UNIVERSIDADE DE SÃO PAULO
FACULDADE DE ODONTOLOGIA DE BAURU

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Effect of cranberry and proanthocyanidin in the inhibition of wear and dentin demineralized organic matrix degradation

Efeito do cranberry e da proantocianidina na inibição do desgaste e da degradação da matriz orgânica da dentina desmineralizada

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Orientador: Prof. Dr. Heitor Marques Honório

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DEDICATÓRIA

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ABSTRACT

ABSTRACT

Effect of Cranberry and proanthocyanidin in the inhibition of wear and dentin demineralized organic matrix degradation

The literature recognizes that one of the most effective strategies in minimizing the dentin tissue loss by erosive agents is the maintenance of its organic matrix, because this acts as a diffusion barrier to acid. However, the collagen tends to be degraded and has been increasingly used to search for agents that inhibit this process. There is evidence that the Cranberry and proanthocyanidin can inhibit matrix metalloproteinases (MMPs) which degrade the dentin collagen, but these agents have not been adequately evaluated in dental erosion. The aim of this dissertation was to analyze the role of Cranberry extract and proanthocyanidin applied as a local gel for inhibiting the dentin demineralized organic matrix degradation and hence minimizing wear of dentin subjected to erosion. To this end, two studies were conducted (article 1 and 2). The first evaluated the effect of different concentrations (placebo, 0.05%, 1%, 5% e 10%) and application time (1 and 5 minutes) of proanthocyanidin gels on dentin erosion. After gels application, the dentin blocks were subjected to 3 erosive cycles per day, during 5 days. Profilometry was used to quantify the dentin loss. In this case, proanthocyanidin not have shown a dose and time-response effect, but the results of this study suggests its efficacy on decreasing the deleterious effects of erosion on the dentin, since different proanthocyanidin gels were able to promote lower dentin wear when compared with the placebo gels. The second study evaluated the effect of different gels (proanthocyanidin, chlorexidine, Cranberry, NaF e placebo) in inhibition wear and demineralized organic matrix (DOM) degradation. Before the treatment, samples were demineralized by immersion in citric acid (0,87 M, 36 h). Then, the studied gels were applied once on dentin for 1 minute. Next, the samples were immersed in artificial saliva containing collagenase obtained from *Clostridium histolyticum* for 5 days. The response variable was depth of dentin loss measured by profilometry. The results of this study showed that Cranberry was able to reduce the dentin wear and collagen degradation, but the proanthocyanidin obtained the best results, confirming its effectiveness in preventing dentin erosion.

Keywords: Dentin. Erosion. Tooth wear.

RESUMO

RESUMO

Efeito do Cranberry e da proantocianidina na inibição do desgaste e da degradação da matriz orgânica da dentina desmineralizada

A literatura reconhece que uma das estratégias mais efetivas na minimização da perda de tecido dentinário promovido por agentes erosivos é a manutenção da sua matriz orgânica, visto que esta atua como barreira de difusão aos ácidos. Contudo, o colágeno tende a ser degradado por metaloproteinases da matriz (MMPs) e tem sido cada vez mais frequente a busca por agentes que possam inibir este processo. Existem indícios de que o Cranberry e a proantocianidina podem inibir as MMPs, porém estes agentes não foram adequadamente avaliados na erosão dentária. Assim, o objetivo desta dissertação foi avaliar o papel do extrato de Cranberry e da proantocianidina aplicados como gel tópico na inibição da degradação da matriz orgânica da dentina desmineralizada e consequentemente na minimização do desgaste da dentina submetida à erosão. Para isso, dois estudos foram conduzidos (artigo 1 e 2). O primeiro avaliou o efeito de diferentes concentrações (placebo, 0,05%, 1%, 5% e 10%) e tempos de aplicação (1 e 5 minutos) da proantocianidina na dentina submetida à ciclagem erosiva *in vitro*. Após a aplicação dos géis, os blocos de dentina foram submetidos a 3 ciclos de des-remineralização por dia, durante 5 dias. A perfilometria foi utilizada para quantificar a perda de dentina. Neste estudo, a proantocianidina não mostrou um efeito dose e tempo/ resposta, mas os resultados deste trabalho sugerem a sua eficácia na redução dos efeitos deletérios da erosão na dentina, uma vez que géis de diferentes concentrações de proantocianidina foram capazes de promover menor desgaste da dentina quando comparado com os géis placebo. O segundo estudo avaliou o efeito de diferentes géis (proantocianidina, clorexidina, Cranberry, NaF e placebo) na inibição do desgaste e degradação da matriz orgânica desmineralizada (DOM). Antes do tratamento, as amostras foram desmineralizadas por imersão em ácido cítrico (36 h a 4ºC). Os diferentes géis foram aplicados sobre a dentina durante 1 minuto. Em seguida, as amostras foram imersas em saliva artificial contendo colagenase do *Clostridium histolyticum*, durante 5 dias. A variável de resposta foi de profundidade de perda de dentina medida por meio de perfilometria. Os resultados deste estudo mostraram que o grupo Cranberry foi capaz de reduzir o desgaste da dentina e a degradação do colágeno, mas a proantocianidina obteve os melhores resultados, confirmando a sua eficácia na prevenção da erosão da dentina.

Palavras-chave: Dentina. Erosão. Desgaste dentário.

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1 INTRODUCTION

1 INTRODUCTION

Tooth wear promoted by erosion can be described as a lesion characterized by structure loss by the action of acids without the involvement of micro-organisms and may involve intrinsic or extrinsic processes (IMFELD, 1996; ECCLES, 1979; ZERO,1996; MEURMAN; TEN CATE, 1996; LUSSI et al., 2004; LUSSI, 2006; MAGALHÃES et al., 2009a). Intrinsic erosion is caused by acids from the digestive system, drug use, pregnancy, chronic indigestion, hiatal hernia, gastroesophageal reflux disease, chronic alcoholism and psychosomatic disorders (vomiting, as part of anorexia or bulimia) (RYTOMAA et al, 1998; O'SULLIVAN et al, 1998; ZERO, 1996; SCHEUTZEL, 1996; BARTLETT, 2006). On the other hand, extrinsic erosion occurs by exogenous acids, including acidic substances, beverages, food or environmental exposure to acid agents (ECCLES, 1979; ZERO, 1996; LUSSI, 2006). Extrinsic processes are quite frequent and several epidemiological and clinical case reports studies have shown the association of dietary habits with dental erosion (ECCLES, JENKINS, 1974; ECCLES, 1979; ECCLES, 1982; MILLWARD et al, 1994; NUNN 1996; WATERHOUSE et al, 2008; MURAKAMI et al, 2010)..

In more advanced cases, dentin can be exposed, which is usually associated with pain and hypersensitivity (O'SULLIVAN et al., 1998; RIOS et al., 2007; RYOTOMMA et al., 1998). At this stage, the treatment becomes more complex, as it is necessary procedures that require more time and cost to the patient. Thus, prevention has an important role in minimizing these problems through simple steps, cheap procedures and less inconvenient for the patients. Therefore, it is necessary understand how erosion occurs in dentin.

Dentin is constituted by 47% of inorganic components (apatite), 33% organic component and 20% of water. Among organic components, 90% consists of Type I collagen and 10% non-collagenous components: phosphoproteins dentin, proteoglicans and glicosaminaglicans (MAGALHÃES et al, 2009a; PASHLEY et al, 2004; SILVERSTONE; HICKS, 1985). The dentin demineralization rate decreases when the amount of degradable collagen increases, thus maintaining this collagen hinders the diffusion of acid into the tissue, minimizing erosion (KLONT, TEN CATE., 1991; KLETER et al, 1994). However, the organic matrix can be degraded mechanically and chemically, contributing to the progression of dentin wear (GANSS, 2004., 2007, HARA et al., 2005). This chemical degradation occurs,

by the enzymes called matrix metalloproteinases (MMPs), which can be activated at pH below 4.5 (TJÄDERHANE et al., 1998).

After an erosive challenge, the drop in pH, as well as demineralization of dentin, exposing the collagen fibrils, active MMPs that degrade this demineralized organic matrix. All this allows the progression of dentin loss. Thus, the application of MMP inhibitors could reduce this dentin loss for subsequent erosive challenges because the organic matrix would function as a protective layer, which would prevent the diffusion of the acid, reducing the progression of erosion (GANSS et al., 2004).

Thus, several studies have investigated the effects of enzyme inhibitors against tooth erosion (KATO et al., 2009; 2010; 2012; 2014; KIM et al., 2011). Inhibiting agents such as chlorhexidine (GENDRON et al., 2009; KATO et al., 2010; KIM et al., 2011) and fluoride (KATO et al., 2010; 2014; BRACKET et al., 2015) have shown excellent results in this process, however the search for natural proteases inhibitors have increasingly attracted the attention of researchers (XIE, BEDRAN-RUSSO, WU, 2008, CASTELLAN et al, 2010; 2011, BEDRAN-RUSSO et al; 2014). The main reasons for this are the lower toxicity of natural agents and minimal side effects.

Among natural agents, Cranberry and Proanthocyanidin's rich agents have been shown a important role. In medical, dental caries and periodontal disease researches, benefits related to Cranberry´s polyphenols were verified. The Cranberry extract has the ability to inhibit the adhesion of *S.sobrinus* to dental tissues of dentin (WEISS EI et al 2004), and reduce the development of dental caries *in vivo* (KOO H et al, 2010). Furthermore, La, Howell and Grenier in 2009, reported that Cranberry was able to inhibit the production of MMPs in some 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 in dental caries and adhesion studies (BEDRAN-RUSSO et al, 2008; 2014; HAN et al 2003, KHADAM et al, 2014, LIU et al, 2014). Moreover, studies emphasize the role of rich agents on proanthocyanidins in dentin biomodification, mainly these agents induce cross-links in dentin collagen (XIE, BEDRAN-RUSSO, WU, 2008, CASTELLAN et al, 2010; 2011, BEDRAN-RUSSO et al; 2014). Thus, at this moment, it is important to evaluate the influence of these agents on dentin subjected to erosion. Additionally, it needed to verify the performance of these agents directly in the inhibition of dentin wear and collagen degradation.

Thus, based on the above, the aim of this dissertation was to analyze the effect of Cranberry and proanthocyanidin in inhibiting wear and DOM degradation of dentine exposed to acid challenges.

2 ARTICLES

2.1 ARTICLE 1

Article 1 - The article listed below was accepeted for publication and cannot be reproduced in this dissertation for copyright reasons.

Boteon, A. P.; Prakki, A.; Buzalaf M.A.R; Rios, D.; Honório, H.M. Effect of different concentrations and application time of proanthocyanidin gels on dentin erosion. **Am J Dent**. Unpublished results.

The manuscript's acceptance e-mail is presented in Annex 1 (page 49).

2. 2 ARTICLE 2

Article 2 - The article presented in this Dissertation was written according to the Journal of Dentistry instructions and guidelines for article submission

Effect of Cranberry and proanthocyanidin in the inhibition of wear and dentin demineralized organic matrix

ABSTRACT

Objectives: The aim of this study was to evaluate the effect of Cranberry and Proanthocyanidin in inhibiting wear and demineralized organic matrix (DOM) degradation.

Methods: Bovine incisors were used in this study and dentin specimens obtained were randomly allocated into 5 groups (n=45): Proanthocyanidin (10%), Cranberry (10%), Chlorhexidine (0,012%), NaF (1.23%), no active compound (P, placebo). Before the treatment, samples were demineralized by immersion in 0.87 M citric acid, pH 2.3 (36 h at 4ºC). Then, the studied gels were applied once over dentin for 1 minute. Next, the samples were immersed in artificial saliva containing collagenase obtained from Clostridium histolyticum for 5 days. The response variable was depth of dentin loss measured by means of profilometry. Data were analyzed by One Way Analysis of Variance (ANOVA) followed by Tukey's test were applied. The level of significance was set at 5%.

Results: Data showed statistically significant difference for the dentin wear and DOM degradation between Proanthocyanidin and the other groups. There was no difference between Cranberry and chlorhexidine groups and no statistical difference was also found between the placebo group and NaF, but both agents presented dentin wear and DOM degradation significantly higher when compared to other groups.

Conclusion: The results of this study showed that Cranberry was able to reduce the dentin wear and collagen degradation, but the proanthocyanidin obtained the best results, confirming its effectiveness in preventing dentin erosion.

Clinical Significance: Cranberry and proanthocyanidin gels could be considered as an alternative therapy for erosion dentin wear and collagen degradation because these agents act to keep the organic matrix intact, which acts as a barrier against the diffusion of the acids from erosion.

Keywords: Collagen. Dentin. Erosion. Preventive dentistry and Wear

INTRODUCTION

Erosive demineralization in dentin is completely distinct that from in enamel, because acids cause a rapid dissolution of mineralized portion, but the organic portion is maintained [1,2]. This organic portion is called demineralized organic matrix (DOM). In dentin erosion, the maintenance of the DOM is important because it acts as a diffusion barrier against erosion acids and the active ingredients diffused by it [2]. Moreover, studies demonstrated that DOM is also resistant against abrasive forces [3].

However, the DOM can be degraded by the host enzyme matrix metalloproteinases (MMPs) present in both saliva and dentin [4,5,6] which are activated in acid pH and degrade the exposed collagen when the pH is neutralized [7,8].

Thus, several studies have investigated the effects of enzyme inhibitors against tooth erosion [9-16]. Inhibiting agents such as chlorhexidine [11,13,16] and fluoride [9,10,15] have shown excellent results in this process, however the search for natural proteases inhibitors have increasingly attracted the attention of researchers [17-19, 20,21]. The main reasons for this are the lower toxicity of natural agents and minimal side effects.

Among natural agents, Cranberry and Proanthocyanidin's rich agents have been shown a important role. In medical, dental caries and periodontal disease researches, benefits related to Cranberry´s polyphenols were verified. The Cranberry extract has the ability to inhibit the adhesion of *S.sobrinus* to dental tissues of dentin [22], and reduce the development of dental caries *in vivo* [23]. Furthermore, La, Howell and Grenier [24] reported that Cranberry was able to inhibit the production of MMPs in some inflamed periodontal tissues and the catalytic activity of MMP-1 and MMP-9. Then, it is important to evaluate its action on dentin subjected to erosion.

One of Cranberry's polyphenols is proanthocyanidin also found in cocoa, grape, and peanuts [19], which demonstrated inhibitory effect on MMPs [17,19, 25-27]. Moreover, studies emphasize the role of proanthocyanidins rich agents in dentin biomodification, mainly these agents induce cross-links in dentin collagen [19-21, 28]. In addition, a recent study showed that proanthocyanidin promoted less wear on dentin subjected to erosion [29]. Thus, at this moment, it is important to evaluate the influence of these agents directly in the inhibition of dentin wear and collagen degradation.

Thus, based on the above, the aim of this study was to evaluate the effect of Cranberry and Proanthocyanidin in inhibiting wear and DOM degradation.

MATERIAL AND METHODS

Experimental design

Bovine incisors were used in this study and dentin specimens obtained were randomly allocated into 5 groups: Proanthocyanidin (10%), Cranberry (10%), Chlorhexidine (0,012%), NaF (1.23%), no active compound (P, placebo). Before the treatment, samples were demineralized by immersion in 0.87 M citric acid, pH 2.3 (36 h at 4ºC). Then, the studied gels were applied once over dentin for 1 minute. Next, the samples were immersed in artificial saliva containing collagenase obtained from *Clostridium histolyticum* for 5 days. The response variable was depth of dentin loss measured by profilometry.

Blocks Preparation

In total, 225 slices of 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 for removal of vestibular and lingual enamel, creating a slice of dentin.

Next, samples were sectioned by diamond disks to get dentin blocks (10x8x4 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 Al2O3 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 blocks surfaces using a scalpel, which determined the area of treatment (1.0 x 0.5-cm) and allowed for accurate repositioning of the stylus. Subsequently, five baseline surface profiles were obtained from each wet blocks (only the excess of water was carefully removed with filter paper) using a profilometer (MarSurf GD 25, Göttingen, Germany) at certain 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 in order to allow reference surfaces for wear analysis.

Treatment

Dentin blocks were demineralized by immersion in 0.87 M citric acid, pH 2.3 (36 h at 4ºC). Next, samples were thoroughly rinsed in de-ionized water (30 sec). Excess water was removed with absorbent paper. After demineralization the nail varnish was removed and five profilometric analysis was performed again at the same sites as the baseline measurements (2^{nd} measure). In sequence, the nail varnish was applied again and specimens were randomly allocated into 5 groups, according to the treatment gel (n = 45 per group), as follows: 10% Proanthocyanidin (Purified Grape Seeds Oligomeric Proanthocyanidins, 1298219, Sigma-Aldrich Co.®, USA), Cranberry (10%), Chlorhexidine (0,012%), NaF (1.23%), no active compound (P, placebo). The studied gels were applied once over dentin for 1 minute. All gels formulations presented essentially the same composition (hydroxyethylcellulose, propyleneglycol, methylparaben, imidazolidinyl urea, and de-ionized water, pH 7.0) except for the active compounds.

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 CaCl2, 0.20 mmol/L MgCl2.6H2O, 4 mmol/L KH2PO4, 30 mmol/L KCl, 0.30 mmol/L NaN3) at a concentration of 100 U/mL, for 5 days (37ºC) [30].

Profilometric Analysis

The dentin blocks were maintained wet until the analysis to avoid shrinkage of the organic layer. Immediately before the profilometric measurement, only the excess of water was carefully removed with filter paper. After the immersion time, the nail varnish was removed and five profilometric analysis was performed again at the same sites as the baseline measurements (3rd measure). The dentin blocks were precisely repositioned in the wells of the profilometer, enabling baseline profiles to match with the final ones. Then, the dentin loss was quantitatively determined using specific software (MarSurf XCR 20, Göttingen, Germany) by calculating the average depth of the eroded surface relative to the baseline surface profiles. The response variables were the dentin wear (difference between 1st and 3rd measures) and total amount of degradeted DOM (difference between 2nd and 3rd measures) (Figure 1).

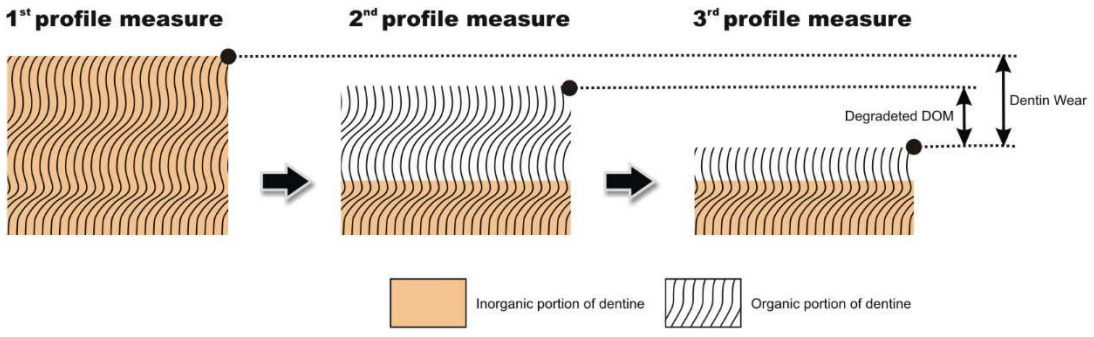

Figure 1- Illustrative scheme of wear analysis

Statistical Analysis

Statistical analysis was performed with SigmaPlot version 12.3 (2011 Systat Software, Germany). The assumptions of equality of variances and normal distribution of errors were checked. Since the assumptions were satisfied, One Way Analysis of Variance (ANOVA) followed by Tukey's test were applied. The level of significance was set at 5%.

RESULTS

The results of dentin wear and DOM degradation are shown in table 1. One Way ANOVA showed statistically significant difference for the dentin wear and DOM degradation between Proanthocyanidin and the other groups. There was no difference between Cranberry and chlorhexidine groups. No statistical difference was also found between the placebo group and NaF, but both agents presented dentin wear and DOM degradation significantly higher when compared to other groups.

Table 1 – Mean (±S.D.) of dentin loss (μm) and DOM degradation for the studied groups.

Groups	Dentin Wear	Degradeted DOM
Proanthocyanidin	110.0356 ± 41.425[a]	68.9623 ± 34.751[a]
Chlorhexidine	154.3072 ± 40.544[b]	115.6512 ± 37.342[b]
Cranberry	159.5203 ± 45.812[b]	120.1921 ± 44.779[b]
NaF	190.0779 ± 46.241[c]	151.5199 ± 40.925[c]
Placebo	208.4750 ± 59.674[c]	161.7586 ± 60.079[c]

* Different lowercase letters indicate statistically significant difference between the groups for the dentin wear and DOM degradation (One Way ANOVA and Tukey's Test, $p<0.05$).

DISCUSSION

Protease inhibitors have been studied for dentin erosion therapy in different vehicles, such as rinsing solutions or gels for topical application. Rinsing solutions are for home use by patients, however their application is not practical, since it is necessary to rinse the solutions immediately after each erosive challenge. On the other hand, gels are for professional use, presuming no need for frequent application. In the present study, cranberry and proanthocyanidin were tested as gels with single application after an erosive challenge of 36 hours. In gastro-esophageal reflux patients, the pH drops below 5.5 for 4.3 minutes during 24 hours [31]. Thus, an erosive time of about 4 minutes with HCl (pH 3.0) might simulate one day of erosion on a clinical situation. When extrapolating this simulation to the present protocol, even knowing that citric acid (pH 2.3) is an extrinsic acid, the dentin was subjected to erosion for 36 hours, which might simulate 540 or more *in vivo* days. In this study, three gels (proanthocyanidin, Cranberry and chlorhexidine) were able to minimize dentin wear when compared to placebo group during the studied period. However, on future studies it is important to evaluate how long the gels protective effect lasts, for predicting the time need for professional reapplication.

It is known that profilometry on eroded dentin does not reflect mineral loss, because there is interference of collagen matrix [3]. In order to verify the exact mineral loss, in the present study the collagen matrix was removed by the action of collagenase [32].

The thickness of DOM was estimated by calculating the difference between the level of the surface after collagen degradation (3rd measure) and the level of the surface after demineralization (2nd measure), similar to was carried out by Schlueter et al, 2012 [33]. Therefore, each dentin block was positioned correctly in the profilometry during three

measures [34]. In addition, the dentin was maintained under wet conditions to avoid the shrinkage of collagen matrix [35].

During the second measure, it was observed that wear was low despite of the challenge (citric acid, pH 2.3, 36h); this is illustrated in figure 1. This fact shows that the surface measured by profilometry was probably the remained collagen matrix, because the wet conditions were maintained [3]. However, after treatment and immersion on artificial saliva with collagenase, the exact wear was revealed, highlighting the placebo group with higher values. What did not occur to the so-called good agents NaF, chlorhexidine, Cranberry and mainly proanthocyanidin. These results indicate that the agents with the ability to inhibit collagenolytc enzymes may have preserved part of the DOM, even after the action of collagenase. This can be due to the maintenance of the DOM itself or to its action on the de – and remineralization process [36].

This was clearly seen in the results of proanthocyanidin, which presents interesting features. Studies show that proanthocyanidin positively affects the demineralization and/or remineralization processes and its remineralizing mechanism seems to be different from fluoride [28]. What happens is the formation of insoluble complex that remains stable in acid pH [37], that further bind to the Ca2 + ions in saliva, thereby enhancing the remineralization [28]. In addition, proanthocyanidin have improved the DOM by its ability to induce cross-links in dentin collagen [19-21, 25, 28]. The relative stiffness of the collagen fibrils depends on the formation of endogenous and exogenous cross-links [38]. Endogenous cross-links happen between the telopeptide and adjacent helical domains of type I collagen molecules. The interaction of proanthocyanidin and collagen results in the induction of exogenous cross-links primarily by hydrogen bonding between the protein amide carbonyl and the phenolic hydroxyl [39] and also covalent and hydrophobic bonds. The relatively large stability of these

cross-links compared with other polyphenols (such as tannins) suggests structure specificity [40], which although encouraging hydrogen binding also create hydrophobic pockets [25].

Another possible reason for the performance of the proanthocyanidin is the concentration used in this present study (10%). Studies showed good results in concentrations of proanthocyanidin less than 10% and its dose-response effect [17].

Cranberry has not achieved the proanthocyanidins' results despite it is being present in Cranberry's composition. This may be due to proanthocyanidin concentration in Cranberry be around the 65% [41] a lower value than purified proanthocyanidin used in this study. However, the Cranberry group showed performance similar to chlorhexidine group, which presents recognized ability to reduce DOM degradation [16,42].

In the present study, NaF group showed no statistical difference from placebo group. This finding is similar to that found by Kato et al, 2010 [9]. These results could be due to the response variable used in these studies, which is dentin wear instead of a direct analysis of the different gels ability to inhibit collagen degradation. Other studies demonstrated the ability of NaF to reduce the collagen degradation [14] and inhibit MMPs [15].

Therefore, further investigations is needed to prove the influence of agents, mainly proanthocyanidin, directly in the inhibition of collagen degradation before indicating its use.

CONCLUSION

Cranberry was able to reduce the dentin wear and collagen degradation similarly to chlorhexidine, but the proanthocyanidin obtained the best performance, showing its effectiveness in preventing dentin erosion.

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3 DISCUSSION

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The importance of DOM maintenance to minimize the progression of dentin erosive lesions was already demonstrated (BUZALAF MA, CHARONE S, TJADERHANE L, 2015; BUZALAF MA, KATO MT, HANNAS AR, 2012). DOM can be degraded by two groups of proteolytic enzymes: metalloproteinases (MMPs) and cysteine cathepsins (TJADERHANE L et al.,2015; ZARELLA BL et al., 2015). In dental erosion, tooth pH is rapidly neutralized by the buffer effect of saliva. Thus, specific inhibitory agents for cathepsins (BRESCHI L et al., 2010) may have an important role in studies of dental caries, rather than erosion. During dental erosion mainly MMPs might be involved in the degradation of DOM, since they are activated in low pH and are still functionally active when the pH is neutralized (ZARELLA BL et al., 2015). The effect of cranberry in MMPs is known (LA VD, HOWELL AB, GRENIER D, 2009), but the same is not true for cathepsins, this fact does not invalidate the choice of gel test, because it is a dentin erosion study.

The literature demonstrates the effectiveness of various agents (LA VD, HOWELL AB, GRENIER D, 2009; BEDRAN-RUSSO et al., 2011; KATO et al., 2010; 2014) with inhibitor effect on DOM collagenolytic enzymes, with emphasis on chlorhexidine (GENDRON et al., 1999; KIM et al., 2011; MAGALHÃES et al, 2009b). Natural products are increasingly investigated for the development of products for oral health because in principle they involve a lower incidence of side effects (XIE, BEDRAN-RUSSO, WU, 2008). Thus, in recent years proanthocyanidins have received special attention since it is a natural product of high biocompatibility, high dentin bioactivity and availability as renewable resources (BEDRAN-RUSSO et al. 2007; 2008; 2014).

Proanthocyanidins demonstrate inhibitory effects on MMPs (XIE, BEDRAN-RUSSO, WU, 2008; BEDRAN-RUSSO et al., 2014) and it would be interesting to know what is the effect on dentin erosion. In the study described in Article 1, the purpose was to investigate the possible dose-response and time-response preventive effect of the application of proanthocyanidin gels against erosive challenges. However, this effect was not found. Four different concentrations (0.05%, 1%, 5% and 10%) and two application times (1 and 5 minutes) were tested in this study. Some studies showed a dose-response effect of fluoride agents (KATO et al., 2014; MEI et al. 2012) and chlorhexidine (GENDRON et al., 1999) in the MMPs inhibition. Kim et al., 2011 observed that higher concentrations of chlorhexidine

showed better effect in the dentin demineralization and remineralization (KIM et al., 2011). However, only one study aimed to evaluate the dose-response effect of grape seed extract (proanthocyanidin rich agent) by means of changes in stiffness of demineralized dentin (BEDRAN-RUSSO et al., 2008). The results showed that the use the extract significantly affected the elastic modulus of dentin, being both concentration and time dependent (BEDRAN-RUSSO et al., 2008). In the study 1, unlike the above mentioned publications (GENDRON et al., 1999; KIM et al., 2011; MEI et al., 2012; KATO et al., 2014) it was not possible to verify a time or dose-response effect in minimizing the wear by different gels applied to dentin and exposed to erosion. This result could be due to the response variable used in this *in vitro* study, which is dentin wear instead of a direct analysis of the different gels ability to inhibit collagen degradation.

However, this is the exact purpose of the study presented in the second article. In this case, it was chosen a methodology that allows the verification of the degraded collagen layer thickness. Thus, dentin blocks were demineralized by citric acid and immersed in artificial saliva with collagenase *Clostridium hystoliticum*. In addition, the blocks were analyzed by profilometry in 3 measures: initial, after demineralization and after degradation by collagenase. Thus, each dentin block was positioned correctly in the profilometry during three measures and the dentin was maintained under wet conditions to avoid the shrinkage of collagen matrix (ATTIN et al., 2009).

In this study, the results of proanthocyanidin were better than the other groups. This is because it presents interesting features. Studies show that proanthocyanidin positively affects the demineralization and/or remineralization processes and its remineralizing mechanism seems to be different from fluoride (XIE, BEDRAN-RUSSO, WU, 2008). However, in the present study, it is most likely to proanthocyanidin have improved the DOM by its ability to induce cross-links in dentin collagen and reinforce the remaining collagen matrix (HAN et al., 2003; XIE, BEDRAN-RUSSO, WU, 2008; CASTELLAN et al., 2010; 2011; BEDRAN-RUSSO et al., 2014).

When comparing the magnitude of wear of articles 1 and 2, it could be observed a large difference. This can be explained by the methodology chosen for each article. In article 1 was made erosive cycling, while in article 2 a single immersion in acid for an extended period (36 hours). Despite this difference, in both articles, the proanthocyanidin obtained wear values significantly lower than the placebo groups, showing its effectiveness in reducing the wear of dentin subjected to erosion.

Cranberry has not achieved the proanthocyanidins' results despite it being present in Cranberry's composition. This may be due to proanthocyanidin concentration in Cranberry be around the 65% (BODET, CHANDAD, GRENIER, 2006) a lower value than purified proanthocyanidin used in this study. However, the Cranberry group showed performance similar to chlorhexidine group, which is already presents ability to reduce demineralization on dentin confirmed (MAGALHÃES et al., 2009b; KIM et al., 2011).

In the second article, NaF group showed no statistical difference from placebo group. This result could be due to the response variable used in these studies, which is dentin wear instead of a direct analysis of the different gels ability to inhibit collagen degradation. Other studies demonstrated the ability of NaF to reduce the collagen degradation (KATO et al., 2012) and inhibit MMPs (KATO et al., 2014).

Within the limitations of these studies, it seems that Cranberry and proanthocyanidin gel are able to prevent dentin wear progression. Thus, Cranberry and proanthocyanidin can be considered as a promising therapy for dentin erosion. However, additional studies are needed to evaluate cranberry gels concentration, application time and ideal frequency for reapplication to assist in the design of clinical trials. In addition, molecular analyzes might enhance the understanding of cranberry effect on MMPs inhibition and DOM maintenance.

4 CONCLUSION

4 CONCLUSION

The first article suggests the efficacy of proanthocyanidin on decreasing the deleterious effects of erosion in dentin, despite the time-response and dose-response do not observed. However, the second article demonstrated the efficacy of proanthocyanidin in preventing dentin erosion; because the dentin wear and DOM degradation were lower than the others groups.

In addition, Cranberry was able to reduce the dentin wear and collagen degradation similarly to chlorhexidine.

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ANNEX

Annex A - Article accepted for publication

De: "Franklin Garcia-Godoy" <godoy@amjdent.com>
Para: "Prof. Dr. Heitor Marques Honório" <heitorhonorio@usp.br>
Enviadas: Terça-feira, 16 de Fevereiro de 2016 23:45:31
Assunto: RE: AJD ANSWER: Effect of different concentrations and application time of proanthocyanidin gels on dentin erosion

Re: Effect of different concentrations and application time of proanthocyanidin gels on dentin erosion.

Prof. Honório :

I am pleased to inform you that your revised manuscript has been accepted for publication in the **American Journal of Dentistry.**

Before publication you will receive page proofs (galleys) for your approval.

Again, thank you for considering the **American Journal of Dentistry** for publication of your work.

Sincerely,

Franklin Garcia-Godoy, DDS, MS, PhD, PhD
Editor

Franklin Garcia-Godoy, DDS, MS, PhD, PhD
Editor, *American Journal of Dentistry*

AMERICAN JOURNAL of DENTISTRY

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