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Effect of Proanthocyanidin-enriched extracts on the inhibition of wear and degradation of dentin demineralized organic matrix

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ABSTRACT

Objectives: The aim of this study was to evaluate the effect of Cranberry and Grape seed-enriched extract gels in inhibiting wear and degradation of demineralized organic matrix (DOM).
Design: 225 dentin specimens obtained from bovine incisors were randomly allocated into 5 groups (n = 45): 10% Grape seed extract gel (GSE), 10% Cranberry extract gel (CE), 0.012% Chlorhexidine gel (CX), 1.23% NaF gel (F), and no active compound gel (P, placebo). Before the treatments, samples were demineralized by immersion in 0.87 M citric acid, pH 2.3 (36 h). Then, the studied gels were applied once over dentin for 1 min. Next, the samples were immersed in artificial saliva containing collagenase obtained from Clostridium histolyticum for 5 days. The response variable for dentin wear was depth of dentin loss measured by profilometry and for collagen degradation was hydroxyproline determination. Data were analyzed by ANOVA followed by Tukey's test and Pearson Correlation Test ($p < 0.05$).
Results: Grape seed extract significantly reduced dentin wear compared to the other groups ($p < 0.05$). Cranberry extract and Chlorhexidine did not differ statistically and were able to reduce wear when compared to NaF and placebo treatments. The hydroxyproline analysis showed that there was no significant difference among groups for all treatments ($p < 0.05$). Correlation analysis showed a significant correlation between the amount of degraded DOM evaluated by profilometry and the determination of hydroxyproline.
Conclusion: Cranberry extract was able to reduce the dentin wear and collagen degradation, likely due to the proanthocyanidin content and its action. Therefore, Cranberry could be suggested as an interesting natural-based agent to prevent dentin erosion.

1. Introduction

Erosive demineralization in dentin is completely distinct from that occurring in enamel, as acids cause a rapid dissolution of minerals, maintaining dentin organic portion (Ganss et al., 2010; Kleter et al., 1994). It is called demineralized organic matrix (DOM). In dentin erosion, maintenance of DOM is important because it acts as a diffusion barrier against erosive acids and the active ingredients released by demineralization (Kleter, Damen, Everts, Niehof, & Ten Cate, 1994). Moreover, studies demonstrated that DOM is also resistant against abrasive forces (Ganss, Hardt, Blazek, Klimek, & Schlueter).

However, the DOM can be degraded by host enzymes such as matrix metalloproteinases (MMPs) and cysteine-cathepsins present in both saliva and dentin (Tjaderhane et al., 1998; Tjaderhane et al., 2015; Zarella et al., 2015). Such enzymes are activated in acidic pH and degrade the exposed collagen in acidic or neutralized pH (Van Strijp, Jansen, De Groot, Ten Cate, & Everts, 2003).

Thus, several studies have investigated the effects of enzyme inhibitors against tooth erosion (Brackett et al., 2015; Gendron, Grenier, Sorsa, & Mayrand, 1999; Kato et al., 2009; Kato, Leite, Hannas, & Buzalaf, 2010; Kato, Leite, Hannas, Oliveira et al., 2010; Kato et al., 2012; Kato et al., 2014; Kim et al., 2011; Magalhães et al., 2009). Although inhibiting agents such as chlorhexidine (Gendron et al., 1999; Kato, Leite, Hannas, Oliveira et al., 2010; Kim et al., 2011; Magalhães et al., 2009) and fluoride (Brackett et al., 2015; Kato, Leite, Hannas, Buzalaf, 2010; Kato, Leite, Hannas, Oliveira et al., 2010; Kato et al.,

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2014) have shown excellent results, the search for natural protease inhibitors have increasingly attracted the attention of researchers (Bedran-Russo, Pashley, Agee, Drummond, & Mieschke, 2008; Bedran-Russo, Castellan, Shinohara, Hassan, & Antunes 2011; Bedran-Russo et al., 2014; Castellan, Pereira, Grande, & Bedran-Russo, 2010; Castellan, Bedran-Russo, Karol, & Pereira, 2011). This is mainly due to the lower toxicity and biocompatibility of natural agents.

Among these natural agents, Cranberry and Grape seed extract compounds are promising. In medical, dental caries and periodontal disease researches, benefits related to Cranberrýs polyphenols have been verified. The Cranberry extract has the ability to inhibit the adhesion of *S.sobrinus* to dentin (Weiss et al., 2004) and to reduce the development of dental caries *in vivo* (Koo et al., 2010). Furthermore, La, Howell, & Grenier (2009) reported that Cranberry was able to inhibit the production of MMPs in inflamed periodontal tissues and the catalytic activity of MMP-1 and MMP-9.

One of Cranberry's polyphenols is Proanthocyanidin, also found in cocoa, grape, and peanuts (Bedran-Russo et al., 2014), which demonstrated inhibitory effect on MMPs (Bedran-Russo et al., 2008; Bedran-Russo et al., 2014; Han, Jaurequi, Tang, & Nimni, 2003; Khaddam et al., 2014; Liu et al., 2014). Moreover, studies have emphasized the role of Proanthocyanidin-rich agents in dentin biomodification, mainly as agents that favor the induction of dentin collagen cross-links (Bedran-Russo et al., 2014; Castellan et al., 2010; Castellan et al., 2011; Xie et al., 2008). In addition, a recent study showed that Proanthocyanidin promoted less wear on dentin subjected to erosive challenge (Boteon, Prakki, Buzalaf, Rios, & Honório, 2017). Thus, it is important to evaluate the influence of these agents directly on the inhibition of dentin wear and collagen degradation.

Therefore, the aim of this study was to evaluate the effect of Cranberry and Grape seed extract in inhibiting wear and DOM degradation. The tested null hypotheses were: there is no difference in dentin wear, DOM degradation and hydroxyproline release of dentin specimens treated with different protease inhibiting agents.

2. Material and methods

2.1. Experimental design

Bovine incisors were used in this study. Obtained dentin specimens were randomly allocated into 5 groups: 45 specimens treated with 10% Grape seed extract gel (GSE), 45 specimens treated with 10% Cranberry extract gel (CE), 45 specimens treated with 0.012% Chlorhexidine gel (CX), 45 specimens treated with 1.23% NaF gel (F), and 45 specimens treated with no active compound gel (P). Before the treatments, samples were demineralized by immersion in 0.87 M citric acid, pH 2.3 (36 h at 4 °C). Then, the studied gels were applied once over dentin for 1 min. Next, the samples were immersed in artificial saliva containing collagenase obtained from *Clostridium histolyticum* for 5 days. The response variable for dentin wear was depth of dentin loss measured by contact profilometry, which was performed in the MarSurf GD 25 (Göttingen, Germany) profilometer equipped with a specific software called MarSurf XCR 20 (Göttingen, Germany). For collagen degradation, the response variable was the quantification of hydroxyproline (Fig. 1).

2.2. Dentin blocks preparation

In total, 225 samples (4 mm thick) were prepared from freshly extracted bovine incisors. We obtained each sample by sectioning the crown longitudinally with 2 parallel diamond disks (XLI 2205, Extec Corp., Enfield, CT, USA), separated by a 4-mm spacer. This allowed the removal of buccal and lingual enamel, creating a slice of dentin. Next, samples were sectioned by diamond disks to get dentin blocks (10 × 8 × 4 mm), which were stored and sterilized in 0.1% thymol solution (pH 7.0) at 4 °C. The surface of the blocks was ground flat with water-cooled carborundum discs (320, 600 and 1200 grades of $Al2O_3$ papers; Buehler, Lake Bluff, IL, USA), and polished with felt paper wetted with 1 μm diamond spray (Buehler®). The blocks were randomly divided into 5 groups. Prior to treatment, identification marks were made on the sample surfaces using a scalpel, which determined the area of treatment (1.0 × 0.5-cm) and allowed for accurate repositioning of the profilometer stylus. Subsequently, five baseline surface profiles were obtained from each wet block (only the excess of water was carefully removed with filter paper) using a profilometer (MarSurf GD 25, Göttingen, Germany) at specific distances from the edge: 2.25, 2.0, 1.75, 1.5, and 1.25 μm. The marks and external dentin surface were covered with nail varnish (Cosmed Commercial and Medicaments' Industry S/A, Barueri, São Paulo, Brazil) in order to produce reference surfaces for the wear analysis.

2.3. Treatment

Dentin blocks were demineralized by immersion in 0.87 M citric acid, pH 2.3 (36 h at 4 °C). Next, samples were thoroughly rinsed in deionized water (30 s). Excess water was removed with absorbent paper. After demineralization, the nail varnish was removed and five profilometric analyses were performed again at the same sites as the baseline measurements (2nd measurement). In sequence, the nail varnish was applied again and specimens were randomly allocated into 5 groups, according to the treatment gel (n = 45), as follow: 10% Grape seed extract (Purified Grape Seeds Oligomeric proanthocyanidins, 1298219, Sigma-Aldrich Co.®, USA), 10% Cranberry extract (Shaanxi M.R Natural Product Co., Ltd, Xi'an, China), 0.012% Chlorhexidine (Pharma Nostra®, Campinas, Brazil), 1.23% NaF (Via Farma®, São Paulo, Brazil), no active compound (Placebo). The studied gels were applied once over dentin for 1 min and subsequently removed with a cotton swab (Boteon et al., 2017; Kato, Leite, Hannas, Buzalaf, 2010; Kato, Leite, Hannas, Oliveira et al., 2010; Kato et al., 2012). All gel formulations presented essentially the same composition (hydroxyethylcellulose, propyleneglycol, methylparaben, imidazolidinyl urea, and de-ionized water, pH 7.0) except for the active compounds (Kato, Leite, Hannas, Buzalaf, 2010; Kato, Leite, Hannas, Oliveira et al., 2010).

Specimens were subjected to collagen degradation by the action of collagenase obtained from *Clostridium histolyticum* (Type VII, Product No. C0773, Sigma-Aldrich, St. Louis, MO, USA) added in artificial saliva (20 mmol/L HEPES, 0.70 mmol/L $CaCl_2$, 0.20 mmol/L $MgCl_2{\cdot}6H_2O$, 4 mmol/L KH_2PO_4, 30 mmol/L KCl, 0.30 mmol/L NaN_3) at a concentration of 100 U/mL, for 5 days (37 °C), without renewal (Ganss, Lussi, Klimek, & Stark, 2004).

2.4. Quantification of hydroxyproline

The collagenolytic activity was assessed by measuring the hydroxyproline content in artificial saliva after incubation with collagenase. Measurement of hydroxyproline in each sample was determined in triplicate, by quantitative analysis, as described previously (Jamall, Finelli, & Que See, 1981), with minor modifications. Briefly, a 0.002 mL aliquot sample (artificial saliva) was resolubilized with 1.198 mL of 50% isopropanol. A 0.2 mL aliquot of reagent A (3.9 mL of 7% chloramine-T solution + 1.1 mL citrate-acetate buffer) was added. After a 10-min interval, 1 mL reagent B (3 mL of Ehrlich's reagent + 8 mL 50% isopropanol) was added. Then, samples were incubated at 50 °C for 90 min (EVLAB; BM/EV. O15; Londrina, PR, Brazil). Hydroxyproline standards, containing 0–16 μg hydroxyproline were prepared in 50% isopropanol. The absorbance was spectrophotometrically read at 558 nm (Ultrospec 2000, Pharmacia Biotech, Cambridge, England) and corrected for the reagent blank. The standard curve had a coefficient of determination of 0.98.