Evidence of Intratubular Biomineralization in a Root Canal Filled with Calcium Enriched Material over 8 Years

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Abstract

This case report describes evidence of intratubular biomineralization in root canal filled with calcium enriched material after 8 years of clinical maintenance. The schematic findings of dentinal tubules were investigated with scanning electron microscopy (SEM) and energy dispersive spectroscopy (EDS). The root canal obturation material was closely adapted to root dentin surface, suggesting the possibility of chemical bonding between the two interfaces. SEM and EDS observation of dentinal tubules showed intratubular biomineralized crystal structures with Ca/P ratio in a range of 1.30–2.12, suggesting bioactive capacity of calcium enriched cement.

Keywords: biomineralization; calcium enriched material; calcium deficient hydroxyapatite; dentinal tubule; energy dispersive spectroscopy; scanning electron microscopy

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1. Introduction

For the root canal treatment of teeth, calcium enriched filling materials such as mineral trioxide aggregate (MTA) have been proposed as an alternative to conventional gutta percha. MTA is highly biocompatible and provides an excellent seal, although it requires an extended setting time and is irretrievable for retreatments. MTA's sealing effects appear to be due to the formation of hydroxyapatite crystals and the biomineralization of dentinal tubules [1], as reported by *in vitro* studies [2-6]. However, the long term clinical evidence for the biomineralization in dentin are missing, and its relevance unclear.

This case report provides the first long-term clinical evidence of the biomineralization of dentin in root canal treated teeth. There was clear evidence of intrabular biomineralization within the dentinal tubules of a human permanent mandibular canine, more than 8 years after an orthograde obturation of the canal with MTA.

2. Clinical Presentation and Treatment

A 43-year-old women presented to the dental clinic with the chief complaint of a fractured right mandibular canine. Clinical and radiographic examination revealed a subgingival fracture of the tooth, and prior treatment of the root canal that was associated with a periapical radiolucency (Fig. 1A). A treatment plan was formulated for retreatment of the canal and restoration of the tooth, and informed consent obtained from the patient.

Local anesthesia was applied, rubber dam isolation obtained, and the previous gutta percha filling material removed from the root canal. Then the canal was prepared with NiTi rotary files (ProTaper F4, Dentsply/Maillefer), and the apical area enlarged with manual stainless steel K files up to #45. During instrumentation, the canal was irrigated copiously with 5.25% sodium hypochlorite (NaOCl). Then the canal was dried with paper points, filled with calcium hydroxide paste as an intracanal medicament, and the access sealed with temporary filling material. After 2 weeks, the root canal was copiously irrigated with 5.25% NaOCl, then immersed in 17% ethylenediaminetetraacetic acid (EDTA) solution (pH 7.2) for 1min, and finally flushed with 5.25% NaOCl, with all irrigants activated ultrasonically (P5 Newtron1 XS; Satelec, Acteon group, Mėrignac, France). Then, the canal was copiously rinsed with sterile distilled water, dried with sterile paper points and obturated with OrthoMTA (BioMTA, Seoul, Korea) as previously reported [1, 7]. The OrthoMTA had been mixed with distilled water by using the OrthoMTA automixer (BioMTA), as recommended by the manufacturer. The paste was introduced into the canal with the OrthoMTA carrier (BioMTA), and applied to the canal wall using OrthoMTA compactor, which has a 25/0.02 tip. The compactor was inserted to working length and rotated with a circumferential filing motion at 60 rpm. Sterile dry ISO-standardized paper point (Meta

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Dental Corp., Chungbuk, Korea) which has a coinciding size of the apical preparation was used to compact the MTA cement at the root canal apex. After obtaining apical plug, additional paper points with increasing sizes according to an increasing coronal taper of the root canal was used to refine the inserted MTA material up to 7 mm level from the apex. When the patient was recalled a week later to confirm appropriate set of intracanal MTA cement, the tooth was prepared for cast post restoration. The tooth was finally restored with gold cast post (Fig. 1B), and full veneer gold crown was placed as an abutment of removable denture in lower arch.

The patient was recalled regularly at 6 monthly intervals and the root canal treatment and restoration of this tooth examined and evaluated. The patient was satisfied and the tooth remained asymptomatic for more than 8 years, until a subgingival fracture occurred (Fig. 1C). Clinical and radiographic examination revealed a deep subgingival fracture with limited remaining tooth structure. A treatment plan was formulated for extraction, and informed consent obtained from the patient.

3. Laboratory Examination and Analysis

For permission to study the extracted tooth, approval was obtained from the Institutional Review Board (IRB) of Seoul National University Dental Hospital, Seoul, South Korea (ERI16003). Following extraction, the tooth was embedded in an acrylic block and apical root segment (5 mm) trimmed with a slow-speed, water-cooled diamond saw (Isomet Low Speed Saw; Buehler, Lake Bluff, IL, USA). This root segment was split longitudinally, washed briefly in distilled water, and sputter coated with platinum for scanning electron microscopy (SEM; S-4700, Hitachi, Tokyo, Japan; 15kV). The interface between the obturation material in the root canal and the surrounding root dentin were carefully examined as well as the intratubular microstructure observation. Elemental composition of intratubular mineralized precipitates were analyzed by using energy dispersive spectroscopy (EDS; 7200-H, Horiba, Northampton, England) to provide qualitative and semi quantitative measurements of atomic calcium and phosphorous to calculate the superficial calcium to phosphorous atomic ratios (Ca/P).

4. Results and Discussion

This case report provides the very first *in vivo* evidence that demonstrates the clinical effectiveness of OrthoMTA as an orthograde root canal filling material. In this case, OrthoMTA was effective as a root canal filling material in a mandibular canine with chronic (asymptomatic) apical periodontitis, which had previously been treated with conventional materials. Careful microscopic and chemical analyses of the OrthoMTA filled

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root canal revealed a tight seal between the material and dentin with evidence of intratubular biomineralization (Fig. 2).

OrthoMTA is mainly composed of tricalcium silicate and contains less heavy metal than the original ProRoot MTA (Dentsply, Tulsa, OK, USA) [8]. Previous *in vitro* studies reported its use as root canal filling material, showing evidences of intracanal mineralization of root canal dentin at the interface of dentin and obturation material [7, 9, 10]. However little was known on in vivo data on this issue. In this regard, this case report presents proof of concept evidence of bioceramic materials as root canal obturation material, with bonding property to dentin surface and inducing intratubular biomineralization.

The bioceramic materials bond to dentine by a process known as alkaline etching (caused by the alkalinity of the cement), and a mineral infiltration zone develops at the interface of the dentine in contact with the material [11]. On this wise, root canal obturation material was closely adapted to the root dentin surface, in accordance with previous studies (Fig.2A) [4-6, 8]. At the interfaces between MTA and root dentin, we could find a very thin intermediate layer where Ca, P, Si were all detected, suggesting the biomineralization of material surface after hydration and the possibility of chemical bonding between the two interfaces. Such structure may be beneficial in material adaptation onto root dentin and sealing capacity at the material-root dentin interface. Since the layer was less than 5 µm in thickness in limited sample, investigations on crystallinity and phase of biomineralized structures other than SEM/EDS was not practicable in this case. Further indepth analysis including XRD analysis or infrared spectrum analysis is required.

SEM analysis clearly demonstrated dentinal tubule orifices occluded with mineralized structures (with Ca/P ratio higher than 1.67), probably resulted from precursor-precipitation phase of the material [5, 6] (Fig.2B). Intratubular mineralization structures were found from orifice along the tubule pathway with Ca/P ratio (range 1.30 – 2.12) (Fig.2C). We speculate that these precipitates, most probably amorphous calcium phosphate, was gradually formed from calcium ions leached from MTA and phosphorous ions in dentinal fluids [12, 13]. Such intratubular mineralization could physically entomb microorganism [9, 14], and also the depletion of intratubular phosphorus might result in the inhibition of E. faecalis since phosphorus ion is essential to the survival of E. faecalis [15]. Noteworthy the Ca/P ratio at the orifice level was relatively higher than deeper tubules, and decreased along the tubular pathway. This is probably affected by the diffusion of calcium ions from material surface. Such biomineralized structures in dentinal tubules around orifices suggest basic microenvironment which is beneficial in inflammation control, whilst those with lower Ca/P make acidic microenvironment inside the tubules which could provoke phase transition of CaP into unstable forms including

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carbonates [16, 17]. Such phase transition would be attributed to decreased permeability when carbonates could interact with fibronectin in dentin matrix [18, 19] but also recurrent periapical pathosis and microleakage of bacterial virulence factors.

This case report has clearly documented intratubular biomineralization by the careful SEM examination and EDS analysis of a root canal that had been filled with MTA for than 8 years. Leaving many other issues aside, this report partly shows clinical evidence of MTA as a root canal filling material in terms of long term stability of intratubular biomineralization. However, further in-depth investigations is required on the crystallinity and phase of biomineralized structures to presume clinical relevance of MTA root canal obturation.

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Author Contributions

Yeon-Jee Yoo, Yoo Sang Lee, Seok Woo Chang, and Kee-Yeon Kum conceived and designed the study; Jun-Sang Yoo performed the treatment and follow up of the patient; Chang Sun Yoo, Yoo Sang Lee, Hyen Sug Kang performed the *in vitro* analysis; Yeon-Jee Yoo, Hiran Perinpanayagam, Soram Oh, Seok Woo Chang, and Kee-Yeon Kum wrote the paper.

Conflicts of Interest

The authors declare no conflict of interest.

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Figure Legends

Figure 1. Intraoral periapical radiographs of the patient's mandibular right anterior dentition. (A) At initial presentation the canine had a subgingival fracture and prior root canal treatment. (B) The root canal was retreated and obturated with calcium enriched material, and the tooth restored with a gold cast post. (C) After 8 years, there was a subgingival root fracture below the level of crestal bone.

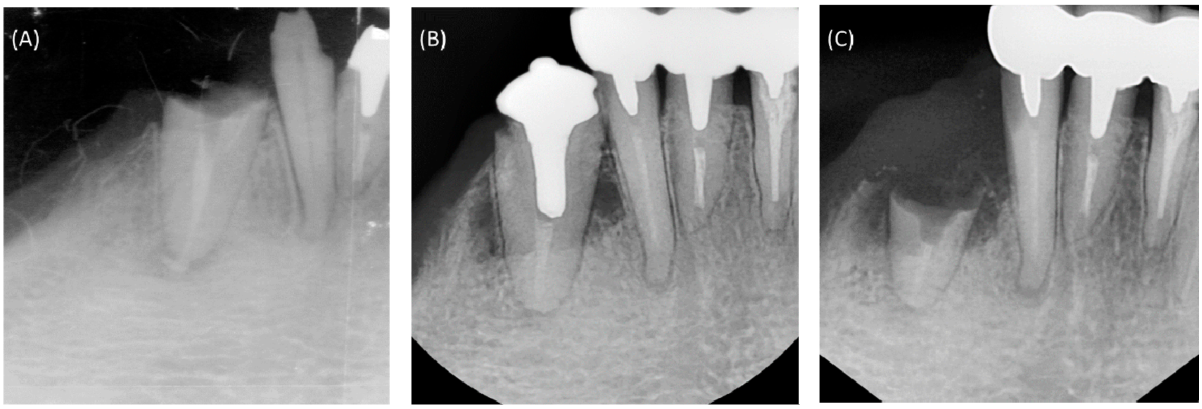

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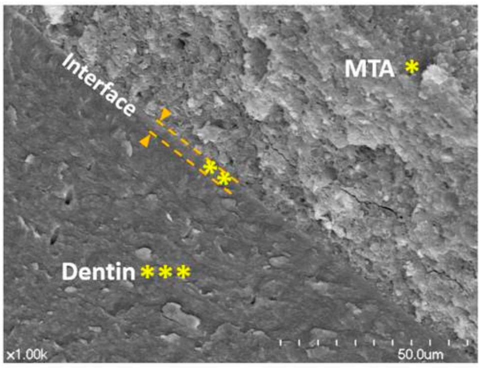
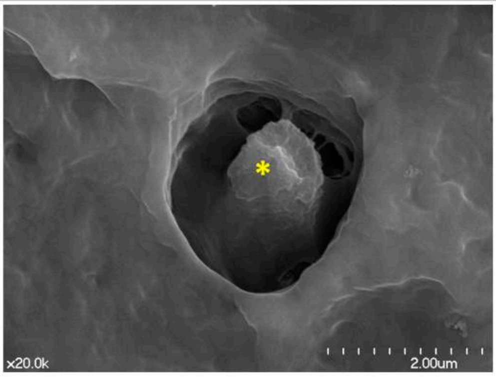
Figure 2. Scanning electron microscopic images that are representative of the root canal dentin and MTA interface. (A) There was an intermediate layer (**) at the interface of MTA (*) and dentin (***) (×1,000). (B) A dentinal tubule that was occluded by a mineralized structure (×20,000). (C) Higher magnification image (×50,000) of yellow boxed area in upper left image showing horizontal cross sectional view of the root (×50). Biomineralized dentinal tubules (arrowheads) at 1.0–2.0 mm distance from root canal. M, mineral trioxide aggregate; D, dentin. Tables show semiquantitative chemical composition showing Ca/P ratio of the pointed areas (*) in each figure.

(A)



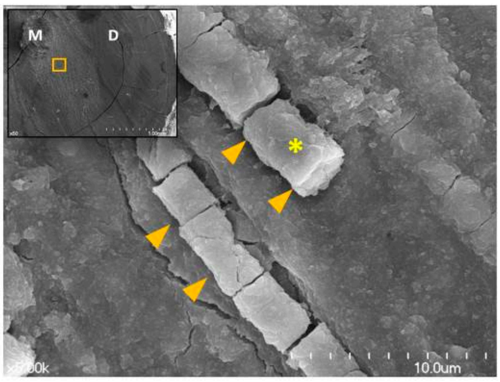
Element	At(%)		
	*	**	***
O	57.35	53.72	54.02
P	N/A	14.41	16.24
Ca	15.16	29.71	26.56
Si	16.23	15.41	N/A
Ca/P ratio	N/A	2.06	1.64

(B)



Element	At(%)
O	41.01
P	19.68
Ca	38.83
Si	N/A
Ca/P ratio = 1.97	

(C)



Element	At(%)
O	53.46
P	18.06
Ca	27.49
Si	N/A
Ca/P ratio = 1.52	