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Mechanism of bioactive molecular extraction from mineralized dentin by calcium hydroxide and tricalcium silicate cement

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ABSTRACT

Objectives. The objective of the present study was to elucidate the mechanism of bioactive molecule extraction from mineralized dentin by calcium hydroxide ($Ca(OH)_2$) and tricalcium silicate cements (TSC).

Methods and results. Transmission electron microscopy was used to provide evidence for collagen degradation in dentin surfaces covered with $Ca(OH)_2$ or a set, hydrated TSC for 1–3 months. A one micron thick collagen degradation zone was observed on the dentin surface. Fourier transform-infrared spectroscopy was used to identify increases in apatite/collagen ratio in dentin exposed to $Ca(OH)_2$. Using three-point bending, dentin exposed to $Ca(OH)_2$ exhibited significant reduction in flexural strength. Using size exclusion chromatography, it was found that the small size of the hydroxyl ions derived from $Ca(OH)_2$ enabled those ions to infiltrate the intrafibrillar compartment of mineralized collagen and degrade the collagen fibrils without affecting the apatite minerals. Using ELISA, TGF-β1 was found to be extracted from dentin covered with $Ca(OH)_2$ for 3 months. Unlike acids that dissolve the mineral component of dentin to release bioactive molecules, alkaline materials such as $Ca(OH)_2$ or TSC released growth factors such as TGF-β1 *via* collagen degradation.

Significance. The bioactive molecule extraction capacities of $Ca(OH)_2$ and TSC render these dental materials excellent for pulp capping and endodontic regeneration. These highly desirable properties, however, appear to be intertwined with the untoward effect of degradation of the collagen matrix within mineralized dentin, resulting in reduced flexural strength.

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1. Introduction

Calcium hydroxide ($Ca(OH)_2$) has been used in dentistry since the 1920s [1] and has long been considered the 'gold standard' for indirect and direct pulp capping procedures [2]. Tricalcium silicate cements (TSCs), introduced in 1993 as a root repair material [3], have been recommended as coronal seal materials for regenerative endodontic procedures [4], and are gradually replacing $Ca(OH)_2$ as endodontic repair materials for dentinogenesis [2,5]. In retrospective studies, the 5-year success rate after direct pulp capping with $Ca(OH)_2$ ranged from 37% to 82% [6]. The first large multicenter randomized clinical trial (RCT) and a most recent RCT both revealed more than 80% success rate using TSCs as the direct pulp capping materials [6,7].

The initial reaction of hydraulic cements such as TSCs with water is hydrolysis and ion exchange [8]. Hydration of the tricalcium silicate particles leads to rapid exchange of Ca^{2+} with H_3O^+ ions from the aqueous mixing solution to form a solid–liquid interface. Reaction of Ca^{2+} ions with OH^- ions derived from water results in the formation of $Ca(OH)_2$ that creates a highly alkaline environment [8]. Hence, $Ca(OH)_2$ and TSCs share similar characteristics that contribute to their successful clinical outcomes, including biocompatibility that facilitates dental tissue wound healing, bactericidal effects and induction of reparative dentinogenesis [9–11]. Recent reports indicate that these regeneration capacities are attributed to the extraction of a cocktail of bioactive molecules that are fossilized within the dentin during tooth development. These bioactive molecules include transforming growth factor-beta 1 (TGF-β1) and vascular endothelial growth factor (VEGF), which provide important signals for progenitor cell recruitment, differentiation and proliferation in angiogenesis and dentinogenesis, thereby contributing to tissue repair and regeneration [9–13]. Because mineralized dentin contains a reservoir of growth factors and other bioactive molecules, recent studies suggest that this hard tissue should be re-categorized as a bioactive matrix instead of a bioinert matrix [13,14]. While the highly alkaline nature of $Ca(OH)_2$ and TSCs is responsible for their extraction ability [9–11]; it also inadvertently affects the strength and fracture resistance of mineralized dentin after prolonged application of these materials [15,16].

The mechanism by which bioactive molecules are extracted from mineralized dentin by $Ca(OH)_2$ and TSC is not completely understood. During dentinogenesis, bioactive molecules secreted by the odontoblasts bind to extracellular matrix components such as collagen [17], to prevent their proteolytic degradation by endogenous enzymes such as matrix metalloproteinases and cysteine cathepsins [18,19]. During dentin mineralization, bioactive molecules–collagen binding complexes are trapped within the mineralized matrix by extrafibrillar and intrafibrillar apatite crystallites [20]. A recently developed model based on size exclusion chromatography sheds light on the potential mechanism of bioactive molecular extraction from mineralized dentin by $Ca(OH)_2$ and TSCs [21]. In that size exclusion model, molecules with molecular weight less than 286 Da can penetrate the intrafibrillar milieu of mineralized collagen fibrils, while molecules with higher molecular weights are excluded [21]. Based on this model, it may be rationalized that highly alkaline inorganic molecules such as $Ca(OH)_2$ may penetrate mineralized dentin and alter the 3-dimensional conformation of tropocollagen molecules. By degrading collagen fibrils from the mineralized dentin, the binding between collagen and bioactive molecules is destroyed, resulting in the release of the bioactive molecules. However, the same process also adversely modified the mechanical properties of mineralized dentin by destroying the organic component of the mineralized matrix and rendered the latter more brittle and susceptible to fracture. Although such a premise appears cogent, it has never been validated. Accordingly, the effects of $Ca(OH)_2$ and TSC on mineralized dentin were evaluated in the present study by the testing of two hypotheses: (1) the small size of the hydroxyl ion released by $Ca(OH)_2$ or TCS allows it to permeate the intrafibrillar compartment of mineralized collagen; and (2) $Ca(OH)_2$ and TSC release bioactive molecules such as TGF-β1 from dentin *via* collagen degradation within the mineralized matrix.

2. Materials and methods

2.1. Preparation of dentin discs and dentin powder

Six hundred and ninety-six extracted caries-free, unrestored third molars were obtained after receiving the patients' consent under a protocol approved by the Human Assurance Committee of Augusta University, with informed consent obtained from the donating subjects with respect to the use of human tissues. The extracted teeth were stored in 0.9% (w/v) NaCl containing 0.02% sodium azide at 4 °C for no longer than one month. A 0.5 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, USA) under water cooling. A dentin slab (3 mm wide, 7 mm long and 0.5 mm thick) was prepared from each of the 96 tooth slices, to be used for different parts of the experiments.

The other 600 teeth were used to create dentin powder according to the authors' previous protocol [21]. Briefly, the apical 4 mm of each root segment was removed. Enamel and pulpal tissues were removed using dental burs in a high-speed hand piece with copious air-water spray, as well as stainless steel files. The dentin specimens were cut into 1–1.5 mm slices with an Isomet saw (Buehler Ltd., Lake Bluff, IL, USA) under water cooling. The tooth fragments were immersed in liquid nitrogen for 15 min and crushed to a fine powder using a cutting mill (Model 3383-L10; Thomas Scientific, Swedesboro, NJ, USA). The powder was sieved through a 300–500 μm mesh sieve and kept frozen at −80 °C until use to prevent degradation of the collagen component.

2.2. Dentin-material assembly

Calcium hydroxide powder (Henry Schein Inc. Melville, NY, USA) was mixed with deionized water to produce a thick slurry according to manufacturer's instructions. The slurry was applied to one surface of a dentin slab. A hydraulic TSC (ProRoot® MTA white version, Dentsply Sirona, York, PA, USA) was mixed with the purified water supplied in a 3:1 pow-

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