Survival of the Apical Papilla and Its Resident Stem Cells in a Case of Advanced Pulpal Necrosis and Apical Periodontitis

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Abstract

Introduction: Apical papilla represents a source of an enriched mesenchymal stem cell (MSC) population (stem cells of the apical papilla [SCAPs]) that modulates root development and may participate in regenerative endodontic procedures in immature teeth with pulp necrosis. The characteristics and phenotype of this tissue in the presence of inflammation are largely unknown. The purpose of this study was to characterize a human apical papilla sample that was isolated from an immature tooth with pulp necrosis and apical periodontitis. **Methods:** Inflamed periapical tissue that included part of the apical papilla (apical papilla clinical sample [CS]) was collected from an immature mandibular premolar previously diagnosed with pulp necrosis and apical periodontitis during an apexification procedure. Harvested cells from this tissue (SCAP CS) were compared with inflamed periapical progenitor cells (IPAPCs) and normal SCAP (SCAP-RP89) in flow cytometry and quantitative osteogenesis experiments. Part of the issue was further processed for immunohistochemistry and compared with apical papilla and coronal pulp sections from normal immature teeth as well as inflamed periapical tissues from mature teeth. **Results:** Similar to SCAP-RP89, 96.6% of the SCAP CS coexpressed the MSC markers CD73, CD90, and CD105, whereas only 66.3% of IPAPCs coexpressed all markers. The SCAP CS showed a significantly greater mineralization potential than both SCAP-RP89 and IPAPCs. Finally, immunohistochemical analysis revealed moderate infiltration of cells expressing the inflammatory markers CD45/68 in the apical papilla CS and prominent CD24, CD105, and von Willebrand factor expression. **Conclusions:** Under inflammatory conditions, human apical papilla was found moderately inflamed with retained SCAP vitality and stemness and increased osteogenic and angiogenesis potential. *(J Endod 2016;■:1–7)*

Key Words

Apical papilla survival, apical periodontitis, characterization, periapical inflammation, regenerative endodontics, stem cell, stem cells of the apical papilla

Significance

The apical papilla may survive despite intense inflammatory infiltrate following pulp necrosis. In this report, SCAPs maintained their stemness and expressed increased osteogenic and angiogenesis potential. Regenerative strategies should focus on promoting the continued survival, recruitment, and differentiation of these cells to achieve predictable guided endodontic repair and regeneration.

The apical papilla consists of the apical portion of the dental papilla and, in conjunction with the Hertwig epithelial root sheath, is responsible for root development (1). Stem cells of the apical papilla (SCAPs) have been shown to have great proliferation and differentiation potential in addition to high motility (2). Studies have highlighted the potential role of SCAPs and the apical papilla in the continuation of root development and regeneration of the pulp-dentin complex (1, 3). Notably, in a pilot experiment, surgically removing the apical papilla resulted in the arrest of root development even in the presence of intact pulp (1). Huang et al (3) further showed that SCAPs have the ability to differentiate into odontoblastlike cells and lead to *de novo* dental pulp regeneration *in vivo*. These findings suggest the importance of maintaining the vitality of the apical papilla in immature teeth as a source of stem cells that contribute to and regulate root development.

In regenerative endodontic procedures (REPs), evoked bleeding from the periapical tissues has been shown to lead to a significant influx of mesenchymal stem cells (MSCs) in the root canal system of both immature and mature teeth (4, 5). Using this step as a method to introduce stem cells into the root canals is a significant concept in regenerative endodontics because it provides access to the most readily available sources of MSCs (ie, apical papilla, periodontal ligament, alveolar bone, and inflamed periapical tissues) for potential dental pulp regeneration (6). In immature teeth, the apical papilla represents an enriched pool of MSCs in direct contact with the tooth apex (2, 7). Even with the odontogenic differentiation potential of SCAPs, REPs do not always result in the formation of dentin and pulplike tissue (8–10). The root canal microenvironment including pulp status and infection control regimens seems to affect the regeneration phenotype (8, 11–16). REPs in teeth with pulp necrosis and harboring bacteria in the root canal system have been associated with

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cementum or bonelike tissue formation, whereas dentin formation was noted in teeth with irreversible pulpitis (11, 12). Importantly, prolonged infection and periapical inflammation may seriously affect the survival of the apical papilla or alter its properties, which is considered crucial for the continuation of root development in immature teeth that undergo regenerative endodontic treatment (1). Despite the importance of the latter, the effect of pulp necrosis and periapical inflammation on the survival of human apical papilla is largely unknown.

Studies have identified unique properties and characteristics of the human apical papilla and SCAPs; however, the included samples were from human immature teeth with normal pulp and periapical tissues (2, 3, 17, 18). These studies showed the distinct histologic and immunohistochemical properties of apical papilla compared with dental pulp as well as characterized SCAP cell lines in terms of gene expression, stemness, and differentiation potential (2, 3, 17, 18). Nonetheless, it is very likely that apical papilla and SCAPs present differently in pulp necrosis and periapical inflammation (1). Hypoxia and inflammation have been shown to affect SCAP proliferation and differentiation in culture (19, 20). Tobias Duarte et al (21) evaluated the histopathological conditions of dental pulp and apical papilla after inducing endodontic infection in a rat model and found that apical papilla remained vital after 90 days of infection yet slightly or moderately inflamed. Despite the current evidence, to date, no study has evaluated human apical papilla from an immature tooth with pulp necrosis and apical periodontitis. The purpose of this study was to report for the first time the characterization of a human apical papilla sample harvested through the canal system of an immature tooth with pulp necrosis being treated with an apexification procedure. We hypothesized that apical papilla and its resident cells retain their vitality under chronic inflammatory conditions.

Materials and Methods

Case Report

A 9-year-old female presented to the Graduate Endodontics Clinic at the University of Texas Health Science Center at San Antonio, San Antonio, TX, for evaluation and treatment of tooth #28. The patient reported tooth sensitivity in the past, approximately 1 year before the appointment, produced by hot/cold drinks, and her complaint at the time of the visit was sensitivity on biting. Tooth #28 presented clinically with extensive occlusal caries, normal probing depths and mobility, and normal soft tissues. Radiographically, tooth #28 presented with a carious lesion extending into the pulp chamber, immature apex, and periapical radiolucency (Fig. 1*A*). Pulp sensibility tests with Endo-Ice (Coltene/Whaledent Inc, Cuyahoga, OH) and an electric pulp test (Electric Pulp Tester; SybronEndo, Glendora, CA) were performed, and negative pulp responses were noted. Periradicular tests revealed that tooth #28 was sensitive to percussion but not sensitive to palpation. Based on the clinical tests, the pulpal diagnosis was determined as pulp necrosis and the periradicular diagnosis as symptomatic apical periodontitis. Both the guardian and patient were informed of the clinical findings and presented the treatment options of regenerative endodontic treatment, apexification with a mineral trioxide aggregate (MTA) plug, extraction, and no treatment. The guardian and patient elected MTA apexification and signed the informed consent. After adequate anesthesia, the tooth was isolated with a rubber dam, and access was made into the root canal system. Pulp necrosis was confirmed clinically upon access. For the chemomechanical preparation, Hedstrom files (H-files, SybronEndo) were used to file the canal

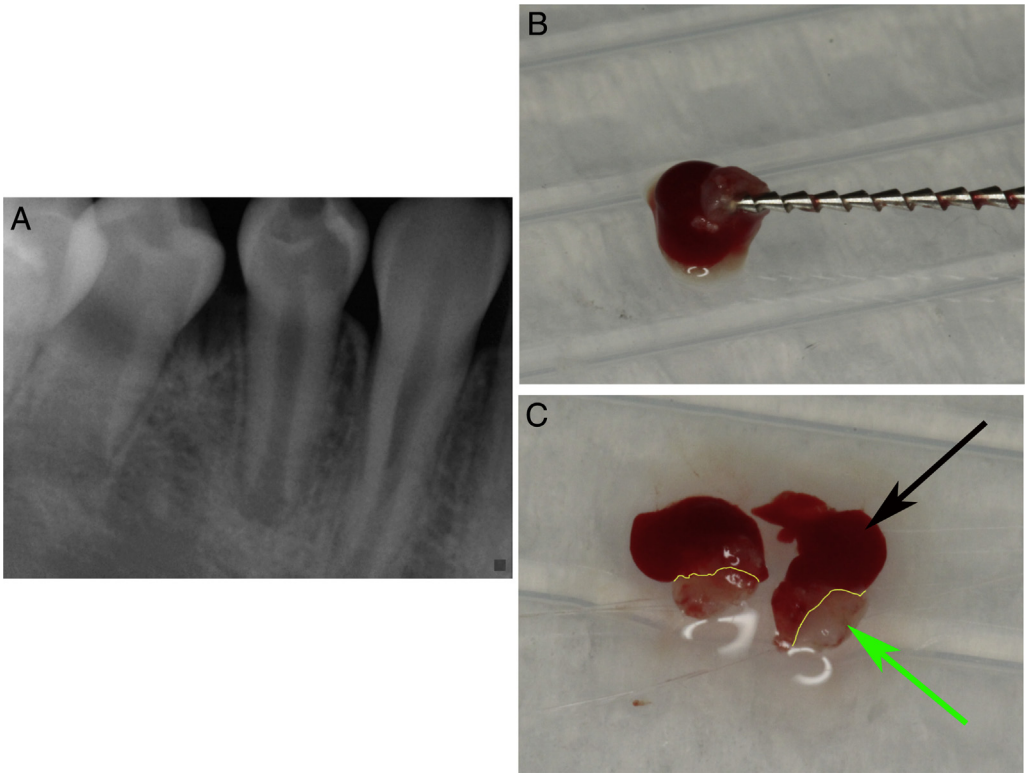

Figure 1. Case report radiograph and clinical photographs. (*A*) A periapical radiograph of tooth #28. (*B*) Periapical tissue was engaged to a H-file after removal from the root canal of tooth #28. (*C*) Tissue was immediately sectioned into 2 pieces, both containing part of the apical papilla (*green arrow*) and part of the inflamed periapical tissue (*black arrow*). The *yellow lines* represent the junction between the 2 distinct tissues.