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Effect of photoactivated riboflavin on the biodegradation-resistance of root-dentin collagen

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ABSTRACT

This study was conducted to evaluate the effect of UVA-activated 1% riboflavin solution on structural integrity; mechanical properties and stability; and collagenase-mediated collagen solubilisation resistance of demineralized root dentin collagen matrix. Root dentin specimens demineralized with 17% EDTA for 7 days were treated with 1% RF for 1 min followed by UVA photo-activation at intensity 7 mW/cm^2 for 1 min. Control specimens were completely devoid of riboflavin and/or UVA treatments. Specimens were challenged with bacterial collagenase type-I solution for different time-periods at 37 °C. Collagen solubilisation resistance was evaluated in terms of hydroxyproline (HYP) liberation. Mechanical characterization of dentin specimens before and after 24 h of exposure to collagenase solution was done in terms of apparent-elastic modulus (E_{appr}) and ultimate tensile strength (UTS). Variations in dentin collagen-network structure with exposure time in collagenase were visualized by TEM. Crosslinking dentin with UVA-activated riboflavin significantly decreased HYP release and increased E_{appr} and UTS compared to control specimens with storage time in collagenase. Moreover, crosslinked specimens showed higher structural resistance to collagenase effect reflected from dense, well-formed collagen fibrils-network with characteristic collagen cross-banding. UVA-activated riboflavin treatment increased collagenase-mediated collagen degradation resistance and enhanced mechanical stability against collagenase challenges of root dentin after EDTA demineralization.

1. Introduction

Dentin constitutes the greatest volume of coronal and root portions of tooth structure, thus its mechanical stability and degradation resistance are crucial aspects of restorative procedures. Dentin, in human teeth, consists of an inorganic phase mainly hydroxyapatite crystals, an organic phase, and water [1]. Makes up approximately 90% of the dentin organic matrix is composed of type I collagen fibrils with the other 10% consisting of proteoglycans and phosphoproteins. Fibrillar type I collagen is heterotrimeric with 3 domains comprising of two α1 chains and one α2 chain that has the non-triple helical NH_2-terminal (N-telopeptide), COOH-terminal (C-telopeptide) and the central triple helical domain [2].

Collagen matrix has been shown to be a significant benefactor to the mechanical strength and integrity of demineralized dentin [3]. In addition, root dentin showed significantly higher ultimate tensile strength than coronal dentin when demineralized with 0.5 M EDTA at a pH of 7.4 for 10 days, however, no difference was found in un-demineralized dentin. Cross-linking between coronal and root dentin collagen fibrils is thought to be significantly different. The differences noted between coronal and root dentin can likely be attributed to the differences between the embryological processes of their formation [4]. Odontoblasts in different locations may possibly exhibit differing site-specific enzyme expression and activation during dentinogenesis [5]. Root dentin does not appear to display peritubular dentin [6], and the proportion of the tubular area appears to be lower [7].

It was reported previously that the structural integrity, mechanical stability and enzymatic degradation resistance of the dentin collagen network play a crucial role in the determination of resin-dentin bond strength and bond durability [8]. Several recent studies have therefore attempted to strengthen and preserve the coronal dentin collagen matrix by cross-linking via auxiliary interactions to enhance mechanical properties and reduce enzymatic degradation, thereby, attempting to improve the durability of the resin-dentin interface [8,9]. In contrary, only few studies probed the effect of various cross-linking agents on the mechanical integrity and resistance to enzymatic degradation of root dentin collagen matrices [10,11].

Riboflavin, also known as Vitamin B_2, is an effective producer of

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oxygen radicals upon activation by ultraviolet-A (UVA) radiation. The production of singlet oxygen species allows for cross-linking of collagen fibrils through the process of photo-oxidation, thereby improving the physio-chemical properties of the collagen scaffolds. Because of its lack of toxicity, collagen cross-linking induced by UVA radiation of riboflavin has been reported as a successful and safe treatment for ophthalmic applications [12,13]. It is thought to work by enhancing the biomechanical properties of collagen matrix [14] and its resistance to enzymatic digestion [15].

In the field of dentistry, it was reported that pre-treatment of demineralized coronal dentin with UVA-activated riboflavin increased bond strength, stabilized the adhesive interface, and inhibited matrix-metalloproteinase (MMP) enzymes [16]. More recently, it was found that pre-treatment of acid-demineralized coronal dentin collagen matrices with UVA-activated 0.1 and 1% riboflavin solutions enhances bulk and surface mechanical stability, structural integrity and collagen resistance to degradation [17]. However, no previous studies investigated the effect of UVA-activated riboflavin on the resistance of root dentin collagen matrices to collagenase-mediated degradation. Therefore, this study aims to investigate the effect of UVA-activated 1% riboflavin solution on the mechanical stability, mechanical properties and resistance to degradation of demineralized root dentin collagen matrices. The null hypotheses were, cross-linking of EDTA-demineralized root dentin with UVA-activated 1% riboflavin would not adversely affect: the ultimate tensile strength (UTS), apparent elastic modulus (E_{appr}) or the hydroxyproline (HYP) release with the storage time in collagenase solution.

2. Materials and Experimental Procedures

Freshly-extracted sound human single-rooted anterior teeth (approved by the Institutional Review-Board of the National University of Singapore) with straight canals, as confirmed by radiography, were collected (n = 72). Teeth were stored in 0.5% chloramine-T solution for 2 weeks and then in distilled water at 4 °C [18] until they were used within one month from extraction time. Chemicals used in this study were purchased from Sigma-Aldrich unless otherwise stated.

2.1. Root Dentin Specimen Preparation and Cross-linking

The crowns of the single-rooted teeth were sectioned and the lengths of all roots were adjusted to 12 mm, using a low speed diamond saw (Isomet; Buehler Ltd., Lake Bluff IL, USA) under water cooling. Patency of each root canal was checked using 15 K-file and the working length (WL) was established 1 mm short of the apex. Cleaning and shaping were performed to the WL with a crown-down technique, using Pro-File nickel-titanium rotary instruments (Dentsply Maillefer, Ballaigues, Switzerland). Each canal was prepared to size 60, 0.06 taper, irrigated alternatively with 3 mL 2.6% NaOCl and deionized water between instrumentation. Dentin specimens (4 from each root) of 0.5 × 1.7 × 6.5 mm (thickness × width × length) were cut with low speed diamond saw under water irrigation, wet-polished to 2500 grit-size and demineralized in 17% EDTA for 7 days [10]. Solutions of 1% riboflavin (RF) were prepared by dissolving riboflavin-5-phosphate in distilled water and kept in light-proof test tubes to avoid premature light activation. The demineralized root dentin specimens were cross-linked with UVA-activated RF as described previously [17]. Briefly, the demineralized root specimens were rinsed in distilled water for 10 min, placed in the 1% riboflavin solution for 1 min, and photo-activated with UVA of 7 mW/cm^2 for 1 min. UVA-light source was used (Dymax, BlueWave, USA; wavelength: 370–410 nm) and placed 5 mm from the specimen surface with a spot size of 7 mm such that the whole dentin specimen was fully irradiated with a single UVA irradiation spot. The control specimens were prepared as above and placed in distilled water without exposure to the UVA light source.

2.2. Collagenase-mediated Collagen Resistance to Degradation

The resistance of the root dentin collagen matrices to enzymatic degradation was evaluated by measuring the hydroxyproline (HYP) liberation with challenging in bacterial collagenase type-I solution for 12, 24 and 48 h at 37 °C. The collagenase solution was prepared by dissolving 100 mg of collagenase type-I in 6 mL of tricine buffer and 3 mL of distilled water [19]. Specimens from the experimental and control groups were further subdivided to be placed in collagenase for 12, 24 and 48 h (n = 9). HYP liberation was measured by an assay kit (Bio-Vision Inc., CA, USA) according to manufacturer's instructions. After exposure to collagenase, a 100 µL aliquot of the supernatant was first collected and hydrolysed in 12 N HCl at 120 °C for 3 h. Next, 10 µL of hydrolysed specimen aliquots from each group were transferred to a 96-well plate and evaporated to dryness under vacuum. After that, 100 µL of chloramine-T buffer reagent was added to each well containing either the experimental specimen or the standard solution, and incubated for 5 min at room temperature. DMAB reagent (100 µL) was then added to each well and incubated for 90 min at 60 °C. A spectrophotometer plate reader (Infinite 200 Tecan, Switzerland) was used to measure the absorbance at 560 nm. The standard curves for the quantity of HYP liberation were generated and the HYP was averaged from triplicate measurements.

2.3. Mechanical Evaluation

The variations in the mechanical properties of the crosslinked and the control specimens before and after 24 h of exposure to the collagenase type I solution were investigated in terms of the apparent-elastic modulus (E_{appr}) and ultimate tensile strength (UTS) [17]. Briefly, for E_{appr} testing, specimens were fixed to a custom-made metallic jig and exposed to a three-point bending test. The span distance between the two metal supports was 2.5 mm. Compressive load was applied at the centre of the specimen perpendicularly at a cross-head speed of 0.5 mm/min through a 50 N load cell fixed to a universal testing machine (Instron, 5848 Microtester, USA). Stress-strain curves were plotted from the corresponding load-displacement curves and the apparent elastic modulus (E_{appr}) was calculated in MPa at 3% maximum strain. For UTS testing, specimens were fixed to a customized tensile metallic-jig by cyanoacrylate adhesive. The load was applied parallel to the longitudinal axis of the specimen at a cross-head speed of 1 mm/min until failure. All measurements were done in air while the specimens were in hydrated state.

2.4. Microscopic Investigation

Selective specimens from the crosslinked and the control groups were placed in the collagenase solution up to 72 h to be later viewed by transmission electron microscope (TEM) to study the variations in demineralized dentin collagen-network structure. At the specific exposure time points (12, 24 and 72 h), the specimens were retrieved and prepared for TEM viewing at different magnifications.

2.5. Statistical Analysis

Data was expressed as mean ± standard deviation and analysed by two-way ANOVA followed by Tukey-Kramer multiple-comparison post hoc tests for pair-wise comparison between the different groups in terms of E_{appr}, UTS and HYP release. $P < 0.05$ was considered significant. The normality of the data was explored through test of normality table (Shapiro-Wilk and Kolmogorov-Smirnov tests) and the normal Q-Q plots.

3. Results

The statistical analysis showed the significant effect and the