Microbial Modulation of Stem Cells and Future Directions in Regenerative Endodontics

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Abstract

Regenerative endodontic procedures (REPs) have been shown to promote the resolution of signs and symptoms of disease and increase survival compared with traditional treatment procedures. However, there is still variable predictability of continued root development and evidence that the tissues formed do not recapitulate the native pulp-dentin complex. There is growing evidence that the apical papilla is capable of surviving prolonged endodontic infection and apical periodontitis and that it represents a rich source of undifferentiated mesenchymal stem cells in REPs. The survival and proper differentiation of stem cells transferred into infected root canals are fraught with challenges. Residual antigens, such as lipopolysaccharides, have been shown to be present in dentin even after adequate chemomechanical debridement. These antigens have a profound effect on stem cell fate by modulating their proliferative capacity and postdifferentiation phenotype. Thus, root canals must be detoxified in addition to disinfection. There is a strong need for translational studies that incorporate all aspects of tissue engineering in endodontics in models that include an existing infection to promote further advancement of the field. This is particularly important to make REPs more predictable when treating immature teeth in young patients. Importantly, regenerative procedures could eventually promote tooth longevity in our aging population. Lessons learned from translational studies that best mimic the clinical challenges could be evaluated in pragmatic clinical trials to determine the effectiveness of these procedures to promote desirable patient-centered outcomes. *(J Endod 2017;43:S95–S101)*

Key Words

Apical Papilla, immature, infection, regenerative, revascularization, stem cells

Significance

The presence of bacteria and their antigens has a profound effect on the final phenotype of stem cells transferred into root canals during regenerative endodontic procedures. Thus, the root canal microenvironment must be detoxified after adequate disinfection to allow for more controlled stem cell fate.

Regenerative endodontic procedures (REPs) are now considered a treatment alternative for immature teeth diagnosed with pulp necrosis. There has been tremendous growth in knowledge regarding the biological principles of these procedures since the foundational studies in the 1960s (1) and the first contemporary case report in 2001 (2). Initial researchers and clinicians advocated intracanal bleeding with the primary goal of forming a blood clot to promote angiogenesis and healing analogous to the process observed in other tissues. However, it became obvious that far more than simple wound healing was being observed in some of the initial published cases. These findings include resolution of pathosis (ie, signs and symptoms of apical periodontitis), gain in root width and root length (3–5), and reestablishment of vitality responses in some cases. In 2011, a clinical study using molecular biology techniques demonstrated REPs in immature teeth, also called revascularization or revitalization procedures, were instead stem cell–based procedures. Later, it was shown that undifferentiated mesenchymal stem cells (MSCs) could also be transferred into root canals after evoked bleeding in mature teeth in adult patients (6). Therefore, these data collectively support the hypothesis that the currently used techniques in REPs are indeed stem cell–based procedures.

Tissue engineering (TE) approaches rely on the interplay of the triad formed by stem cells, scaffolds, and growth factors (7). Interestingly, currently used REPs have incorporated TE principles because the fibrin network in a blood clot, used as a vehicle for stem cell delivery, can be considered a scaffold as well as dentin. Growth factors have been found to be present and released from dentin and platelets (8). Therefore, the intentional manipulation of these 3 components should be considered a TE approach in REPs. The advantages of this "cell-free" approach include low cost, minimal risk for the patient, and acceptable clinical outcomes. Conversely, disadvantages include the inability to control transferred stem cell numbers and phenotypes and the lack of control in scaffold composition and growth factor content. It is noteworthy that clinicians have incorporated other forms of scaffolds, in addition to a blood clot, that include platelet-rich plasma, platelet-rich fibrin, collagen scaffolds, and exogenous growth factors with similarly clinically acceptable outcomes (5,9–12). Despite these outcomes, there is still poor predictability of continued root development, and the evidence of tissue formation does not resemble the native pulp-dentin complex at the histologic level (13–17).

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0099-2399/$ - see front matter

http://dx.doi.org/10.1016/j.joen.2017.07.012

A major challenge to be overcome in REPs is the presence of polymicrobial infections in teeth with pulp necrosis (18, 19). The role of a persistent infection is not to be ignored because the eradication of invading microorganisms and their induced tissue inflammation represents the primary goal of endodontic therapies. Similar to other tissues, persistent infections in root canal systems are not conducive to healing and must be first debrided and disinfected. Indeed, the surgical debridement of infected tissues is a common practice in medicine before healing and regeneration or repair (20). However, endodontic infections are not limited to soft tissue, which is readily accessible for debridement. Instead, in addition to soft tissue, polymicrobial infections extend far into the dentinal tubules in both planktonic and biofilm forms, presenting a major challenge for both conventional root canal therapy and REPs (21, 22). Histologic examination of a failed case treated with an REP illustrates this issue, which is not entirely surprising because persistent infections are also a major reason for failure after conventional endodontic treatments (15). Importantly, there is strong evidence that agents used in disinfection alter stem cell viability and differentiation potential, change the bioavailability of endogenous growth factors, and modify scaffold properties (8,23–28). There has been a significant effort dedicated to elucidate how to achieve maximum disinfection with a minimal detrimental effect on the regenerative potential of the recruited stem cells (28). Tissue engineering in endodontics should have the premise that a successful disinfection protocol that is biocompatible with host cells is a prerequisite for regeneration, and any regenerative effort, albeit sophisticated, will be futile without it. Thus, in regenerative endodontics, the classic triad of tissue engineering described by Langer and Vacanti becomes a quartet with the addition of the overencompassing disinfection and detoxification requirement (Fig. 1).

Infection and Stem Cell Viability

Dental pulp has elaborate defense mechanisms against infections with a robust microbial pattern recognition system that, in addition to immune cells, include odontoblasts, fibroblasts, and dense nociceptive innervation (29–34). In addition, there is outward fluid of dentinal fluid containing immunoglobulins against invading microorganisms and their antigens, an elaborate arteriole-venule shunt mechanism (35), and a specialized niche of stem/progenitor cells with the capacity to participate in cellular defenses and modulate the overall response against microorganisms (36–38). Despite these apparently redundant defense mechanisms, the dental pulp often succumbs to an overwhelming infection that may be facilitated by direct damage to these cellular processes by a traumatic injury and the lack of collateral circulation.

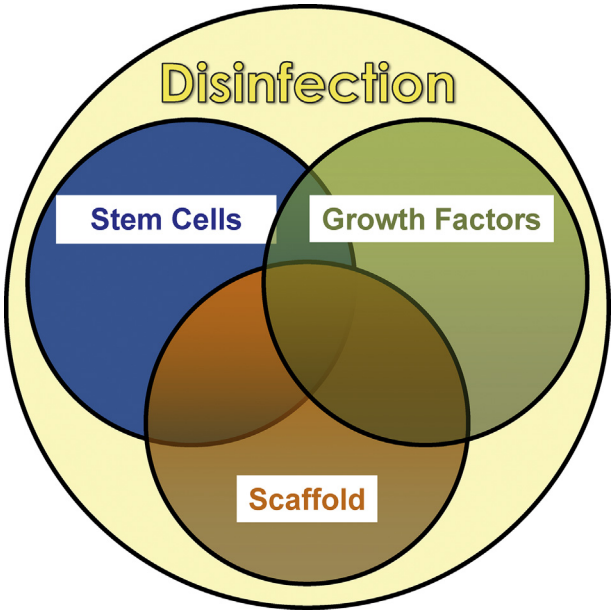

Figure 1. The quartet of tissue engineering in endodontics. Disinfection is a fundamental part of regenerative endodontics, interacting with the interplay between stem cells, scaffolds, and growth factors.

The outcome of dental trauma on pulp vitality and tooth survival is strongly associated with the stage of root development, the age of the patient, and the severity of the traumatic injury (39, 40). These important studies highlight the intrinsic capacity of repair and regeneration of the dental pulp. This is particularly evident in cases of reimplantation of immature teeth in which a significant proportion of teeth reattain vitality. It has been hypothesized that revascularization is greater with a larger apical foraminal diameter because of favoring the ingress of newly formed blood vessels and the anastomosis of apical vessels of the damaged pulp vasculature. Equally important is the presence of the apical papilla in immature teeth. The cells contained in this distinct structure are derived from the dental papilla formed since the initial stage of tooth development (41). It represents a rich source of MSCs that are known to be responsible for root developed under the influence of the Hertwig root sheath through an intricate epithelial-mesenchymal interaction. The fate of the apical papilla after pulpal infections was completely unknown until recently.

It is intuitive to assume that the apical papilla tissue undergoes liquefaction necrosis in tandem with the dental pulp because these 2 tissues are interconnected. However, there is growing evidence that the apical papilla and its rich resident stem cell population are capable of surviving infection and advanced apical periodontitis. Thus, the apical papilla appears to have features that make it particularly suited to not only survive infection but also to react to the insult in a fashion that is favorable for repair or regeneration. The role of the niche in the survival of stem cells of the apical papilla may be explained by its low vascularity (42) (Fig. 2*A* and *B*). There is a significantly lower number and density of blood vessels in the apical papilla compared with the neighboring dental pulp (42). Instead, the most vascularized dental tissue in intimate contact with the apical papilla is the dental follicle, which is richly vascularized. Therefore, the dental papilla appears to have low metabolic demands with cells when in the quiescent stage, acquiring nutrient and gaseous exchanges primarily by diffusion from neighboring tissues. An animal study supports this hypothesis because viable apical papilla tissue was still visualized 90 minutes after pulp exposure of rat molars (43). This is particularly remarkable because advanced apical periodontitis and pulp necrosis are known to happen in as little as 14 days after exposure in this animal model. In another animal model, pulp exposures were made in immature canine molars, and apexification or REPs were performed followed by histologic evaluation (44). The apical papilla was also found to be still present and histologically distinct from the other tissues despite advanced pulpal infection and endodontic procedures (44). Therefore, animal models of REPs have shown the presence of a vital periapical apical papilla despite complete pulp necrosis.

A recent clinical study reported harvesting of the apical papilla through the canal space of a premolar with a history of a long-standing infection and intraoral swelling (45). The tissue was retrieved from its anatomic location with an endodontic file with the aid of an operating microscope by an endodontist. The tissue