J Endod 2017;■:1–5)*

Key Words

Antibiotics, bacteria, endodontic infections, pulp regeneration

Significance

Considerable amount of information is now available on the methods of root canal disinfection, in preparation for regenerative endodontic procedures. This paper discusses the importance of optimal root canal disinfection, as well as recent evidence for the efficacy of the different protocols, materials, and concentrations that have been investigated.

Dental pulp revascularization after irreversible pulpitis or pulp necrosis is not a new concept or practice. For decades, it has been known that, in the absence of infection, in immature teeth or a widened apical foramen, the dental pulp can be promoted to grow back into the root canal. This may occur after devitalization caused by pulp extirpation (1), trauma and replantation (2, 3), or autotransplantation (4). There is also older experimental evidence in animal models that show in extracted apicoectomized and replanted or autotransplanted teeth vascular networks reach the pulp horns within 30 days (5–7). The pulp in similarly treated teeth with mature apex became necrotic. Other animal model work showed success of revascularization with collagen/calcium phosphate gel scaffolds (8). One animal study showed success of revascularization after complete removal of the original pulp tissue after 1 week of cryopreservation (9). In this study, revascularization was similar in immature or mature apicoectomized teeth.

In addition to the immature or wide apical foramen, which allows adequate vascular supply to the tissue, the key element for success of these procedures has always been attributed to the absence of infection (10). Infection within the pulp space is thought to disrupt the ability of host tissues to develop within the pulp space, in the way that wound healing occurs in many other tissues. In addition, the potent antimicrobial agents that are needed to disinfect the pulp space are insufficient to sterilize the root canal space and may interfere with the growth of host connective tissues.

The change in thinking on this issue came about in the first decade of the 21st century. Several published case reports and case series showed that strong antimicrobial therapy with minimal mechanical root canal preparation may control the infection and promote clinically successful mineralization and maturation of the root (11–13). The publication of clinical evidence for the success of revascularization or revitalization in cases with previous infection has been accompanied by a large amount of preclinical research into the feasibility of pulp regeneration with a multitude of materials, protocols, and approaches, the objective of which has been to maximize clinical success and regenerate a tissue as close phenotypically as possible to the original pulp and dentin. At this time, pulp regeneration procedure codes are available, and many dentists, primarily endodontists and pediatric dentists, perform these procedures routinely. Both the American Association of Endodontics (14) and the European Society of Endodontology (15) have published guidelines to help practitioners stay current with the advancing scientific literature. In addition, case reports have

From the Department of Endodontics, School of Dentistry, University of North Carolina, Chapel Hill, North Carolina.

Address requests for reprints to Dr Ashraf F. Fouad, Department of Endodontics, School of Dentistry, University of North Carolina, 1098 First Dental Building, CB# 7450, Chapel Hill, NC 27599-7450. E-mail address: afouad@email.unc.edu

0099-2399/$ - see front matter

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http://dx.doi.org/10.1016/j.joen.2017.06.010

recently been published on expanding the use of these protocols to mature teeth (16), retreatments (17), cases with root resorption (18, 19), and cases with complex congenital anomalies such as dens invaginatus (20–22). These publications demonstrate an acceptance of pulp regenerative techniques into the mainstream procedures in dentistry and recognition of this modality as an option for the management of a variety of endodontic situations.

However, important fundamental questions remain unanswered in this field. For example, it is not known which procedures, materials, and protocols provide the most effective disinfection of root canals of immature teeth while maintaining biocompatibility with periapical tissues and the least biological alteration of dentin. The qualitative and quantitative extent of bacterial disinfection necessary to allow revitalization is not clear. The exact disinfection and/or tissue engineering protocols that are required to promote the regeneration of actual pulp and dentin are not known. The following discussion will discuss recent advances in addressing these areas and describe areas of ongoing research.

Microbial Pathogenesis of Pulp Space Infection in Immature Teeth

It is generally accepted that teeth erupt into the oral cavity about 2 to 3 years before complete radiographic maturation of the root apex. Teeth with an immature apex appear to have a higher healing capacity and resistance to disease than teeth with a mature apex. For example, the pulp in immature teeth that are subjected to luxation injuries such as extrusion or lateral luxation has a much higher chance for survival than that in mature teeth (23). The pulp in intruded or avulsed mature teeth has no chance of survival, but for immature teeth the current guidelines call for observation and monitoring for spontaneous revascularization (23–25).

During the time between tooth eruption and apical maturation, 3 main etiologic factors may result in pulp necrosis and subsequent root canal infection: caries, trauma, or congenital anomalies. Microbial proliferation and invasion of the dental tissues are different in these conditions. In cases of caries and congenital anomalies of the crown, such as dens evaginatus and dens invaginatus, the progression of disease occurs from a coronal source of infection in an apical direction. The progression of the infection in cases with an immature apex is likely slower than that in mature teeth, given the higher collateral circulation in immature teeth. In these teeth, this progression of the infectious process may occur to a higher degree in one region of the pulp more than another or in some canals but not others in multirooted teeth. For example, in cases with dens evaginatus, a common etiology for pulp necrosis in mandibular premolars of adolescent patients of Asian heritage, the tooth may have no response to pulp testing, periapical radiolucency, and a draining sinus tract but have bleeding upon access preparation (26, 27). This bleeding may be related to purulence and infection but occasionally occurs before any canal manipulation and may simply be related to severe inflammation together with the localized areas of infection in these cases.

The pathogenesis of pulp necrosis in immature teeth that receive traumatic injuries may be different. In cases of avulsion or luxation injuries, the bacterial invasion of the pulp space likely occurs from multiple directions, including lateral canals, the apical foramen, and coronal fractures if present. In these cases, the pulp is likely devitalized at the time of injury and thus loses much of its capacity to resist microbial advances. The monitoring period, during which clinicians determine whether the pulp will spontaneously revascularize, represents a race between the growth of a vital tissue with a functional host response against bacterial infection and the growth of the bacterial biofilm. It would be beneficial to intervene therapeutically before a formidable biofilm is formed. However, clinical diagnosis in these cases is hampered by the unreliability of pulp testing after traumatic injuries and the difficulty of establishing whether a periapical radiolucency is part of the tooth follicle that is a source of stem cells (28, 29), a transient apical breakdown (24, 30), or a developing periapical lesion. The most definitive sign of an infection at this early stage, and the risk of delaying intervention, is the development of infection-related (previously inflammatory) root resorption, which can destroy the root at a fairly fast rate (31, 32).

The microbiology of immature teeth with necrotic pulp and apical periodontitis has not been adequately studied. One study revealed the endodontic pathogens commonly found in teeth with an immature apex (Table) (33). Interestingly, of the specific species targeted in this study, the most prevalent species in this group of 15 teeth was *Actinomyces naeslundii*. This organism is capable of forming periapical colonies that resist host responses and is identified in biopsies after surgical treatment. This is important because the apex is wide open in these cases, and the canal microflora can easily be translocated to the apical lesion spontaneously or during canal preparation. However, it is recognized that this study was limited in its examination of the microflora present, and the presence of *Actinomyces* spp in the root canal is not predictive of apical actinomycosis.

In general, given the limited data available on the microbiology of endodontic infections in immature teeth thus far, it can only be surmised that endodontic pathogens present in these cases are similar to those found in cases with mature apex. However, this microflora may be influenced or modulated, at least in part, by the more robust host responses found in the periapex of immature teeth.

Concerns with Traditional Antimicrobial Strategies for Immature Teeth Undergoing Regenerative Procedures

In traditional root canal treatment, strong antimicrobials are used to disinfect the infected root canal and eliminate necrotic tissues. The prevention of further infection or growth of residual bacteria is primarily achieved through interappointment antimicrobial medicaments and final obturation and sealing of the root canal environment.

Sodium hypochlorite is perhaps the most potent endodontic antimicrobial agent (34), and its use at a concentration of 1.25%–6% usually results in about a 40%–60% reduction in the number of teeth with residual cultivable bacteria (35–37). However, sodium hypochlorite has a number of deleterious effects on dentin, which could interfere with the regenerative process. For example, it interferes with stem cell attachment to dentin (38) and abrogates the ability of dentin-based growth factors to be effective in mediating pulp regeneration (39). Fortunately, these effects of sodium hypochlorite could be moderated and/or ameliorated by the application of 17%

TABLE 1. Prevalence of Specific Bacterial Species Detected in Necrotic Pulps of 15 Immature Teeth

Microorganism	Percentage
Actinomyces naeslundii	67
Porphyromonas endodontalis	33
Parvimonas micra	33
Fusobacterium nucleatum	33
Porphyromonas gingivalis	26
Prevotella intermedia	26
Tannerella forsythia	20
Filifactor alocis	13
Treponema denticola	13

Data from Nagata et al (33).

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