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Long-term evaluation of the stability of dentin matrix following treatments with aqueous solutions of titanium tetrafluoride at different concentrations

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ABSTRACT

Objective: The purpose of this study was to investigate the effects of aqueous solutions of different concentrations of titanium tetrafluoride (TiF_4) on dentin matrix stability up to six months.
Design: Dentin specimens prepared from fifteen nonerupted molars were demineralized and randomly distributed into groups: 2.5% TiF_4, 4% TiF_4, 1000 ppm NaF, and control (distilled water). The modulus of elasticity (ME) and dry masses of the dentin matrix were determined at baseline, and up to 6 months following treatment. Collagen solubilization was estimated by hydroxyproline (HYP) quantification in the simulated body fluid used to store the dentin specimens.
Results: The 2.5% TiF_4 group presented higher ME immediately after treatment, and at 3 and 6 months storage, whereas 4% TiF_4 showed higher means at 3 and 6 months ($p < 0.001$). No significant differences were observed among the groups over time ($p = 0.9325$). However, the 2.5% TiF_4 group showed significantly higher ME than the control group, immediately after treatment. All the groups presented significantly higher mass change immediately, compared with 3 and 6 months ($p < 0.0001$). Except for the 4% TiF_4 group, HYP release was higher in the first quarter ($p = 0.0152$), when no significant differences were found among the groups. In the second quarter, the means were significantly higher in the 2.5% TiF_4 and 4% TiF_4 groups. The group treated with 2.5% TiF_4 had a statistically higher HYP release than the control group.
Conclusion: An aqueous solution of 2.5% TiF_4 increases the immediate stiffness values, but does not stabilize the collagenous dentin matrix.

1. Introduction

Adhesion mechanisms are widely used in restorative dentistry. The adhesive interface is susceptible to degradation over time, particularly the hybrid layer. The degradation of the hybrid layer is triggered by host-derived matrix-bound proteases, such as matrix metalloproteinases (MMPs) and cathepsins (Breschi et al., 2008; Liu et al., 2011; Scaffa et al., 2017; Tersariol et al., 2010). Adhesion strategies use either prior acid-etching with conventional systems, or acidic monomers of the self-etching systems, creating micromechanical retention via a hybrid layer composed of resin and dentin of low stability (Tjäderhane, 2015). The loss of resin from the inter-fibrillar spaces and the disorganization of the collagen fibrils calls for the use of therapies focused on maintaining the stability not only of the resin but also of the dentin organic components (Castellan, Bedran-Russo, Karol, & Pereira, 2011; Lee & Sabatini, 2017).

The biomodification of dentin is a biomimetic approach to enhance and reinforce the dentin by locally altering the biochemistry and biomechanical properties (Bedran-Russo et al., 2014). Biodegradation of the dentin matrix can be evaluated by *in vitro* models using endogenous or exogenous proteases, and by determining collagen solubilization by hydroxyproline release (Bedran-Russo, Yoo, Ema, & Pashley, 2009; Castellan, Pereira, Grande, & Bedran-Russo, 2010; Hiraishi et al., 2013).

Although the use of titanium tetrafluoride (TiF_4) at 2.5% and 4% is not considered a biomimetic approach, it has been investigated for potential application as a dentin pretreatment to modify the dentin (Basting et al., 2015, 2017; Bridi, Amaral, França, Turssi, & Basting, 2013; Devabhaktuni & Manjunath, 2011; Domingues et al., 2014; Dündar, Ozcan, Cömlekoglu, & Sen, 2011; Torres et al., 2017; Tranquilin et al., 2016). TiF_4 forms a vitreous layer of titanium dioxide on the dentin surface, resulting from the bonding between the titanium and the oxygen from the phosphate group of dentin (Sen & Büyükyilmaz, 1998). During TiF_4 hydrolysis, the titanium ion has a

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great affinity for oxygen, with a strong tendency to form titanium phosphate complex (titanium ion reacts with the oxygen atom of the phosphates of the tooth structure). The "vitreous" or "glaze-like" dentin surface (Sen & Büyükyilmaz, 1998) has not been found to influence the bond strength values of total or self-etch adhesive systems (Basting et al., 2017; Bridi et al., 2013; Devabhaktuni & Manjunath, 2011; Domingues et al., 2014; Torres et al., 2017; Tranquilin et al., 2016). Furthermore, when used as a pretreatment on the demineralized dentin, TiF_4 has provided greater immediate bond strength to conventional adhesive systems (Tranquilin et al., 2016). However, there is limited understanding of the interaction of TiF_4 with the dentin matrix.

In addition to the increased fluoride uptake of dental hard tissues by TiF_4, the formation of an acid-stable surface layer providing mechanical protection to the surface could reduce dentin demineralization, and also stabilize the dentin matrix, according to the time applied and the concentration used. Thus, the aims of this study were to evaluate the long-term effect of TiF_4 on the modulus of elasticity of demineralized dentin, and the susceptibility of type I dentin collagen to biodegradation by endogenous and exogenous proteases. The null hypotheses to be tested were that there were no differences 1) in the modulus of elasticity; 2) in the amount of hydroxyproline released and 3) in the resistance to collagenase digestion of dentin matrices treated with different concentrations of TiF_4 and stored in simulated body fluid up to 6 months, in comparison with the control group.

2. Materials and methods

2.1. Dentin specimen preparation

Fifteen extracted sound human molars with no enamel cracks or malformations were selected for this study (Institutional Review Board Committee of the University of Illinois at Chicago, protocol # 2011-0312). The teeth were stored at −20 °C for no more than 6 months. Debridement procedure with scalpel blades and periodontal curettes was performed. A total of 60 dentin specimens (1.7 × 0.5 × 6.0 mm) were obtained as previously described (Bedran-Russo et al., 2009). After preparation, the beams were stored individually in a 2 ml saline solution containing sodium azide 0.01%. A dimple was made on one end of the surfaces to serve as orientation for repeated measurements to be performed on the same surface.

Dentin specimens were fully demineralized in 10% phosphoric acid (H_3PO_4) (Ricca Chemical Company, Arlington, TX, USA) for 5 h at room temperature. After demineralization was complete, the beams were rinsed profusely with distilled water to remove the acid, transferred to individually labeled containers, and placed overnight inside a desiccator containing anhydrous calcium sulfate at room temperature. The initial dry mass was measured with an analytical balance (XP6, Mettler Toledo Inc, Columbus, OH, USA), and immediately afterwards, the beams were rehydrated with distilled water for 1 h.

2.2. Preparation of treatment solutions

Treatment solutions included fresh preparations of TiF_4 (Sigma Aldrich, Saint Louis, MO, USA), TiF_4 P.A. (pro-analyses) dissolved in distilled water to final concentrations of 2.5% (w/v; pH 1.4) and 4% (w/v; pH 1.2), and NaF (Fisher Scientific, Fair Lawn, NJ, USA) dissolved in distilled water to a concentration of 1000 ppm of F (w/v; pH 6.7).

2.3. Apparent modulus of elasticity

The demineralized dentin specimens were randomly divided into four groups (n = 15), and immersed in 100 µl of treatment solution for 1 h, according to the assigned experimental group: 2.5% TiF_4, 4% TiF_4, 1000 ppm NaF (sodium fluoride), and distilled water (control). The demineralized dentin beams were assessed at baseline and after 1 h in their respective treatment solutions. The apparent modulus of elasticity was determined in a three-point bending flexural test with a 1-N load cell on a universal testing machine (EZ Graph, Shimadzu, Kyoto, Japan) at a crosshead speed of 0.5 mm/min (Castellan et al., 2010). All measurements were performed with specimens fully immersed in distilled water. The modulus of elasticity (Bedran-Russo, Pashley, Agee, Drummond, & Miescke, 2008) was calculated by displaying displacement (D) during compression in millimeters, and then calculating it at the maximum strain of 3%, using the following formula: $D = eL^2/6T$, where "e" is strain, "L" is support span, and "T" is thickness of the specimen. Then, the modulus of elasticity (E) of the specimens was expressed in MPa (Mega Pascals) and calculated using the following formula: $E = PL^3/4DbT$, where "P" is the maximum load, "L" is the support span, "D" is the displacement, "b" is the width of the specimen, and "T" is the thickness of the specimen.

After the treatment, the beams were rinsed with distilled water and the apparent modulus of elasticity was verified immediately. Measurements were performed before treatment (baseline), immediately following treatment, and 3 and 6 months after treatment. The beams were placed individually in Eppendorf tubes containing 1000 µl of simulated body fluid (SBF), and stored at 37 °C. The SBF solution contained 50 mmol/L HEPES; 5 mmol/L $CaCl_2{\cdot}2H_2O$; 0.001 mmol/L $ZnCl_2$; 150 mmol/L NaCl; and 3 mmol/L sodium azide at pH 7.4 (Carrilho et al., 2009).

2.4. Dentin matrix mass change

After measuring the apparent modulus of elasticity following treatment, the beams were transferred to individually labeled containers and placed in a desiccator containing anhydrous calcium sulfate overnight, again. As described before, the demineralized dentin beams were weighed before (M1) and after (M2) dentin treatment, with a precision of 0.01 mg. Evaluation of weight mass change (Wmc) was determined as the percentage of gain or loss in mass for each specimen, based on the following formula: Wmc (%) = ((M2 × 100)/M1)-100. The weight was measured after demineralization, after treatment, after 3 months and after 6 months.

2.5. Biodegradation by endogenous protease: hydroxyproline assay

Collagen solubilization was estimated by detection of hydroxyproline (HYP) in the storage media of the dentin specimens used to perform the stiffness test at 6 months. SBF (1000 µl) was collected every 2 weeks for a 6-month aging period. Since bacteria growth was impaired by adding sodium azide to this solution, the presence of collagen peptides (HYP) was detected by endogenous enzymatic activity. SBF was pooled to evaluate the HYP released up to 3 months and from 3 to 6 months.

The HYP assay followed a standard protocol (Reddy & Enwemeka, 1996) with some modifications. SBF collected and pooled from fifteen specimens treated with 2.5% TiF_4, 4% TiF_4, 1000 ppm NaF and distilled water was lyophilized and resuspended in 0.2 ml deionized water. Small aliquots of 0.01 ml were hydrolyzed in 2N NaOH at 120 °C for an hour, and mixed with 0.056 M chloramine T reagent for 25 min at room temperature. Next, 1 M Erlich's reagent was used to develop color at 60 °C for 40 min. Absorbance was measured at 550 nm in a 96-well plate spectrophotometer reader (Spectramax Plus, Molecular devices, Sunnyvale, CA, USA). Reference values for several HYP concentrations (0.5, 1, 2, 3, 4, 5 µg/mL) were generated using 1 µg/mL OH-L-proline in 1 ml deionized water.

2.6. Biodegradation by exogenous protease

2.6.1. Specimen preparation

A total of 180 dentin specimens (1.7 × 0.5 × 1.7 mm) were obtained as previously described (Bedran-Russo et al., 2009) from fifteen recently extracted sound human molars. After preparation, the beams