First Evidence for Regeneration of the Periodontium to Mineral Trioxide Aggregate in Human Teeth

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Abstract

Introduction: The aim of this study was to elucidate whether the use of mineral trioxide aggregate (MTA) in endodontic therapy in human teeth leads to the same regeneration of the apical tissues as observed in animals. **Methods:** Four human teeth were identified in a policlinic that had been treated endodontically with MTA and had to be extracted for other reasons than just endodontic failure. All teeth were processed for histologic and one for immunohistochemical analyses to analyze the histologic response of the periapical structure to the former treatment with MTA. **Results:** All identified teeth showed clinical and radiographic signs of healing at the time of extraction. In the histologic evaluation, all teeth showed a layer of cementlike tissues at least on the MTA surface. Further double immunofluorescence analyses for collagen type I and type III revealed protein expression and colocalization of the 2 proteins, implicating formation of periodontal ligamentlike tissue, presumably fibers. **Conclusions:** Histologic healing of the human periodontium to MTA corresponds to the healing pattern shown in animal studies. Cementlike tissues were formed on the surface of MTA, which proves regeneration of the periodontal ligament. *(J Endod 2017;■:1–8)*

Key Words

Cementum, endodontic therapy, mineral trioxide aggregate, periodontal ligament, regeneration

Significance

This article provides first evidence for regeneration of the periodontium to MTA in human teeth. The histologic and immunohistologic evaluation shows regeneration of cementlike tissues and periodontal ligamentlike tissues in teeth treated with MTA. Therefore MTA provides the most favorable mode of healing in clinical endodontic application toward a *restitutio ad integrum* of the periapical tissues.

Ultimate healing of the periodontium after therapy of inflamed periapical tissues of endodontic origin is the regeneration of a healthy periodontal ligament (PDL) with surrounding sound alveolar bone. Mineral trioxide aggregate (MTA) seems to be a material of choice for retrograde root-end fillings after endodontic surgery (1–3) but also for orthograde endodontic treatments of teeth with open apices (4) because of its favorable properties concerning its sealing ability (5), biocompatibility (6), and histologic healing of surrounding alveolar tissues (1, 2). A series of *in vitro* studies showed a superior sealing ability of MTA compared with other retrograde filling materials, such as intermediate restorative material (IRM), SuperEBA (Harry J Bosworth Co, Skokie, IL), and amalgam (5, 7). Moreover, studies have shown that MTA is not cytotoxic (8) and provides a favorable environment for PDL fibroblast adhesion as well as for PDL fibroblasts and cementoblast growth (6, 9). Histologic animal studies have revealed that MTA frequently initiates superior healing quality of the resected dentin surfaces (1, 10, 11). Using materials like IRM or SuperEBA provides the ability to heal toward the filling material, in best cases without signs of inflammation in the PDL space, but without regeneration of the PDL (1, 10). Only bioceramic root repair material shows comparable regenerative properties with MTA regarding the PDL (2).

Although animal studies have shown the superior healing properties of MTA toward the regeneration of the PDL, proof of the same favorable histologic response after endodontic therapy performed with MTA stands out in humans. A systematic review of the histologic responses of the periodontium to MTA in 2013 could only identify histologic animal studies (12). To our knowledge, any histologic proof of the reaction of the surrounding tissues after endodontic application of MTA in human teeth is lacking to date. This is mainly because of massive ethical implications associated with obtaining histologic samples of healed teeth in humans. Although teeth without signs of healing will certainly not contribute to showing the healing potential of MTA toward PDL regeneration in humans, it is very rare that a clinically and radiographically healed tooth has to be removed so that a histologic sample can be obtained.

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Therefore, we have documented all cases of endodontic surgery performed with MTA in the outpatient clinic of the Dental Academy for Continuing Professional Development, Karlsruhe, Germany, and followed up these cases until the rare event that a tooth had to be removed for other reasons than endodontic failure.

The purpose of this study was to elucidate whether the use of MTA in human teeth leads to the same regeneration of the apical tissues as observed in animals and to analyze the mode of regeneration, if any, of the PDL.

Methods

A total of 4 patients were identified, each having 1 tooth that was formerly treated with MTA (Angelus MTA; Angelus Dental Products Industry S/A, Londrina, Brazil) and had to be extracted. Three teeth underwent root-end surgery, and 1 tooth was treated because of dental trauma. In the last case, MTA was placed as an apical and lateral barrier in a tooth with an open apex and signs of advanced lateral root resorption. All teeth showed clinical and/or radiographic healing of the periapical tissues at the time of extraction. Tooth characteristics and the reasons for extraction are summarized in Table 1.

All teeth were carefully luxated and removed to prevent damage of the root tips with the adhering tissues as much as possible. For obvious ethical reasons, it was not possible to obtain a resection of surrounding bone tissue including the tooth. After extraction, the teeth were immediately fixed in 10% formalin. Subsequently, the teeth were processed for histologic and immunohistochemical analysis.

Antibodies and Reagents/Chemicals

The mouse monoclonal antibody against collagen type I (ab90395) and the rabbit polyclonal antibody against collagen type III (ab7778) were purchased from Abcam (Cambridge, UK). Donkey antirabbit or antimouse immunoglobulin G and Alexa Fluor 488 (Dianova, Hamburg, Germany) or Alexa Fluor 568 (Germany) were used as secondary antibodies. Fluoromount-G was purchased from Biozol (Eching, Germany).

Histologic Analysis

Three teeth were embedded in acrylic resin (Technovit 4071; Heraeus Kulzer, Wehrheim, Germany). The teeth were then cut in sections using a nondecalcifying sawing-grinding technique (13, 14) along their coronoapical axis so that the area of interest containing the MTA was exactly in the middle of the section plane. Sections of 100- to 150-μm thickness were ground with a grinding machine to sections of 7 μm with grain sizes up to P1200 Fédération Européenne des Fabricants de Produits Abrasifs classification. The sections were then stained with toluidine blue. If possible, more lateral sections were cut and evaluated if they contained MTA in the section plane. The apical region was photographed with a digital camera (CFW 1312M; Scion, Frederick, MD) mounted to a microscope (Axiophot 2; Zeiss, Jena, Germany).

All apical surfaces were quantified for newly formed cementlike tissue (CLT) and PDL-like tissue at the root tips with ImageJ of the Fiji distribution (National Institutes of Health, Bethesda, MD) (15). For quantitative analyses, the apical region of every histologic section was photographed with an overlapping series of pictures at 20× magnification. All pictures were photo stitched with Hugin (Version: 2016.0.0.3b4e2790cb90 built by Jan Dubiec) for complete panoramas of the apical surfaces of the roots. For metric analyses, ImageJ was set to the correct micrometer per pixel relation at 20× magnification. The apical surfaces of the roots were measured as well as the surface of MTA and the surface of CLT in micrometers. Then, the rates of coverage

TABLE 1. Case Characteristics of Investigated Mineral Trioxide Aggregate (MTA) Treatments

Case no.	Tooth	Reason for treatment	Type of treatment	Reason for extraction	Age of patient at time of treatment in years	Time from treatment to extraction in months	Clinical healing	Radio-graphic healing	No. of histologic sections with MTA surface	Type of histologic evaluation
1	13	Persistent apical periodontitis	Root-end surgery with retrograde MTA application	Subgingival tooth fracture	80	43	Complete	Complete	3	Undecalcified sawing/grinding with toluidine staining
2	16	Persistent apical periodontitis	Root-end surgery with retrograde MTA application	Persistent apical periodontitis with clinical symptoms mesiobuccal root	41	25	Partially	Partially (distobuccal and palatal roots healed)	2	Undecalcified sawing/grinding with toluidine staining
3	46	Persistent apical periodontitis	Root-end surgery with retrograde MTA application	Persistent apical periodontitis with clinical symptoms mesial root	51	26	Partially	Partially (distal root healed)	2	Undecalcified sawing/grinding with toluidine staining
4	21	Root resorption after dental trauma	Orthograde placement of MTA as artificial apexification	Persistent signs of infection	20	21	Partially	Partially (apical healing but lateral advancing resorption)	2	Decalcified paraffin sectioning and H&E and immohistochemical staining for collagen type I and III

H&E, hematoxylin-eosin.