Effects of Calcium Silicate–based Materials on Collagen Matrix Integrity of Mineralized Dentin

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Abstract

Introduction: Calcium silicate–based materials (CSMs) are used in various endodontic procedures. The present study examined whether prolonged contact of mineralized dentin with recently commercialized versions of these materials adversely affects dentin collagen matrix integrity. Methods: Dentin slabs prepared from extracted human third molars (7 × 3 × 0.3 mm) were divided into 3 groups on the basis of the material to which dentin was exposed (MTA Plus, Biodentine, untreated control dentin slabs) and the time period of exposure (24 hours, 1, 2, and 3 months; n = 6). Hydroxyproline assay was performed on each group’s supernatant to quantify the collagen extraction amounts of each group per time period. Data were analyzed with two-factor repeated-measures analysis of variance and Holm-Sidak pair-wise comparisons (α = 0.05) to determine the effects of material and aging time on collagen extraction. Dentin slabs from the 3 months of aging group were demineralized for transmission electron microscopy examination of collagen matrix ultrastructural changes. Results: Material (P = .002), aging time (P < .001), and their interactions (P = .007) significantly affected the amount of hydroxyproline (pg/mg of mineralized dentin) extracted from mineralized dentin and were significantly correlated by power regression models. Collagen degradation was identified from the surface of dentin slabs that were in direct contact with CSMs. Conclusions: Prolonged contact of mineralized dentin with CSMs has an adverse effect on the integrity of the dentin collagen matrix. However, the amount of collagen extracted was limited to the contact surface. Clinicians can continue to apply CSMs in endodontic procedures; however, caution is advised when these materials are applied to thin dentinal walls. (J Endod 2012;38:829–833)

Key Words
Calcium silicate, collagen, hydroxyproline, transmission electron microscopy

Use of calcium silicate–based materials (CSMs) in dentistry became popularized (1–5) with the advent of mineral trioxide aggregate (MTA) in 1993 as a root-end filling material (6). Other developed clinical applications of MTA include root perforation repair (2–4, 7), pulp capping (2–4, 8–11), pulpotomy (3, 4), dens in dente cases (12), internal resorption (12), apexification or as an apical barrier for teeth with necrotic pulps and open apexes (2–4, 9, 12–14).

These numerous clinical applications of MTA are due to its significant inherent advantages, which include biocompatibility (2, 4), sealing ability (4, 6), regenerative capabilities (15, 16), and antibacterial characteristics (5). The antibacterial properties of CSMs are due to the release of calcium hydroxide (Ca(OH)2) on surface hydrolysis of the calcium silicate components (3, 17). However, on prolonged contact with dentin, Ca(OH)2 has been shown to adversely affect strength and fracture resistance (18–21). White et al (18) also found that bovine root dentin was weakened after 5 weeks of exposure to MTA. Further research is needed to determine the biochemical effects of CSMs on the collagen ultrastructure because dentin toughness is attributed to its collagen matrix (22, 23).

Other noted shortcomings of MTA such as difficult handling characteristics (24), long setting time (25, 26), high cost (2), and potential of discoloration (2) led to the development of new CSMs such as Biodentine (Septodont, Saint Maur des Fossés, France) and MTA Plus (Prevest-Denpro, Jammu City, India). Biodentine is a bioactive (27) dentin substitute composed of powder components of tricalcium silicate, calcium carbonate, and zirconium oxide and a water-based liquid containing calcium chloride as the setting accelerator and water-reducing agent (28). MTA Plus has a finer particle size than other commercially available MTA versions (50% of the particles finer than 1 μm) and comes with a salt-free water-soluble polymer gel as the mixing vehicle to improve its washout resistance (29).

With the development of these new commercially available products and the potential for decreasing dentin toughness, this study was designed to analyze the effects of Biodentine and MTA Plus on the collagen matrix integrity of mineralized dentin. The null hypothesis tested was that there are no changes in the amount of hydroxyproline extracted from mineralized dentin over time when the 2 CSMs are placed in direct contact with human dentin.
Materials and Methods

Mineralized Dentin Slabs and CSMs

One hundred forty-four extracted caries-free, nonrestored third molars were obtained after receiving patients' consent under a protocol approved by the Georgia Health Sciences University Human Assurance Committee. A 0.3-mm-thick tooth slice was obtained from the mid-coronal portion of each tooth by using a slow-speed saw (Isomet; Buehler Ltd, Lake Bluff, IL) under water cooling. A dentin slab (3 × 7 × 0.3 mm) was prepared from each tooth slice. Ninety-six slabs were randomly assigned to 8 experimental groups to be placed in contact with either of the 2 CSM groups for 4 designated aging times. The other 48 slabs were used as controls for each aging time.

MTA Plus and Biodentine were mixed according to manufacturers' instructions and placed in 3 × 7 × 2 mm silicone molds inside a 100% relative humidity chamber until set. For Biodentine, liquid from the single-dose container was emptied into the powder-containing capsule and triturated by using a capsule-mixer for 30 seconds, with a final setting time of 10 minutes. MTA Plus was hand-mixed by using a 3:1 powder-liquid ratio to achieve a putty-like consistency, with a final setting time of 1.2 hours.

Aging of Dentin Slabs in Contact with Set CSMs

Each mineralized dentin slab was first placed in anhydrous CaSO4 (W. A. Hammond Drierite Co Ltd, Xenia, OH) and weighed to constant mass (weighed half-hourly for 8 hours) by using a XP2U ultra-microbalance (Mettler Toledo, Highstown, NJ; repeatability at nominal load 0.00025 mg). Each slab was then rehydrated in a solution of 0.9% NaCl and 0.02% NaN3. Two rehydrated dentin slabs were placed in contact with a set CSM block with only one side of each slab exposed to the CSM to simulate contact of the material with crown/root dentin in a clinical scenario. Each dentin-CSR assembly was aged in a polypropylene microcentrifuge tube containing 400 μL of storage medium at 37°C for 24 hours, 1 month, 2 months, or 3 months. Twelve dentin slabs were placed into 6 vials (n = 6) for each material per aging time (2 materials × 4 aging times = 8 experimental groups). The storage medium consisted of 50 mmol/L HEPES buffer, 5 mmol/L CaCl2·2H2O, 0.001 mmol/L ZnCl2, 150 mmol/L NaCl, and 3 mmol/L Na3 to prevent bacterial growth (pH 7.2) (30). There were 4 control groups, 1 for each designated aging time (n = 6). No CSM was used in the control groups; dentin slabs were placed in the storage medium and aged as previously described.

Hydroxyproline Assay

Assessment of solubilized collagen peptides from the mineralized dentin slabs was performed by using hydroxyproline assay (31) to determine the amount of hydroxyproline present in the storage medium (30, 32, 33). The rationale for using hydroxyproline as a relative index of the amount of solubilized collagen peptide fragments is that 90% of the dry mass of demineralized dentin collagen consists of type I collagen, and that type I collagen contains 9.6 mass% hydroxyproline, whereas most other proteins contain no hydroxyproline (34). At the end of each designated aging time, the pH values of the storage media were measured by using a pH meter. Then, 200 μL of vortexed storage medium was collected from each vial and placed in an individually labeled ampule and diluted with an equal volume of 12 N HCl to give a final concentration of 6 N HCl. The ampules were flame-sealed (Amplumatic; Biosciences Inc, Allen-town, PA), and the media were hydrolyzed at 120°C in an oil bath for 18 hours. After hydrolysis, the ampules were opened and placed in glass desiccators containing Drierite and NaOH pellets to trap the water and HCl vapor released from the hydrolysates as they were evaporated to dryness. The hydroxyproline content of each hydrolysate was analyzed spectrophotometrically at 558 nm by using a Shimadzu Model UV-A180 spectrophotometer (Tokyo, Japan) in the transmission mode. The amount of hydroxyproline released from solubilized collagen peptide fragments was determined from a regression equation derived from absorbance values obtained from known concentrations of HCl-hydrolyzed hydroxyproline standards. The amount of hydrolyzed hydroxyproline was expressed as pg/mg of dehydrated mineralized dentin.

Statistical Analyses

One-way analysis of variance was first used to examine whether differences existed among the data obtained from the 4 control groups (24 hours, 1 month, 2 months, and 3 months) and those obtained from the 2 experimental groups at 24 hours. This analysis was performed after
validating the normality (Shapiro-Wilk test) and homoscedasticity assumptions (modified Levene test) of the data sets.

Because there were no statistically significant differences in the amount of extracted hydroxyproline among the 4 control groups and the 2 experimental groups after 24 hours of aging, the 24-hour results of the 2 CSM groups were used for statistical evaluation of the 8 experimental groups. Data were analyzed with two-factor repeated-measures analysis of variance to examine the effects of material and aging time and the interaction of those 2 factors on hydroxyproline content (normality assumption satisfied, \( P = .787 \); homoscedasticity assumption satisfied, \( P = .787 \)).
Transmission Electron Microscopy

Two dentin slabs from each of the 3-months-of-aging groups (control, no CSM; MTA Plus; and Biodentine) were completely demineralized in 0.5 mol/L ethylenediaminetetraacetic acid. Demineralized slabs were fixed in Karnovsky’s fixative, post-fixed with 1% osmium tetroxide, dehydrated in an ascending ethanol series (50%–100%), transitioned through propylene oxide, and embedded in epoxy resin. Ninety-nanometer-thick sections of the dentin slabs were prepared, stained with 2% uranyl acetate and Reynold’s lead citrate, and examined by using a JEM-1230 transmission electron microscope (JEOL, Tokyo, Japan) at 110 kV.

Results

The pH values of the storage media were 12.75 ± 0.09 and 12.49 ± 0.06 for MTA Plus and Biodentine, respectively, after 3 months of aging in the presence of the respective CSM (n = 6). Both material (P = 0.02) and aging time (P < 0.001) significantly affected the amount of hydroxyproline extracted from mineralized dentin (Fig. 1). The interaction of these 2 factors was also statistically significant (P = 0.007). For both CSMs, the amount of hydroxyproline extracted after 1 month of aging was significantly higher than that extracted after 24 hours (P < 0.05); the amount extracted after 3 months was also significantly higher than that extracted after 1 month (P < 0.05). For each CSM the increase in the amount of hydroxyproline with aging time was significantly correlated by a power regression model (Fig. 2; P < 0.05).

Minimal collagen degradation was observed from the surface of demineralized dentin slabs in the control group after 3 months of aging (Fig. 3A and B). By contrast, a zone of collagen degradation could be identified from the surface of dentin slabs that was in direct contact with CSMs. For MTA Plus, partially degraded collagen fibrils could be identified from the 1- to 2-μm-thick degradation zone (Fig. 3C and D). For Biodentine, collagen fibrils were completely broken down into microfibrils within the 5-μm-thick degradation zone (Fig. 3E and F).

Discussion

The results of this study do not support the null hypothesis that there are no changes in the amount of hydroxyproline extracted from mineralized dentin over time when the 2 CSMs are placed in direct contact with human dentin. The alkaline pH of both CSMs’ storage media after 3 months is mostly likely due to the production of Ca(OH)2 because this by-product is formed on reaction of CSMs with water and even with atmospheric humidity.

The composition of human dentin is 70 wt% mineral, 20 wt% organic, and 10 wt% water, with carbonated apatite as the inorganic component and type I collagen fibrils primarily as the organic constituent (23). The results shown in Figures 1 through 3 could be due to a disruption in the link between the carbonated apatite crystals and the collagen fibrils in dentin because of the Ca(OH)2 released in CSMs (20).

Usually, organic matters such as collagen molecules, fossilized growth factors, and endogenous matrix metalloproteinases within mineralized collagen fibrils in dentin are protected from degradation by extracellular and intracellular apatite crystals. Such a protective mechanism enables these organic matters to be preserved for thousands of years. For example, collagen could be detected by biochemical means from archaeological remains of human and animal bones (35). Both collagenases (molecular weight, 68–130 kDa) and matrix metalloproteinases associated with collagen degradation (molecular weight, 42–105 kDa) are bulky, high-molecular-weight molecules; their sizes preclude them from penetrating collagen molecules that are protected by intracellular apatite crystals. Apparently, both sodium hypochlorite (molecular weight, 74.4 Da) and Ca(OH)2 (molecular weight, 56.1 Da) alter the elastic modulus of the mineralized collagen fibrils and alter the 3-dimensional conformation of tropocollagen. Conceivably, a diffusion gradient of these molecules must have existed, which prevents their further inward diffusion into the underlying heavily mineralized dentin. Thus, the integrity of the subsurface collagen fibrils was preserved.

Even though increased amounts of hydroxyproline-containing collagen fragments were collected in the assays over time with both CSMs, these amounts are very small in respect to the amount of dentin (picograms of hydroxyproline versus milligrams of dentin). In accordance with these miniscule amounts of hydroxyproline, only surface degradation of collagen was observed by transmission electron microscopy for dentin exposed to the CSMs.

In conclusion, CSMs are still valid materials for clinical use in dentistry. However, caution is advised when CSMs are used to obturate root canals with thin dentinal walls and in filling the full length of the canal to prevent collagen degradation that might lead to root fracture.

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References