Effects of fluoride and Aloe vera tooth gel in artificial white spot lesions in vitro

Efeito dos cremes dentais à base de flúor e Aloe vera na lesão artificial de mancha branca in vitro

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ABSTRACT

Objective
The aim of this study was to evaluate the effects of toothbrushing using a fluoride toothpaste and Aloe vera tooth gel on artificial white spot lesions through the Knoop microhardness (KHN) analysis.

Methods
Sound bovine enamel samples (2 mm/diameter and 2 mm/depth) were prepared and immersed in artificial white spot lesion for 24 h. The preparation of artificial white spot lesions was performed by pH-cycling process. The samples were randomly divided into two groups (n=20), according the dentifrice used: containing fluoride (Colgate Total 12) or Aloe vera (Forever Bright Aloe Vera Toothgel). The top surface of samples was submitted to 10,000, 25,000, 50,000 and 100,000 brushing cycles (200 g load) in an automatic brushing machine with abrasive slurry. The KHN analysis were evaluated at baseline, after immersion in artificial white spot lesion and after 10,000, 25,000, 50,000 and 100,000 cycles of brushing. Data were analyzed by two-way repeated measures ANOVA and Tukey tests (p=0.05).

Results
The KHN values significantly increased after brushing cycles compared to demineralized means. No significant differences showed for dentifrice factor (p=0.263). However, there were statistically significant differences between groups in cycles of brushing times (p=0.0001).

Conclusion
The toothpastes (containing fluoride or Aloe vera) were effective in increasing the superficial microhardness of artificial white spot lesions.


RESUMO

Objetivo
Avaliar o efeito da escovação simulada usando creme dental com flúor e Aloe vera em lesão de mancha branca artificial in vitro, através da análise da microdureza Knoop (KHN).

Métodos
Amostras de esmalte bovino (2 mm/diâmetro e 2 mm/altura) foram preparadas e imersas em solução artificial de cárie por 24 h. O preparo para indução de lesão de mancha branca foi realizado por processo de ciclagem de pH. As amostras foram aleatoriamente divididas em dois grupos (n=20), de acordo com o dentífrico utilizado: creme dental contendo flúor (Colgate Total 12) ou creme dental à base de Aloe vera (Forever Bright Aloe Vera Toothgel). A superfície das amostras foi submetida a 10.000, 25.000, 50.000 e 100.000 ciclos de escovação simulada (200 g/peso) em uma máquina de escovação. A análise de microdureza (KHN) foi realizada inicialmente, após o período de imersão em solução artificial de mancha branca e após 10.000, 25.000, 50.000 e 100.000 ciclos de escovação. Os dados foram submetidos aos testes RM-ANOVA dois fatores e Tukey (p=0.05).

Resultados
Os valores de KHN aumentaram significativamente após os ciclos de escovação, quando comparado aos valores após a imersão em solução desmineralizante. Não houve diferenças estatisticamente significativas entre os cremes dentais (p=0.263). Entretanto, em relação aos ciclos de escovação, houve diferenças significativas (p=0.001).

Conclusão
Os cremes dentais (contendo flúor ou Aloe vera) foram efetivos no aumento da microdureza superficial em lesão artificial de mancha branca.

INTRODUCTION

The dental caries is a pathological process of microbiological etiology resulting in the local destruction of dental tissues by the dissolution of the mineral phase (mainly constituted by hydroxyapatite crystals) by organic acids from the bacterial fermentation. The bacteria that have been associated to caries are the Streptococcus of mutans groups (which include the species Streptococcus mutans and Streptococcus sobrinus), Lactobacillus spp. and among others microorganisms favored by the cariogenic conditions promoted by Streptococcus of mutans groups, such as yeasts Candida albicans, capable of surviving and proliferating in acid media. Charaterized by an imbalance of the demineralization-remineralization processes (DE-RE), the caries disease is initiated by DE of the enamel surface. The acids Fermented by the cariogenic bacteria are diffused within the enamel and dissociated in hydrogen ion (H+), promoting the reduction of the pH of the medium, as well as the dissolution of calcium phosphate from the tissues, consequently with structural loss.

The visible initial alterations are displayed as opaque areas (white spots) in enamel, subject to RE. Researchers have demonstrated the development and stopping of initial lesions of white spot in human enamel and the possibility of their RE. Artificial caries lesions may be reproduced in vitro on enamel surface, simulating the main histological features of natural caries. Compared to the natural lesions of the subsuperficial deepness, the initial lesions of white spots are more easily reproducible and because of its homogeneity, they enable the use of several areas of surface for laboratorial tests of RE.

In vitro models of pH cycling enable to: evaluate the alterations in the tooth structure promoted by the mineral gain or loss; simulate the natural process of caries occurring within oral cavity; and assess the anticaries potential of fluoride dentifrices and mouthrinses. According to previous studies discussed about the benefits of Aloe vera tooth gel in oral conditions, the effects of toothbrushing using Aloe vera based dentifrice on the initial caries lesions must be reported.

Thus, the aim of this study was to evaluate the effects of toothbrushing using a fluoride toothpaste and Aloe vera tooth gel on artificial white spot lesions through the Knoop microhardness analysis. The null hypothesis tested was that the toothbrushing using Aloe vera tooth gel is not able to promote the same effect of fluoride toothpaste on artificial white spot lesions.

METHODS

Samples preparation

Forty sound bovine incisors were used to obtain enamel samples. The bovine incisors were obtained following a protocol approved by University Ethic Research Committee. The enamel cylinders (3 mm/diameter and 2 mm/height) were obtained using a trephine bur (3 mm in
diameter) with 1 mm of enamel and 1 mm of dentin. The
dentin surfaces were performed with 600 grit aluminum
oxide abrasive disks (Extec, Enfield, CT, USA) to standardize
the depth of the samples. The enamel surfaces were polished
in a polishing device (DP-10, Panambra Industrial e Técnica,
São Paulo, Brazil) using a sequence of 1200, 2400 and 4000
grit aluminum oxide abrasive disks (Extec, Enfield, CT, USA).
All specimens were stored in deionized water at 4°C, up to
the time of initial microhardness analysis.

Artificial white spot lesion
The artificial white spot lesions were prepared
according to the protocol proposed by Queiroz and others18.
The demineralizing solution consisted of 0.05 M acetate, 1.3
mM of calcium, 0.78 mM of phosphate and 0.0315 ppm F,
adjusted to a pH of 5.0. The pH assessment was performed
through a pHmeter (DM-20, Digimed, SP, Brazil).

The dentinal base of each sample was painted with
two layers of an acid-resistant nail varnish (Risqué, Cosmed
Indústria de Cosmético e Medicamento, SP, Brazil), except
the enamel surface. The samples were kept in individual
supports (Eppendorf, SP, Brazil) with 2 ml of demineralizing
solution for 24 h, at 37°C. After this period, the samples
were washed thoroughly, and stored into deionized water
for 24 h, at 37°C, up to the time of the second surface
microhardness analysis.

Brushing protocols
The enamel surfaces were subjected to brushing
abrasion in an automatic brushing machine (Odeme
Biotechnology, SC, Brazil) which imparted reciprocating
motion to 6 soft and straight bristle toothbrush heads
(Curaropix 5460 Ultra Soft, Curaden Swiss do Brasil Imp.
Exp. Ltda, SP, Brazil). This machine provides linear brushing
movements across the samples at a speed of 120 cycles per
min at 37°C, with a double pass of the brush head over the
surface, under a vertical load of 200 g with dentifrice slurry.
The dentifrice slurry consisted of dentifrice and deionized
water, in a ratio of 1:3, by weight19. The dentifrices employed
and their compositions were specified in Table 1.

Table 1. Composition of the dentifrices tested.

<table>
<thead>
<tr>
<th>Dentifrice</th>
<th>Composition</th>
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<tbody>
<tr>
<td>Colgate Total 12</td>
<td>Sodium monofluorophosphate (1450 ppm), sodium hydroxide, 0.3% triclosan,</td>
</tr>
<tr>
<td>(Colgate Palmolive Co.)</td>
<td>sodium lauryl sulfate, moist, thickener, sorbitol, water and hydrated silica.</td>
</tr>
<tr>
<td>Forever Bright Aloe</td>
<td>Aloe barbadensis gel, sorbitol, hydrated silicaglycerin, sodium lauryl sulfate,</td>
</tr>
<tr>
<td>Vera Tooth gel (Forever living</td>
<td>sodium benzoate and bee propolis, fluoride-free.</td>
</tr>
<tr>
<td>products Brazil Ltda.)</td>
<td></td>
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</tbody>
</table>

The top of enamel surfaces were submitted to
10,000 cycles of brushing for 90 min, 25,000 cycles of
brushing for 4 h 15 min, 50,000 cycles of brushing for
7 h 30 min and a total of 100,000 cycles of brushing
for 15 h, simulating 4.2 years of brushing in oral
cavity20. After each cycles of brushing, the samples were
kept into deionized water at 37°C, up to the time to
microhardness analysis.

Knoop microhardness measurements
The microhardness measurement was performed with a microhardness tester (FM-700, Future
Tech Company, Tokyo, Japan), Knoop tip, under 50 g
load for 15s21. Three indentations were performed 100
µm apart from each other, at the surface of enamel
samples. The means values were determined as Knoop
Hardness Number (KHN) and their increase or reduction
means, respectively, the remineralization (mineral gain)
or demineralization (mineral loss) of the enamel22.

The Knoop microhardness was measured onto
the sound enamel surface (at baseline time), after
the artificial white spot lesion (DE) and after 10,000, 25,000,
50,000 and 100,000 cycles of brushing.

Statistical analysis
The experimental variables under study were
dentifrices (Forever Bright Aloe Vera Tooth gel and
Colgate Total 12) and times (at baseline, after artificial
white spot lesion-DE, after 10,000, 25,000, 50,000 and
100,000 cycles of brushing). The variable response was
the average of three microhardness measurements.

Data were submitted to statistical analysis using
the computer program Minitab (version 16.1, Minitab,
2010), Statistix (version 8.0, Analytical Software, 2003)
and Statistica (version 9.1, StatSoft, 2010). The inferential
statistics consisted of two-way repeated measures
ANOVA (dentifrices and times), followed by Tukey’s test.
The level of significance was the conventional value of
5% (p<0.05).

RESULTS

The KHN means values and standard deviation
is shown in Table 2. Regarding the different times, the
KHN values significantly increased after brushing cycles
compared to DE means. The application of RM-ANOVA
showed significant differences for the time factor
(p=0.0001). However, no significant differences showed
for dentifrice factor (p=0.263).
The DE groups were statistically different at brushing cycles. Colgate and Aloe vera tooth gel showed the similar KHN values at DE time. Also, it was observed that after 10,000 cycles of brushing, both groups showed higher KHN values than DE time.

There were statistically significant differences between groups in cycles of brushing times. Aloe Vera tooth gel exhibited KHN values higher than those of Colgate at 10,000, 25,000 and 100,000 cycles of brushing.

**DISCUSSION**

Aloe vera (*Aloe barbadensis*) used as a gel has complex mixture of chemical composition. The synergic effect of these components in Dentistry is still not clear. Its gel is composed of 98-99% of water and 1-2% of active components (anthraquinones, naftoquinones, flavonoids, polysaccharides, essential amino acids and vitamins), which are highly variable according to the specie and growth conditions of the plant.

Among the essential amino acids Aloe vera gel, arginine is the most abundant. Within the gel, it is also found the salicylic acid, uronic acid and galacturonic acid; fructose, mannose, glucose and other hydrolysable sugars; enzymes as oxidase, amylase and catalase; sodium, potassium, calcium and magnesium. The hard and mineralized enamel surface is porous and may enable the passage of ions, such as sodium, potassium, magnesium and calcium. Therefore, the remineralizing capacity of the Aloe vera based dentifrice, constituted of 35% of pure gel, could be attributed to the deposition of arginine associated to the calcium on the enamel surface.

When the enamel surface is exposed to acids formed by bacterial metabolism, the minerals are removed from the hydroxyapatite crystals, causing in increase in intercrystalline spaces and porosity in the surface. The visible initial alterations are displayed as opaque areas (white spot lesions), which can be remineralized.

In previous studies, the enamel surface submitted a demineralization solution for 24 hours showed decrease in brightness of surface and microscopically some changes on its surface similar to initial caries lesion.

In this present study, white spot lesions in enamel were artificially produced by demineralizing solution (pH=5), instead of oral isolates of *S. mutans*. The biofilm on the enamel surface was initially removed by the polishing of the specimens, and brushing cycles prevented the establishment of a new biofilm. Both dentifrices employed in this study showed in their composition sodium lauryl sulphate that has an emulsifier action and promotes a moderate antiplaque effect. Additionally, the Aloe vera based dentifrice has propolis in its composition, which also has an antiplaque potential. Moreover, some authors concluded that Aloe vera gel, at optimum concentration in either dentifrices or mouthrinses, is effective in preventing the caries diseases.

The results of Knoop microhardness in this study demonstrated that Aloe vera based dentifrice (Forever Bright Aloe Vera Toothgel) is as effective as the fluoride dentifrice (Colgate Total 12) on artificial white spot lesions in enamel. These findings are in agreement with a previous study that observed Aloe vera toothpaste gel (Forever Bright) was equally effective as two popular commercial toothpastes. Thus, the null hypothesis of the present study was rejected.

Regarding brushing cycles, it is observed that after 10,000 cycles of brushing in both dentifrices were significantly effective on artificial white spot lesions compared to the demineralized group. At 50,000, 100,000 cycles of brushing,

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**Table 2. Means (±SD) of KHN and Tukey tests for the dentifrices and times.**

<table>
<thead>
<tr>
<th>Times</th>
<th>Colgate Total 12</th>
<th>Forever bright aloe vera tooth gel</th>
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<tbody>
<tr>
<td></td>
<td>Mean ±SD</td>
<td>Mean ±SD</td>
</tr>
<tr>
<td>Baseline</td>
<td>257.0±80.4BC</td>
<td>305.5±75.8&lt;sup&gt;AB&lt;/sup&gt;</td>
</tr>
<tr>
<td>DE</td>
<td>147.9±28.5E</td>
<td>149.4±43.3&lt;sup&gt;C&lt;/sup&gt;</td>
</tr>
<tr>
<td>10,000 cycles</td>
<td>165.1±57.6DE</td>
<td>193.5±61.4&lt;sup&gt;DE&lt;/sup&gt;</td>
</tr>
<tr>
<td>25,000 cycles</td>
<td>190.5±51.0DE</td>
<td>195.8±62.8&lt;sup&gt;DE&lt;/sup&gt;</td>
</tr>
<tr>
<td>50,000 cycles</td>
<td>286.8±65.0AB</td>
<td>218.6±45.3&lt;sup&gt;C&lt;/sup&gt;</td>
</tr>
<tr>
<td>100,000 cycles</td>
<td>293.3±47.6AB</td>
<td>329.8±77.7&lt;sup&gt;A&lt;/sup&gt;</td>
</tr>
</tbody>
</table>

Note: Different letters means significant differences among groups (p<0.05).
of brushing, the fluoride dentifrice differs significantly from the Aloe vera tooth gel. Colgate Total 12 showed KHN values greater than baseline values, remaining these values without significant differences until 100,000 cycles. After 100,000 cycles of brushing, both dentifrice - Aloe vera and Colgate Total 12 - exhibited microhardness values as same as the baseline time, which showed the effectiveness of the both dentifrice in treatment of white spot lesion. In a previous clinical trials, Aloe vera toothpaste did not show any significant differences from fluoridated dentifrices for the control and reduction of dental plaque and gingivitis.11

It is highlighted that fluoride and Aloe vera are incompatible because the therapeutic potential of Aloe vera is lost together with fluoride. Aloe vera based dentifrice is also less abrasive than the fluoride dentifrice.17 However, the majority of the antimicrobial effects of commercially available toothpastes can be attributed to their fluoride content, in the form of sodium monofluorophosphate (a concentration of 500–1,000 ppm). The Aloe vera tooth gel used in this study has no added fluoride content but it exhibits the same antimicrobial effect on artificial white spot lesions that conventional toothpaste with fluoride content.

It is known that the fluoride applied onto the tooth surface reduces the enamel solubility in acid medium, which inhibits demineralization and accelerates remineralization. Additionally, it has bactericidal and bacteriostatic effects depending on its concentration, but the excessive ingestion of fluoride during the period of tooth formation causes fluorosis.

Dental fluorosis is a result of excessive fluoride ingestion during enamel development, which for the permanent teeth occurs in childhood between 2 to 8 years old.27 The use of fluoride in preventive dentistry has been the most effective in carious lesions, but it also associated with the increasing prevalence of dental fluorosis in many countries. Some authors reported that there were chances of mild fluorosis in children, especially younger than 6 years, who ingested large amounts of fluoride from reconstituted concentrate infant formulas and fluoridated dentifrice.18-30.

Therefore, to minimize the risk of developing fluorosis while maximizing the caries-prevention, the Aloe vera based dentifrice (without fluoride) would be an alternative to oral hygiene, especially in children younger than 6 years old. More studies should be done in order to clarify the mechanism of remineralization of Aloe vera tooth gel on the demineralized enamel surface.

Thus, Aloe vera based dentifrice is effective in preventing the white spot lesion as same as a conventional fluoride dentifrice and it would be an option for toothbrushing in children under the age of six.

CONCLUSION

Based on the methodology proposed, it can be concluded that the toothpastes containing fluoride or Aloe vera were effective in increasing the superficial microhardness of artificial white spot lesions.

Collaborators

TM SILVA participated in methodology, statistical analysis and the writing of the paper. BM FONSECA participated in the development of the methodology, laboratorial tests and in the writing of the manuscript. ALLS SALES collaborated in the laboratory part and writing of the paper. P HOLLEBEN collaborated during experimental and statistical analysis, as well as writing part. MC VALERA was responsible for the research proposed, methodological development and the writing of this study. MAM ARAUJO was also responsible for this study and participated during the experimental design and writing of the paper.

REFERENCES


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