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Dissertação

Avaliação de diferentes protocolos de simulação de erosão ácida em dentes bovinos

Ayumi Batista Kodama

Pelotas, 2017

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Avaliação de diferentes protocolos de simulação de erosão ácida em dentes bovinos

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“.... Tenha tempo para os Sonhos, eles conduzem sua carruagem para as estrelas. Ele, a pessoa mais importante que conheci. Ele me ensinou uma coisa: ‘’Tudo no final sempre dá certo, se ainda não deu, é porque ainda não chegou o final”

Autor desconhecido

Notas preliminares

A presente dissertação foi redigida segundo o Manual de Normas para trabalhos acadêmicos da UFPel, adotando o nível de descrição em capítulos não convencionais. Disponível no endereço eletrônico: http://sisbi.ufpel.edu.br/arquivos/PDF/Manual_Normas_UFPel_trabalhos_acad%C3%AAmicos.pdf.

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Resumo

KODAMA, Ayumi Batista. **Avaliação de diferentes protocolos de simulação de erosão ácida em dentes bovinos**. 42f. Dissertação (Mestrado em Odontologia – Área de Prótese Dentária) – Programa de Pós-Graduação em Odontologia, Universidade Federal de Pelotas, Pelotas 2017

A erosão dental é definida como a perda cumulativa e irreversível de tecido dentário duro em decorrência de um processo químico-mecânico que é comumente associada às desordens alimentares, refluxo gastroesofágico ou consumo exagerado de alimentos excessivamente ácidos. Em casos de refluxo do conteúdo gastroesofágico, o conteúdo gástrico contendo entre outras substâncias o ácido clorídrico (HCl), que apresenta pH reduzido, chega à cavidade bucal e pode ser capaz de provocar perda de conteúdo mineral das superfícies dentárias. Embora alguns estudos tenham sido realizados acerca da simulação da erosão dentária em laboratório, não existe até o momento nenhum protocolo de simulação que seja considerado padrão-ouro. Desta maneira, o objetivo do presente estudo foi avaliar diferentes metodologias para simular *in vitro* o efeito da erosão dentária utilizando protocolos mecânicos, químicos e químico-mecânicos. A erosão ácida simulada foi testada em 6 diferentes grupos experimentais e 1 grupo controle. Após o tratamento foram feitas análises relacionadas à rugosidade superficial, microdureza, alteração topográfica da superfície (MEV) e semi-quantificação por espectroscopia por dispersão de energia (EDS). Os valores de rugosidade foram maiores para os grupos que foram imersos 96h, com diferenças estatisticamente significativas quando comparados aos demais grupos. Em relação aos grupos que utilizaram a solução de HCl, a simulação química ou mecânica-química não influenciou os resultados de dureza independente da profundidade, com exceção de

20μm de profundidade para o grupo HCl (químico-mecânico/96h) e 30μm de profundidade para o grupo HCl (químico-mecânico/108min). Os grupos químicos apresentaram os menores valores de microdureza Knoop na maior profundidade avaliada (grupo HCl (químico/108min): 54,28 e grupo HCl (químico/96h): 44,30), indicando uma maior penetração de ácidos. Ao avaliar as características macroscópicas da superfície dos espécimes, o grupo HCl (químico-mecânico/108min) foi o que apresentou o padrão superficial de perda mineral mais semelhante ao encontrado clinicamente. Nas imagens em MEV apenas os grupos que simulavam HCl durante 96h tiveram os túbulos dentinários expostos. O grupo HCl / químico-mecânico / 108min apresentou a zona de transição entre esmalte e dentina. Os outros grupos pudemos observar apenas a superfície do esmalte. As percentagens de peso e átomos de Cálcio e Fósforo tenderam a diminuir entre os grupos avaliados, com exceção do grupo HCl / químico / 108min. O tempo dos desafios erosivos influencia a rugosidade, a dureza de Knoop e a topografia superficial de lesões simuladas de erosão dentária. O uso de suco de maçã como solução não produziu lesão semelhante à erosão. Os processos químico-mecânicos foram mais capazes de remover o esmalte devido à simulação erosiva. A imersão químico-mecânica com solução de HCl com ciclos de desmineralização / remineralização totalizando 108min apresentou resultados macroscópicos mais semelhantes aos encontrados em lesões de erosão in vivo.

Palavras-chave: erosão dentária; abrasão; desgaste dentário; desordem alimentar; suco gástrico.

Abstract

KODAMA, Ayumi Batista. **Evaluation of different dental erosion simulation protocols in bovine teeth**. 42f. Dissertation (Master Degree in Dentistry – Prosthodontics) – Programa de Pós-Graduação em Odontologia, Faculdade de Odontologia, Universidade Federal de Pelotas, Pelotas, 2017.

Dental erosion is defined as a cumulative and irreversible loss of hard dental tissue in the process of a chemical-mechanical process that is commonly associated with eating disorders, gastroesophageal reflux, or excessive consumption of excessively acidic foods. In cases of reflux of the gastrointestinal content, the gastric content among other hydrochloric acid (HCl) substances, which has a reduced pH, reaches the oral cavity and may be capable of causing loss of mineral content of the dental surfaces. Some of the studies were performed based on the simulation of dental erosion in the laboratory, there is currently no simulation protocol that is considered gold standard. In this way, the objective of the present study to evaluate different methodologies to simulate in vitro the effect of dental erosion using mechanical, chemical and chemical-mechanical protocols. Simulated acid erosion was tested in 6 different experimental groups and 1 control group. Surface analysis, microhardness, surface topographic alteration (SEM) and semi-quantification by energy dispersive spectroscopy (EDS) were made. Roughness values were higher for the groups that were immersed 96h, with statistically significant differences when compared to the other groups. In regard to the groups that used HCl solution, the chemical or chemical-mechanical simulation did not influenced the results of depth-independent hardness, with the exception of 20μm depth for HCl / Chemical-mechanical / 96h group and 30 μm depth for HCl / Chemical-mechanical / 108min group. The chemical groups had the lowest values of Knoop microhardness in the deepest depth (HCl / Chemical / 108min group: 54.28 and HCl / Chemical / 96h group: 44.30), indicating a higher penetration of acids. When assessing the macroscopic characteristics of the surface of specimens, the HCl / Chemical-mechanical / 108min group was the one that presented the surface pattern of mineral loss more similar to that found clinically. In SEM images, only the groups that simulated with HCl for 96h presented dentin tubules exposed. The HCl / chemical-mechanical group / 108min presented the transition zone between enamel and dentin. The other groups we could observe just enamel surface. The percentages of weight and atoms of Calcium and Phosphorus tended to decrease between the evaluated groups, except for the group HCl / Chemical / 108min. The time of erosive challenges influences the roughness, Knoop hardness and surface topography of simulated dental erosion lesions. The use of apple juice as a solution did not produced erosion-like lesion. Chemical-mechanical processes were more able to remove enamel due to erosive simulation. Chemical-mechanical immersion with HCl solution with cycles of demineralization/remineralization totaling 108min presented macroscopic results more similar to those found in in vivo erosion lesions.

Keywords: tooth erosion, abrasion, tooth wear, eating disorder, gastroesophageal reflux, gastric juice.

Lista de Figuras

Lista de Tabelas

Lista de Abreviaturas e Siglas

ANOVA	Análise de Variância
SiC	Carbeto de Silício
g	Grama
gF	Grama-força
ºC	Grau Celsius
h	Hora
MPa	Megapascal
µm	Micrômetro
MEV	Microscópio eletrônico de varredura
ml	Mililitro
mm	Milímetro
mm^2	Milímetro quadrado
mM	Milimolar
mW/cm^2	Miliwatt por centímetro quadrado
min	Minuto
N	Newton
KHN	Número de dureza Knoop
grit	Número de grãos por centímetro quadrado
pH	Potencial Hidrogeniônico
RS	Rio Grande do Sul
rpm	Rotação por minuto
Ra	Rugosidade aritmética - média
s	Segundo

Sumário

1 Introdução

Erosão dental é a perda cumulativa e irreversível de tecido dentário duro em decorrência de um processo químico-mecânico, não causado por bactérias (Carvalho *et al.*, 2015). Sua etiologia é multifatorial, tendo a ação do ácido como o seu principal fator. É caracterizada pela perda da morfologia natural e contorno das superfícies dentárias, os sinais típicos podem ser identificados desde o achatamento das cúspides, até os casos onde se nota ausência total de anatomia, com concavidades mais largas e profundas na superfície oclusal (Carvalho *et al.*, 2015). Pacientes com este tipo de patologia, normalmente apresentam facetas de desgaste nas faces palatinas dos dentes anteriores superiores e superfícies oclusais dos molares inferiores, devido à maior exposição aos ácidos (de Carvalho Sales-Peres *et al.*, 2014).

Segundo o estudo de Jaeggi e Lussi (Jaeggi and Lussi, 2006; Jaeggi and Lussi, 2014), a presença do desgaste erosivo pode começar tão logo a primeira superfície dental chegue à cavidade e sofra exposição a ácidos extrínsecos (derivados da dieta) ou intrínsecos (derivados de regurgitação – bulimia e anorexia nervosa ou refluxo gastroesofágico) presentes na cavidade bucal. Mais recentemente tem sido dada maior atenção a esta condição, sendo observado aumento na prevalência entre adultos jovens (mulheres) e crianças, uma possível causa deste aumento tem relação com as mudanças sociais e culturais, associadas ao aumento do consumo de alimentos com pH ácido como também associado ao vômito auto induzido, o qual acaba por expor a dentição aos desafios ácidos e aumentar o risco para erosão dental (Hermont *et al.*, 2013; Salas *et al.*, 2015).

A dissolução das estruturas minerais dos dentes ocorre principalmente em função da presença do ácido clorídrico (HCl), o qual apresenta um pH extremamente ácido (~1,6), levando à perda do equilíbrio na cavidade bucal e interferindo na capacidade da saliva em manter seu mecanismo de ação para o processo de remineralização das superfícies dentárias. Com esta solubilização dos tecidos, a ação mecânica de escovação, dentre outras, podem ser responsáveis por promover uma remoção destas superfícies desmineralizadas, culminando na perda das estruturas dentárias (Schlueter *et al.*, 2012). Sendo bastante importante a participação do cirurgião-dentista junto ao tratamento multidisciplinar destes pacientes, esta tem sido enfatizada na literatura, tanto para detecção precoce de sinais clínicos característicos da erosão, a fim de evitar danos irreversíveis às estruturas dentárias, quanto para evitar o desenvolvimento de complicações sistêmicas mais graves, no caso das desordens alimentares (Johansson *et al.*, 2012; Hermont *et al.*, 2013; Conviser *et al.*, 2014; Uhlen *et al.*, 2014).

Nos casos em que os dentes de um determinado paciente se encontram em estágios avançados de erosão, cabe ao profissional lançar mão de tratamento reabilitador com o intuito de restabelecer forma e função para a dentição afetada. Por outro lado, além destes tratamentos apresentarem altos custos e serem extensos, podem ter falhas catastróficas ou exigirem manutenção por longos períodos (Ranjitkar *et al.*, 2012). Além disso, existem poucos estudos clínicos que abordem qual o material/técnica restauradora é mais indicado para estes casos, com acompanhamento em longo prazo, o que pode dificultar a escolha do profissional pelo tratamento restaurador.

O efeito de substâncias extrínsecas como refrigerantes, ácido cítrico, suco de laranja, já são amplamente conhecidos pela sua influência significativa no desgaste dental por erosão (Ehlen *et al.*, 2008; Tedesco *et al.*, 2012; Hellwig and Lussi, 2014; Gravelle *et al.*, 2015). Outros autores avaliaram substâncias que fossem capazes de reduzir o processo de erosão, como o uso do leite (Magalhaes *et al.*, 2014) e chá verde (Buzalaf *et al.*, 2012; De Moraes *et al.*, 2016). Também encontramos informações sobre o efeito de substâncias intrínsecas, como o efeito do ácido clorídrico na cavidade bucal, em associação a enzimas digestivas, pepsina e tripsina, como coadjuvantes ao desenvolvimento da erosão (Schlueter *et al.*, 2010; Schlueter *et al.*, 2012). Recentemente, um artigo de Sulaiman *et al*.,(Sulaiman *et al.*, 2015) avaliou o impacto do desafio erosivo com ácidos gástricos nas propriedades ópticas e na superfície de zircônia monolítica. Em 2011, Schellis et al. (Shellis *et al.*, 2011), indicaram uma sugestão de metodologias e modelos a serem seguidos para a reprodução da erosão *in* *vitro* e *in situ,* através do resumo da discussão ocorrida no Workshop de metodologia na pesquisa de erosão, em Zurique, 2010. Abordaram aspectos relacionados ao tipo de agente erosivo, indicando que a concentração e pH das soluções devem ser relevantes ao tipo de pesquisa, se erosão intrínseca ou extrínseca; o controle da temperatura deve ser padronizado e indicam um sistema de agitação para controle da taxa de fluxo da solução erosiva, para simular as condições clínicas. Não chegaram a um consenso quanto à duração dos desafios, apenas que eles eram de curta duração e que deveriam ser adaptados ao tipo de substrato a ser utilizado – dente humano ou bovino – que podem ser diferentes quanto à suscetibilidade ácida.

No entanto, embora tenham feitas inúmeras sugestões e considerações, e ainda que se tenha uma diversidade de estudos sobre o efeito da erosão na superfície dental e nos materiais restauradores, os artigos presentes na literatura ainda se limitam a reproduzir a erosão dental apenas por métodos químicos (geralmente imersão em solução ácida), o que se apresenta como uma condição diferente da observada no ambiente oral. Desta maneira, a literatura se ressente de dados sobre a reprodução da erosão dental por uma metodologia padronizada envolvendo ação químico-mecânica, representativa dos diferentes eventos naturais que ocorrem na cavidade bucal e que reproduza lesões semelhantes às encontradas clinicamente e que possam servir de base para testes laboratoriais de diferentes materiais restauradores de modo a possibilitar a indicação e/ou desenvolvimento de materiais específicos para procedimentos adesivos nestes substratos.

Com isso, o objetivo deste estudo é comparar diferentes protocolos que simulem a erosão dental, avaliando o padrão de erosão dos grupos, submetidos à ação mecânica, química e químico-mecânica, com diferentes tempos, a fim de indicar uma metodologia padronizada que possa mimetizar a superfície erodida em condições mais próximas às encontradas clinicamente. Neste trabalho, iremos utilizar dentes bovinos, usados como alternativa pela maior facilidade de serem obtidos, e por apresentar características estruturais morfológicas e dureza, tanto para esmalte quanto dentina, semelhantes aos dentes humanos, assim como número de túbulos dentinários e matriz orgânica (Schilke *et al.*, 2000; Correa *et al.*, 2003). Além de ser considerado um substituto confiável utilizado em diversos testes *in vitro* como erosão dental, dureza, adesão(Laurance-Young *et al.*, 2011; Soares *et al.*, 2016). A dentina esclerótica, caracterizada pela obliteração dos túbulos dentinários pela deposição de dentina peritubular, em função de causas multifatoriais, dentre elas a erosão dental, também apresenta padrão semelhante ao dente humano (Camargo *et al.*, 2008). A hipótese nula testada foi de que o tempo e o método de simulação (química, mecânica ou químico-mecânica) não afetaria as características de rugosidade superficial, micro dureza, topografia de superfície e composição química da dentina de dentes bovinos.

2. Capítulo 1

Evaluation of different protocols for simulation of dental erosion in bovine teeth

Running head: In vitro dental erosion

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Abstract

Objectives: To compare different laboratory protocols of simulated dental erosion lesion in bovine teeth in order to suggest a standardized method to reproduce erosion-like lesions.

Methods. Samples were prepared from bovine teeth with 0.5mm of remaining enamel. Specimens were randomly assigned into 7 groups, differing by solution type (apple juice or HCl), exposure time (108 min or 96h) and simulation process (chemical, mechanical, or chemical-mechanical). Surface roughness, Knoop microhardness at different depths and surface topography was analyzed by SEM and EDS. Data were tabulated and analyzed by ANOVA and Tukey test (α=0.05).

Results. Roughness values were higher for the groups that were immersed 96h. The chemical groups presented the lowest Knoop microhardness values in the highest depth, indicating a higher penetration of acids. When assessing the macroscopic characteristics, chemical-mechanical simulation with HCl and 108 min presented a similar pattern to that found clinically. In SEM images, only the groups that simulated with HCl for 96h presented dentin tubules exposed. The HCl / chemical-mechanical group / 108min presented the transition zone between enamel and dentin. The other groups we could observe just enamel surface. Calcium and Phosphorus tended to decrease between the evaluated groups.

Conclusion: Solution type, exposure time, and simulation process influenced all evaluated properties of the specimens. Chemical-mechanical immersion with HCl solution simulated during 108min presented macroscopic results more similar to those found in erosion lesions.

Significance. This study suggests a protocol to reproduce dental erosion lesions in laboratory with similar macroscopic characteristics to those observed in vivo.

Keywords: tooth erosion, abrasion, tooth wear, eating disorder, gastroesophageal reflux, gastric juice.

1. Introduction

Dental erosion is the cumulative and irreversible loss of dental hard tissues as a result of a chemical-mechanical process, not caused by bacteria.[1] Its etiology is multifactorial, with an acid action as its main factor. Acids may be extrinsic (dietary) or intrinsic (derived from episodes of regurgitation or gastroesophageal reflux) present in the oral cavity.[2] Lesions are characterized by loss of natural morphology and contour of dental surfaces, typical signs may be identified from cusp flattening to cases where there is a complete absence of anatomy, with deeper concavities on the occlusal surface.[1] Patients with this type of pathology usually presents wear facets on the palatal surfaces of the upper incisors and occlusal surfaces of the mandibular molars due to increased exposure to acids.[3]

More recently, a wider attention has been given to this condition, and an increase in the prevalence among young adults and children has been observed.[2-4] A possible cause of this increase is related to social and cultural changes associated with increased consumption of acidic pH foods and beverages. By exposing the dentition to acidic challenges and increasing the risk for dental erosion.[4, 5] In severe conditions, dental erosion can occur due to eating disorders, considered a relatively common disorder in young women, which, although the low prevalence, presents a high morbidity rate.[6-8]

The dissolution of mineral structures of the teeth occurs mainly due to the presence of hydrochloric acid—HCl—which presents an extremely acidic pH (~1.6), leading to the loss of pH balance in the oral environment and interfering in the buffer capacity of saliva to maintain the process of remineralization of dental surfaces. With the tissues dissolution, the mechanical action of brushing the teeth, among others, may be responsible for promoting a removal of these demineralized surfaces, culminating in the loss of dental structures.[7]

Several studies have been carried out in an attempt to simulate dental erosion using different solutions (soft drinks, citric acid, fruit juice and hydrochloric acid), methods (solution or mechanical immersion), and simulation times (varying from 4 cycles of 22.5 minutes to 96h) were used.[9-15] Although there is a diversity of studies on the effect of erosion on the dental surface, the literature resents data on the reproduction of dental erosion by a standardized method, mainly involving a chemical-mechanical process, which simulates the erosion events in a similar way they occur in the oral environment and that should reproduce the dental erosion lesions similar to those found clinically and that can serve as basis for *in vitro* tests.

Therefore, the objective of this study is to compare different protocols that simulate dental erosion, evaluating the erosion pattern of the groups, submitted to mechanical, chemical and chemical-mechanical action, with different times and solutions, in order to indicate a standardized method that can simulate the eroded surface in conditions closer to those found clinically. The null hypothesis tested was that the time, solution, and simulation method (chemical, mechanical or chemical-mechanical) would not affect the characteristics of surface roughness, Knoop microhardness, surface topography and chemical composition of the dentin of bovine teeth.

2. Materials and Methods

2.1 Study design

This in vitro 2 x 2 x 3 factorial study (n=10) evaluated three factors: two times (108 min or 96 hours), two solutions (apple juice plus apple pulp and HCl) and three methods (chemical, mechanical and chemical-mechanical) divided into 7 groups according to the dental erosion simulation protocol. The division of groups is shown in Table 1.

Table 1– Division of the groups according to the adopted protocols for dental erosion simulation.

Groups	**Time**
CONTROL	-
HCl / Chemical	108 min
HCl / Chemical-mechanical	108 min
Apple juice / Chemical-mechanical	108 min
HCl / Chemical	96h
HCl / Chemical-mechanical	96h
Apple juice / Chemical-mechanical	96h

2.2 Sample preparation

The bovine teeth were previously analyzed under an optical microscope (10X magnification, SMZ-1 Zoom Stereomicroscope, Nikon GmbH, Dusseldorf, Germany) in order to exclude faulty or cracked teeth. The teeth included were stored in distilled water under refrigeration after being cleansed with water and pumice stone and the aid of a low-rotating rubber cup. They were then submitted to transversal cuts, in the region of the cement-enamel junction, separating the crown from the root. The specimens were extracted from the middle-central portion of the crown, with each tooth originating a 6x6mm square specimen.

After these cuts, the specimens had their surfaces ground and polished, with #600; #1500; #2000 and #2500 grit silicon carbide (SiC) sandpapers, until a thickness of 0.5mm of enamel was achieved (the region where the cut with the pulp chamber was the reference).

All specimens were included in acrylic resin following a standard size of 15x30x12mm, according to the format suitable to fit the spaces offered by the Rub & Roll testing machine.[15] A control group was included without any chemical-mechanical simulation.

2.3 Solution preparation

The HCl solution was used to simulate the presence of hydrochloric acid of the stomach that contact dental surfaces due to gastroesophageal reflux. The solutions were prepared based on Schlueter et al.[7], the HCl solution was prepared by dissolving 0.5g NaCl in 99.5mL of distilled water, adjusted with 6 mol L^{-1} of HCl until the pH reached 1.6, according to the normally found pH on the stomach after a meal. The saline mineral solution for storage of the specimens was composed of 4.08 mM H_3PO_4, 20.10 mM KCl, 11.90 mM, Na_2CO_3, and 1.98 mM $CaCl_2$ (pH 6.7, chemicals from Merck, Darmstadt, Germany). The apple juice plus apple pulp (pH 3.7) was produced on the day of each experiment according to a previous study.[15]

2.4 Erosive process simulation

The simulation of the erosive process of the specimens was done between two periods of time, and with two different solutions. As there is no recommended standard to reproduce dental erosion lesions, we adopted the time of immersion according to recent studies found in the literature: The groups that passed by the 108min challenge were carried out following the protocol of immersion time in the solutions suggested by Schlueter et.al.[7] In such protocol the specimens were submitted to six cycles of demineralization of 2 minutes during 9 days, totalizing 108 min of test. Where the number of six cycles represents the vomiting episodes times that regularly occur with bulimic patients per day. And the demineralization period is associated to the time that the pH of the saliva declines after an acid attack.[13] The groups that were immersed in the same solutions but during 96 hours, according to Sulaiman, et al, and represents 10 years of clinical function.[16]

The groups that passed by chemical process just were immersed in the HCl solution for the indicated periods. And to do the chemical-mechanical challenge, simulated in association with the Rub & Roll equipment.[15] A velocity of 30rpm was used, and a rubber base, simulating the periodontal ligament, of 1mm corresponding to an impact force of 30N. After each intervention the specimens were washed in distilled water for 1 minute and stored in saline mineral solution for 1 hour, as well as until the next day of experiment. All specimens were stored at 37ºC. The time established for immersion corresponds to the time of pH decline in the saliva after receiving an acid attack; and the number of times, episodes occurring during the day, based on clinical reports.[7, 13, 14]

2.5 Roughness

The surface roughness of the specimens was evaluated after the acid erosion simulation with the SJ-201 rugosimeter (Mitutuyo, Tokyo, Japan), with cut-off value=0.25 and the reading zone 3 times the cut-off value, at room temperature. Three readings were made for each specimen and the mean (average) and the median roughness value (Ra, μm) was determined from the arithmetic mean between the three measurements.[17]

2.6 Knoop Microhardness

The specimens were sectioned on their long axis, ground and polished with #600, #1200, #1500, #2000-grit SiC sandpapers. The hardness values were obtained in order to identify the depth that the acid would have reached and influenced the hardness of the dentin. The first measurement was made shortly after the passage of the enamel/dentin junction, and the next ones were following an imaginary vertical column towards the center of the tooth. In each half of the sectioned specimen, 5 indentations were made at depths of 10, 20, 30, 40 and 100μm until they reached the middle third of the specimen (pulp chamber region). They were evaluated using a microdurometer (FM-700; Future-Tech Corp., Tokyo, Japan) under the Knoop hardness test and applied load of 5g for 5 seconds.

The test was performed by a single operator and the equipment was previously calibrated at each measurement, also adapted in an environment that did not produce vibrations during the readings.

2.7 Macroscopic analysis of the specimens

Photographs of each group were made after the erosive challenges in order to evaluate the macroscopic aspect of the evaluated protocols in order to check if the macroscopic surface of the tested groups were similar to an eroded tooth. The images were made using a professional camera (Canon Rebel Xsi, Canon, USA) with macro lens and round flash.

2.8 Scanning electron microscopy and EDS analysis

Two specimens from each group was selected for SEM evaluation (JEOL JSM-6610LV), which received specific preparation for the analyzes: after the polishing with the abovementioned sequence of SiC sandpapers, the polishing was done with diamond solutions in decreasing granulation of 3; 1; 0.25, and polyester felt disc at the speed of 100rpm for 1 minute and in the granulation of 0.1μm for 20 minutes. Between each stage the specimens were cleaned in an ultrasonic tank for 1 minute, and at the end were immersed for 1 hour in a 1: 1 water-ethanol solution. Afterwards, the specimens were individually deposited in sterile collecting pots with silica gel particles.

A metallic coating in gold was done and the images were captured in magnification of 500x and 1500x of the surfaces of contact with the solution and also of the sectioned area (long-axis of the tooth): enamel, dentin next to the dentin-enamel junction. The EDS analysis (JEOL JSM-6610LV - Thermo Scientific Ultra Dry) was made at the magnification of 1500x, observing the percentage of Calcium (Ca), and Phosphorus (P) ions in the regions of the sectioned parts observed in the SEM.

2.9 Statistics analysis

The results were tabulated and submitted to Analysis of Variance (ANOVA), since the data presented a normal distribution according Shapiro-Wilk test for normal distribution. Differences between the groups were analyzed using *post-hoc* Tukey test with a significance level of 95%.

3. Results

3.1 Roughness

The time of exposure to the substances tested significantly influenced the roughness values (Table 2). The roughness values were higher for the groups that were immersed 96h, with statistically significant differences when compared to the other groups (control and 108 min), except for the group that used apple juice for 96h, where its roughness (Ra = 0.668) was similar to the 108 minute groups. Both groups submitted to long exposure of HCl presented the highest roughness values (HCl / Chemical / 96h Ra=0.897; HCl / Chemical-mechanical / 96h: Ra=0.900).

Table 2. Average values of roughness and standard deviations according to test groups.

Groups	Roughness	
	Average$^{\#}$(SD)	**Median§(SD)**
Control	0.151(0.10)^{A}	0.119(0.05)^{A}
HCl / Chemical / 108 min	0.297(0.13)^{AB}	0.279(0.13)^{AB}
HCl / Chemical-mechanical / 108 min	0.355(0.16)^{AB}	0.343(0.14)^{AB}
Apple juice / Chemical-mechanical / 108 min	0.310(0.07)^{AB}	0.309(0.07)^{AB}

HCl / Chemical / 96h	0.897(0.41)^{C}	0.867(0.42)^{C}
HCl / Chemical-mechanical / 96h	0.900(0.52)^{C}	0.812(0.54)^{C}
Apple juice / Chemical-mechanical / 96h	0.668(0.35)^{BC}	0.623(0.37)^{BC}

Statistic performed considering the value of the average obtained in the 3 measurements of the roughness of each specimen in the group.
§Statistics performed considering the value of the median obtained in the 3 measurements of the roughness of each specimen in the group.
** Different letters indicate statistically significant differences between the groups (Tukey post-hoc test with $\alpha < 0.05$)

3.2 Knoop Microhardness

The Knoop microhardness results of the different groups are presented in Table 3, where it is possible to observe that the use of apple juice as solution in the erosive protocol did not present statistical difference when compared to the control group for all evaluated depths, with the exception of the chemical-mechanical / apple juice / 96h group. In regard to the groups that used HCl solution, the chemical or chemical-mechanical simulation did not influenced the results of depth-independent hardness, with the exception of 20µm depth for HCl – chemical-mechanical – 96h group and 30 µm depth for HCl – chemical-mechanical – 108min group.

The chemical groups had the lowest values of Knoop microhardness in the greatest depth (HCl / chemical / 108min: 54.28 and HCl / chemical / 96h: 44.30), indicating a higher penetration of acids. Followed by both chemical-mechanical groups immersed for 108min and 96h in HCl solution, which presented higher Knoop microhardness values, respectively (68.89 and 52.50), but without statistically significant difference when compared to those groups.

Table 3. Average values of Knoop microhardness in each depth evaluated and their respective standard deviations according to the test groups.

Groups*	**Microhardness**				
	Depth10µm (SD)	**Depth 20 µm (SD)**	**Depth 30 µm (SD)**	**Depth 40 µm (SD)**	**Depth 100 µm (SD)**
Control	76.93(9.49)^{A}	72.15(11.23)^{A}	72.51(8.65)^{A}	71.77(7.10)^{A}	79.66(13.15)^{A}
HCl / Chem / 108 min	47.05(9.73)^{B}	50.05(13.94)^{BC}	56.04(20.96)^{AB}	55.82(17.06)^{B}	54.28(22.23)^{B}

HCl / Chem-mec/ 108 min	51.91(6.23)B	55.10(6.68)B	57.45(10.56)AC	55.35(12.83)BC	68.89(12.19)ABC
Apple juice / Chem-mec / 108 min	66.84(9.81)AC	56.58(18.17)AE	64.70(9.50)A	71.86(7.53)AC	77.46(7.85)A
HCl / Chem / 96h	35.17(7.78)B	36.76(9.17)CD	39.76(7.76)B	43.26(6.00)B	44.30(17.69)B
HCl / Chem-mec/ 96h	38.21(15.01)B	39.56(14.97)BDE	44.61(13.60)BC	46.62(12.62)B	52.50(22.98)BC
Apple juice / Chem-mec / 96h	56.16(11.47)BC	65.41(12.21)AB	64.61(13.86)A	67.86(11.23)AC	79.49(16.02)A

* Specific group definition can be found in Table 1. ** Different letters indicate statistically significant differences between the groups at same depth (Tukey post-hoc test with $\alpha < 0.05$)

3.3 Macroscopic analysis of the specimens

When assessing the macroscopic characteristics of the surface of specimens of different groups, it was possible to observe a different pattern of tooth wear. The control group (Figure 1-A) presents the initial situation in which all the specimens were before being submitted to the erosive challenges. The group that underwent demineralization cycles with hydrochloric acid, and remineralization, totaling 108 minutes of erosive challenge was the one that presented the surface pattern of mineral loss more similar to that found clinically, once the enamel was partially and evenly removed (Figure 1-C). The HCl groups that passed by the 96h challenge had practically all their enamel surface solubilized; this pattern was associated with a more severe form, which could be related to an excessively harmful erosive challenge. Also, the use of apple juice for 96h caused an alteration in the color of the dental substrate (Figure 1-G).

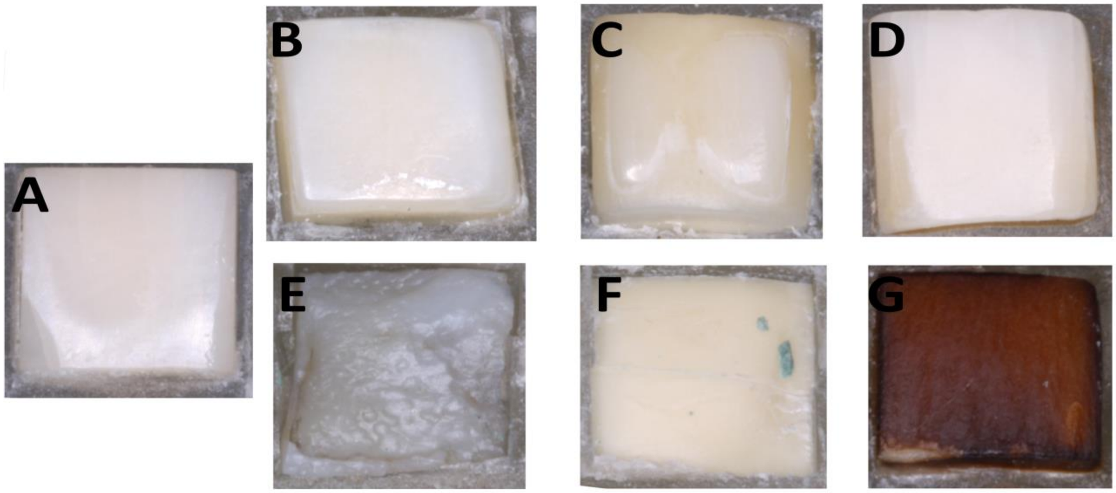

Figure 1 – Pictures for the macroscopic analysis of the specimens. Control group (A); HCl – Chemical – 108min (B); HCl – Chemical-mechanical –108 min (C) presenting mineral loss more similar to the pattern found clinically; Apple Juice – Chemical-mechanical –108 min (D); HCl – Chemical –96h (E) and 6 – HCl – Chemical-mechanical –96h (F) present the more severe wear;Apple Juice – Chemical-mechanical –96h, with color change in dental substrate.

3.4 Scanning electron microscopy (SEM) and EDS analysis

According to Figure 2, it can be observed that in the groups exposed to HCl for 96 hours, chemical and chemical-mechanical (E and F, respectively) presented the highest wear pattern, exposing the dentin portion. In such groups, we observed a great number of tubules, indicating a greater depth reached by the acid. Images B, C, D and G present a most conservative pattern of wear, where only enamel is observed on its surface, being more similar to the control group, which was not exposed to any challenge.

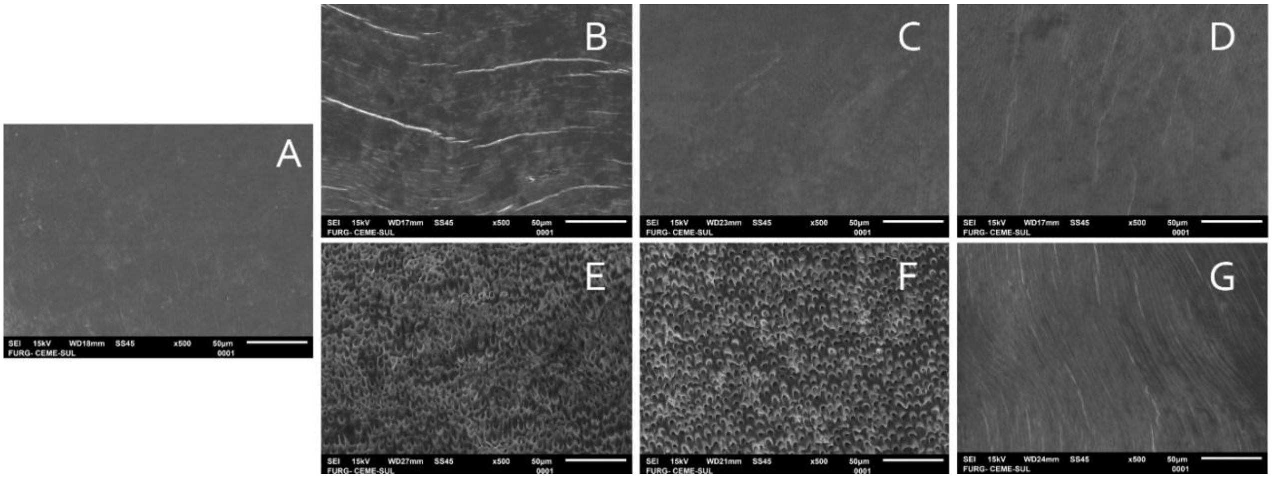

Figure 2. SEM images made with 500x the magnification in region of dental enamel that was exposed to the acid. **A** – Control group, which was not challenged. **B**, **C**, **D** and **G**, only enamel structures could be observed. **E** and **F** presents as remnant only dentin structure, evidencing the enamel loss in this groups.

Figure 3 shows a longitudinal view of the exposed area in the HCl / chemical-mechanical group / 108min at different depths enamel, enamel/dentin junction and dentin.

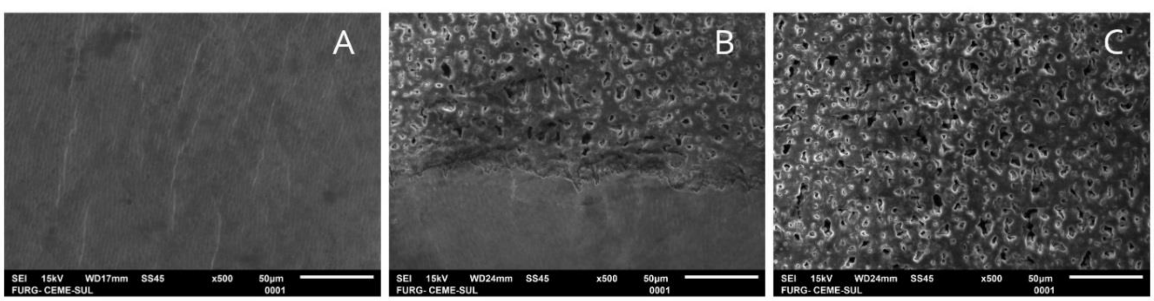

Figure 3 – Images from the surface of the group that underwent the challenge of immersion in HCl alternated with remineralizing solution (HCl / chemical-mechanical / 108min). It is observed the transition zone between the enamel and dentin showing an initial and less severe wear. **A,** Enamel surface. **B**,. Enamel/dentin junction. **C**, Deep dentin.

Table 4 present the weight and atoms percent observed through EDS analysis. The percentages of weight and atoms of Calcium and Phosphorus tended to decrease between the evaluated groups, except for the HCl – chemical – 108 min group, which presented a slight increase for both, in comparison to the control group. The lowest values were found in the groups that underwent the challenge time of 96 hours, followed by the group HCl – chemical-mechanical – 108min. The HCl – chemical-mechanical – 96h group had the highest losses. The weight and percentage of oxygen atoms increased in all groups, except for HCl – chemical – 108 min group, which showed a slight reduction. The weight of carbon in HCl – chemical-mechanical – 96h group was the one that obtained the highest value.

Table 4 – Weight and Atom percent (%)of Carbon (C), Oxygen (O), Calcium (Ca), and Phosphorus (P) ions in the regions of the sectioned parts observed in the SEM at 1,500x magnification, observed through EDS analysis.

Groups	EDS Analysis			
	Weight %			
	C	O	P	Ca
Control	6	44.32	16.22	33.45
HCl / chem / 108min	4.37	42.48	16.94	36.21
HCl / chem-mec / 108min	5.24	59.58	5.11	30.06
Apple juice / chem-mec / 108min	5.93	53.95	7.56	32.55
HCl / chem / 96h	5.21	59.33	5.54	29.92
HCl / chem-mec / 96h	9.49	72.13	-	18.37
Apple juice / chem-mec / 96h	5.14	65.19	0.7	28.96
	Atom %			
Control	10.79	59.86	11.31	18.03
HCl / chem / 108min	8.13	59.42	12.22	20.22
HCl / chem-mec / 108min	8.6	73.36	3.25	14.78
Apple juice / chem-mec / 108min	10.03	68.51	4.95	16.5
HCl / chem / 96h	8.57	73.17	3.53	14.73
HCl / chem-mec / 96h	13.73	78.22	-	7.95
Apple juice / chem-mec / 96h	8.16	77.64	0.42	13.77

4. Discussion

Erosion-induced lesions have been simulated in several studies with the use of only chemical solutions in different concentrations and compositions.[14, 16] In order to obtain a more similar simulation of the dental erosion process, some studies indicate the temperature monitoring (37ºC) and use a liquid agitator motor.[18] However, these methods may not correspond closely to dental erosion lesions observed clinically. It is well known that dental erosion is a chemical-mechanical process,[1] and thus, there is no standardized method, indicated to reproduce dental erosion in laboratory environment. In this way, the objective of this study was to test different dental erosion protocols, in order to promote the totally enamel wear until the dentin in a way to get more similar lesions, including the associated use of the chemical-mechanical process, compare them and suggest a protocol that could result in a similar condition to those found clinically. The chemical-mechanical simulation was enabled by the use of a new testing machine to simulate oral ageing, the Rub&Roll,[15] this device is simple to use, is not technically demanding and allows to simulate more than a single aspect of normal clinical challenges e.g., chewing loads, or abrasive loads. It is possible to use several types of solutions, including hydrochloric acid, without damaging the apparatus and the mechanical cycling together. The specimens are embedded in a cylinder that allows the control of rotation speed, simulating the chewing speed, and also the simulation of the periodontal ligament membrane by the use of rubber pads below each sample. It also has a rubber rod that rotates in the opposite direction, promoting mechanical contact similar to abrasion.

To represent the extrinsic lesions from acid drinks we use the apple juice plus apple pulp, that presents a low pH (3.7) and some abrasiveness. The use of the pulp plus the juice was indicated by Rubben et al.[15] to promote an erosive-abrasive wear more intense than just the fruit juice.

The null hypothesis tested in this study, that time, solution, and different processes would not influence the results of roughness, Knoop hardness and surface evaluation, was partly rejected. In relation to the surface roughness, the use of HCl and apple juice for 96 hours resulted in higher values of surface roughness, regardless of the process (chemical, or chemical-mechanical). However, it is important to note that neither the type of solution (HCl or apple juice) nor the process significantly influenced the surface roughness of the groups submitted to 108 min erosive challenge and apple juice / mechanic / 96 h group. The only difference found in these groups is that the exposure time of 96h presented significant higher values of surface roughness than the control group, while the 108 min groups did not. Sulaiman et al.[16], when assessing the HCl effect on surface of different types of ceramic, found a significant interaction between the surface of the specimens and the acid solution, presenting an increase in surface roughness after acid challenge. To do so, they performed erosive challenges using HCl in cycles of 96 hours, in order to simulate 10 years of clinical function. Although the results were favorable to test the erosive effect in ceramics, in our study the time of 96 hours was excessive for the dental structures, provoking destructive lesions, more severe than the expected for simulated dental erosion lesions. These lesions can be explained by the lack of protective factors during the development of the cycle, that can be found in an artificial saliva or some mineral solution able to raise the pH and do the buffering of the acid important to the oral homeostasis.[19] The buffer capacity of saliva is an important factor, which plays a role in dental remineralization and in the maintenance of salivary pH,.do not let to be always below its critical level (5.5).[20]

In the study by Schlueter et al.[7] they presented found results similar to those of this study, having a positive correlation with the impact of the chemical-mechanical process where the effect of the brushing action led to higher values of mineral loss compared to the control group. The enzymes were indicated as coadjuvants in the progression of erosion, acting together to reduce the stability of the organic matrix, when in chronic exposure in cases of vomiting and gastroesophageal reflux, and thus facilitating mineral loss.

In regard to Knoop microhardness, it can be noted that the use of apple juice as a solution did not significantly influence the dentin hardness at the different depths evaluated, except for the superficial layer (10μm) for the group submitted to 96h of erosive challenge. In general, the lowest values of hardness were found in the groups that used HCl solution, regardless of time (108min or 96h) and the process (chemical or chemical-mechanical). Tedesco et al.[10] evaluated the erosive effect of carbonated beverages in association with the presence or absence of cariogenic challenge in primary tooth enamel, through Knoop microhardness. The loss of mineral tissue was assessed through transversal cut of the exposed surface and the evaluation at different depths, until the end of the enamel layer. All the beverages tested reduced the hardness values, as well as for the group that had the presence of the associated cariogenic challenge. These results are in agreement with those found in the present study since low pH solution influenced the microhardness of the specimens.

In the SEM images, it was possible to make a qualitative analysis of the structural alterations that occurred in the dental tissue according to the different challenges tested.[21] In our study, the groups of HCl that underwent the chemical and chemical-mechanical challenge of 96h had the greatest morphological alteration, presenting deep areas of exposed dentin. However, they extrapolated the expected results, due to the severity of the challenge. The HCl / chemical-mechanical group / 108min presented the transition zone between enamel and dentin, in this group the specimens had contact with the acid and chemical-mechanical action, and then they received baths in a remineralizing solution. This made this group closer to the wear patterns that occur in clinical reality. Since saliva neutralizes acid and calcium and phosphate cause remineralization.[22] Through the EDS, a semi-quantitative analysis of the minerals lost through the comparison between Calcium and Phosphorus ions, before and after the challenges, was performed. Erosion is associated with greater release of calcium from the affected surfaces, causing the hydroxyapatite crystals to dissolve in contact with acid.[23, 24] The estimated mineral loss measured by calcium ions that were released from hydroxyapatite and thus verified the erosive potential of acid on the dental surfaces found in the EDS analysis of the present study are corroborated by findings of previous studies.[23, 24] The groups that had greater exposure to acids (96h) had lower weight and percentage of calcium and phosphorus atoms, indicating their dissolution and thus exhibiting a greater erosive potential. Also, it is possible to observe that both groups that underwent 96 hours of erosion challenge with HCl presented the lowest values of calcium and phosphorus ions, which can be observed macroscopically in Figure 1 E and F. The association of chemical-mechanical erosive challenge with a reduced time (108min) resulted in a smaller reduction of such ions, but this demineralization was more similar (Figure 1; C) to those observed in *in vivo* erosion lesions.

Considering all tested protocols, it was possible to observe that the process, time and types of processes caused different wear patterns. The constant exposure time to the HCl in the dental surface can be extremely aggressive, culminating in the complete dissolution of the enamel layer and subjacent dentin. As well as the association of the mechanical method with chemical challenge, through abrasion, it becomes a significant additional factor in the process development and pattern of mineral loss, leading to further wear. To avoid the color change found in the apple juice / 96h group another kind of solutions (orange juice, and other acids such as malic, acetic or lactic) could be more appropriated to reproduce the extrinsic erosion. The method that reproduced the most similar macroscopic characteristics of dental erosion in this study was observed when a chemical-mechanical process was used with HCl solution during 108 min , presenting a more homogeneous demineralization surface and thus more similar to the cases found in the oral environment.

The *in vitro* nature of this study is a limitation, despite of we have proposed a new method we still cannot reproduce in laboratory all the events that we find in the oral cavity. Another one is related to the use of healthy bovine tooth as a control. To better establish comparative parameters of the erosion simulation *in vitro* with those observed in the oral environment, it would be ideal to use a human tooth that has undergone dental erosion by natural process as a control, in order to compare the different protocols to what occurs clinically. However, obtaining eroded tooth for this purpose is difficult due to some ethical aspects. Additionally, the use of proteolytic enzymes, cited as coadjutants in the erosion process, were not part of the tested erosion challenges. For future studies, the associated use of these enzymes in demineralization cycles would be interesting.

The importance of the participation of a dentist in a multidisciplinary treatment of patients presenting dental erosion has been emphasized in the literature,[5, 8, 25, 26] both for early detection of clinical signs, in order to avoid irreversible damage to dental structures, and to avoid development of more severe systemic complications in the case of eating disorders. In this context, the creation of standardized protocols for the simulation of dental erosion lesions in a laboratory environment could allow us to predict different erosion situations, simulating clinical lesions. Moreover, it can help us to develop techniques and materials that allow could perform better in regard to erosion resistance and adhesion to dentin of teeth that present moderate or severe erosion lesions.

5. Conclusion

Within the limitations of this study, it is possible to conclude that:

- Chemical-mechanical processes were more able to remove enamel due to erosive simulation. And chemical-mechanical immersion with HCl solution with cycles of demineralization/remineralization totaling 108min presented macroscopic results more similar to those found in *in vivo* erosion lesions, while 96h simulating resulted in extremely damaging and unreal erosion lesions.

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7. References

[1] Carvalho TS, Colon P, Ganss C, Huysmans MC, Lussi A, Schlueter N, et al. Consensus report of the European Federation of Conservative Dentistry: erosive tooth wear--diagnosis and management. Clin Oral Investig. 2015;19:1557-61.
[2] Jaeggi T, Lussi A. Prevalence, incidence and distribution of erosion. Monogr Oral Sci. 2006;20:44-65.
[3] de Carvalho Sales-Peres SH, Araujo JJ, Marsicano JA, Santos JE, Bastos JR. Prevalence, severity and etiology of dental wear in patients with eating disorders. Eur J Dent. 2014;8:68-73.
[4] Salas MM, Nascimento GG, Vargas-Ferreira F, Tarquinio SB, Huysmans MC, Demarco FF. Diet influenced tooth erosion prevalence in children and adolescents: Results of a meta-analysis and meta-regression. J Dent. 2015;43:865-75.
[5] Hermont AP, Pordeus IA, Paiva SM, Abreu MH, Auad SM. Eating disorder risk behavior and dental implications among adolescents. Int J Eat Disord. 2013;46:677-83.
[6] Smink FR, van Hoeken D, Hoek HW. Epidemiology of eating disorders: incidence, prevalence and mortality rates. Curr Psychiatry Rep. 2012;14:406-14.
[7] Schlueter N, Glatzki J, Klimek J, Ganss C. Erosive-abrasive tissue loss in dentine under simulated bulimic conditions. Arch Oral Biol. 2012;57:1176-82.
[8] Uhlen MM, Tveit AB, Stenhagen KR, Mulic A. Self-induced vomiting and dental erosion--a clinical study. BMC Oral Health. 2014;14:92.
[9] Ehlen LA, Marshall TA, Qian F, Wefel JS, Warren JJ. Acidic beverages increase the risk of in vitro tooth erosion. Nutr Res. 2008;28:299-303.
[10] Tedesco TK, Gomes NG, Soares FZ, Rocha RO. Erosive effects of beverages in the presence or absence of caries simulation by acidogenic challenge on human primary enamel: an in vitro study. Eur Arch Paediatr Dent. 2012;13:36-40.
[11] Hellwig E, Lussi A. Oral hygiene products, medications and drugs - hidden aetiological factors for dental erosion. Monogr Oral Sci. 2014;25:155-62.
[12] Gravelle BL, Hagen Ii TW, Mayhew SL, Crumpton B, Sanders T, Horne V. Soft drinks and in vitro dental erosion. Gen Dent. 2015;63:33-8.

[13] Schlueter N, Ganss C, Hardt M, Schegietz D, Klimek J. Effect of pepsin on erosive tissue loss and the efficacy of fluoridation measures in dentine in vitro. Acta Odontol Scand. 2007;65:298-305.
[14] Schlueter N, Hardt M, Klimek J, Ganss C. Influence of the digestive enzymes trypsin and pepsin in vitro on the progression of erosion in dentine. Arch Oral Biol. 2010;55:294-9.
[15] Ruben JL, Roeters FJ, Montagner AF, Huysmans MC. A multifunctional device to simulate oral ageing: the "Rub&Roll". J Mech Behav Biomed Mater. 2014;30:75-82.
[16] Sulaiman TA, Abdulmajeed AA, Shahramian K, Hupa L, Donovan TE, Vallittu P, et al. Impact of gastric acidic challenge on surface topography and optical properties of monolithic zirconia. Dent Mater. 2015;31:1445-52.
[17] Valente LL, Peralta SL, Ogliari FA, Cavalcante LM, Moraes RR. Comparative evaluation of dental resin composites based on micron- and submicron-sized monomodal glass filler particles. Dent Mater. 2013;29:1182-7.
[18] Shellis RP, Ganss C, Ren Y, Zero DT, Lussi A. Methodology and models in erosion research: discussion and conclusions. Caries Res. 2011;45 Suppl 1:69-77.
[19] Fenoll-Palomares C, Munoz Montagud JV, Sanchiz V, Herreros B, Hernandez V, Minguez M, et al. Unstimulated salivary flow rate, pH and buffer capacity of saliva in healthy volunteers. Rev Esp Enferm Dig. 2004;96:773-83.
[20] Meurman JH, ten Cate JM. Pathogenesis and modifying factors of dental erosion. Eur J Oral Sci. 1996;104:199-206.
[21] Schlueter N, Hara A, Shellis RP, Ganss C. Methods for the measurement and characterization of erosion in enamel and dentine. Caries Res. 2011;45 Suppl 1:13-23.
[22] Featherstone JD, Lussi A. Understanding the chemistry of dental erosion. Monogr Oral Sci. 2006;20:66-76.
[23] Bartlett DW, Coward PY. Comparison of the erosive potential of gastric juice and a carbonated drink in vitro. J Oral Rehabil. 2001;28:1045-7.
[24] Grenby TH, Mistry M, Desai T. Potential dental effects of infants' fruit drinks studied in vitro. Br J Nutr. 1990;64:273-83.
[25] Johansson AK, Norring C, Unell L, Johansson A. Eating disorders and oral health: a matched case-control study. Eur J Oral Sci. 2012;120:61-8.

[26] Conviser JH, Fisher SD, Mitchell KB. Oral care behavior after purging in a sample of women with bulimia nervosa. J Am Dent Assoc. 2014;145:352-4.

3. Considerações finais

Dentro das limitações deste estudo, é possível concluir que:

- Os processos químico-mecânicos foram melhores para remover o esmalte dental, devido à simulação erosiva;
- A imersão químico-mecânica em solução de HCl com ciclos de desmineralização e remineralização totalizando 108 minutos, apresentaram os resultados macroscópicos mais semelhantes aos encontrados nas lesões in vivo.

Referências

BUZALAF, M. A., KATO, M. T., et al. The role of matrix metalloproteinases in dental erosion. **Adv Dent Res**, v.24, n.2, p.72-76, 2012.

CAMARGO, M. A., MARQUES, M. M., et al. Morphological analysis of human and bovine dentine by scanning electron microscope investigation. **Arch Oral Biol**, v.53, n.2, p.105-108, 2008.

CARVALHO, T. S., COLON, P., et al. Consensus report of the European Federation of Conservative Dentistry: erosive tooth wear--diagnosis and management. **Clin Oral Investig**, v.19, n.7, p.1557-1561, 2015.

CONVISER, J. H., FISHER, S. D., et al. Oral care behavior after purging in a sample of women with bulimia nervosa. **J Am Dent Assoc**, v.145, n.4, p.352-354, 2014.

CORREA, M. D., NETTO, C. A., et al. Estudo morfológico comparativo entre dentina bovina e humana ao MEV. **Revista da Pós-Graguação**, v.10, p.312-316, 2003.

DE CARVALHO SALES-PERES, S. H., ARAUJO, J. J., et al. Prevalence, severity and etiology of dental wear in patients with eating disorders. **Eur J Dent**, v.8, n.1, p.68-73, 2014.

DE MORAES, M. D., CARNEIRO, J. R., et al. Effect of green tea as a protective measure against dental erosion in coronary dentine. **Braz Oral Res**, v.30, 2016.

EHLEN, L. A., MARSHALL, T. A., et al. Acidic beverages increase the risk of in vitro tooth erosion. **Nutr Res**, v.28, n.5, p.299-303, 2008.

GRAVELLE, B. L., HAGEN II, T. W., et al. Soft drinks and in vitro dental erosion. **Gen Dent**, v.63, n.4, p.33-38, 2015.

HELLWIG, E. and LUSSI, A. Oral hygiene products, medications and drugs - hidden aetiological factors for dental erosion. **Monogr Oral Sci**, v.25, p.155-162, 2014.

HERMONT, A. P., PORDEUS, I. A., et al. Eating disorder risk behavior and dental implications among adolescents. **Int J Eat Disord**, v.46, n.7, p.677-683, 2013.

JAEGGI, T. and LUSSI, A. Prevalence, incidence and distribution of erosion. **Monogr Oral Sci**, v.20, p.44-65, 2006.

JAEGGI, T. and LUSSI, A. Prevalence, incidence and distribution of erosion. **Monogr Oral Sci**, v.25, p.55-73, 2014.

JOHANSSON, A. K., NORRING, C., et al. Eating disorders and oral health: a matched case-control study. **Eur J Oral Sci**, v.120, n.1, p.61-68, 2012.

LAURANCE-YOUNG, P., BOZEC, L., et al. A review of the structure of human and bovine dental hard tissues and their physicochemical behaviour in relation to erosive challenge and remineralisation. **J Dent**, v.39, n.4, p.266-272, 2011.

MAGALHAES, A. C., LEVY, F. M., et al. Inhibition of tooth erosion by milk containing different fluoride concentrations: an in vitro study. **J Dent**, v.42, n.4, p.498-502, 2014.

RANJITKAR, S., KAIDONIS, J. A., et al. Gastroesophageal reflux disease and tooth erosion. **Int J Dent**, v.2012, p.479850, 2012.

SALAS, M. M., NASCIMENTO, G. G., et al. Diet influenced tooth erosion prevalence in children and adolescents: Results of a meta-analysis and meta-regression. **J Dent**, 2015.

SCHILKE, R., LISSON, J. A., et al. Comparison of the number and diameter of dentinal tubules in human and bovine dentine by scanning electron microscopic investigation. **Arch Oral Biol**, v.45, n.5, p.355-361, 2000.

SCHLUETER, N., GLATZKI, J., et al. Erosive-abrasive tissue loss in dentine under simulated bulimic conditions. **Arch Oral Biol**, v.57, n.9, p.1176-1182, 2012.

SCHLUETER, N., HARDT, M., et al. Influence of the digestive enzymes trypsin and pepsin in vitro on the progression of erosion in dentine. **Arch Oral Biol**, v.55, n.4, p.294-299, 2010.

SHELLIS, R. P., GANSS, C., et al. Methodology and models in erosion research: discussion and conclusions. **Caries Res**, v.45 Suppl 1, p.69-77, 2011.

SOARES, F. Z., FOLLAK, A., et al. Bovine tooth is a substitute for human tooth on bond strength studies: A systematic review and meta-analysis of in vitro studies. **Dent Mater**, v.32, n.11, p.1385-1393, 2016.

SULAIMAN, T. A., ABDULMAJEED, A. A., et al. Impact of gastric acidic challenge on surface topography and optical properties of monolithic zirconia. **Dent Mater**, v.31, p.1445-1452, 2015.

TEDESCO, T. K., GOMES, N. G., et al. Erosive effects of beverages in the presence or absence of caries simulation by acidogenic challenge on human primary enamel: an in vitro study. **Eur Arch Paediatr Dent**, v.13, n.1, p.36-40, 2012.

UHLEN, M. M., TVEIT, A. B., et al. Self-induced vomiting and dental erosion--a clinical study. **BMC Oral Health**, v.14, p.92, 2014.