

Comparison of the Remineralizing Effect of Brushing with *Aloe vera* versus Fluoride Toothpaste

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Eur J Dent:2021;5:133–138

Abstract

Keywords

- ▶ *Aloe vera*
- ▶ remineralization
- ▶ fluoride
- ▶ toothpaste
- ▶ SEM-EDX
- ▶ prevention
- ▶ dentistry

Objectives The aim of the present *in vitro* study is to compare the remineralization brushing effect of three toothpastes and *Aloe vera* (AV) gel.

Materials and Methods Forty sound extracted teeth were placed in a demineralizing solution for 4 days and randomly assigned to four groups: group A: 1,450-ppm fluoride toothpaste; group B: AV nonfluoridated toothpaste; group C: AV 1,000-ppm fluoridated toothpaste; and group D: AV gel. A 3-minute pH cycling was performed twice a day for each group for 12 days. Specimens were analyzed before and after by scanning electron microscope—energy dispersive X-ray.

Statistical Analysis The outcomes were analyzed by Kolmogorov–Smirnov’s tests, repeated-measures analyses of variance followed by univariate analyses, and Bonferroni’s multiple comparisons tests to compare the calcium-to-phosphorus (Ca:P) ratio within time among toothpaste groups.

Results Following remineralization, the Ca:P ratio increased in all groups. The difference of the Ca:P ratio was not significant between groups C, D, and A. The mean ratio was significantly lower in group B (p -value = 0.026).

Conclusions The AV gel demonstrated a remineralization capacity equal to that of the 1,450-ppm fluoride toothpaste. In contrast, fluoride-free AV toothpaste showed a lower remineralization efficiency. Further studies are required to understand its mechanism.

Introduction

Dental caries is a public health issue that affects a large number of people around the world. It is mainly caused by a dynamic process characterized by a succession of both alternating demineralization and remineralization periods. The lesion progresses when the demineralization favoring pathological factors such as fermentable carbohydrates, cariogenic bacteria, and salivary dysfunctions win over the protective factors such as antibacterial agents, remineralizing ions, and efficient saliva.¹

Fluoride has been proven to reverse and prevent the carious process by inhibiting demineralization, enhancing remineralization, and considering its antibacterial

properties.^{2–6} Since the 1950s, fluoride has been considered the gold standard in caries prevention. However, emerging epidemiological data show a worrying trend: Caries rate is stagnating or even increasing in some population groups notwithstanding the regular use of fluoride toothpastes in these countries.^{7–10} In the era of holistic dentistry and after fluoride has been classified as a chemical neurotoxicant, some safety concerns arose regarding the use of highly concentrated fluoride products.¹¹ More appropriate are the thriving concerns that children nowadays are exposed to fluoride from multiple sources, putting them at risk of developing dental fluorosis.¹²

Considering the narrow “dose gap” between caries depletion advantages and fluoride adverse toxic effects, public health officials have limited fluoride concentrations in

DOI <https://doi.org/10.1055/s-0040-1716597>
ISSN 1305-7456.

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over-the-counter toothpastes to within 1,000 and 1,500 ppm. As for children younger than 6 years, this dose is even lower and considered less than the effective remineralization level of early carious regions.¹³ Evidently, the need for newer remineralization agents arises to complement to fluoride effect and to enhance its remineralization efficacy on the carious lesions.¹⁴ The actual tendencies aim to shift toward natural products to allay the safety concerns, thanks to their fewer side effects,¹⁵ and their effectiveness in the field of dentistry.

Numerous medicinal plant extracts have shown the ability to inhibit the formation of dental biofilms by reducing pathogen adhesion to tooth surface, a primary event in caries formation.¹⁶ Among all the different herbs used in the dental field, modern science corroborates *Aloe vera* (AV)'s antimicrobial properties. Studies suggest that AV (*Aloe barbadensis*) extract is appropriate for treating gingivitis and oral infections since it inhibits plaque formation and bacterial growth.^{17,18}

A cactus-like plant, AV encompasses 360 species containing 75 active ingredients such as vitamins, enzymes, minerals, sugars, lignins, saponins, salicylic acids, and amino acids.¹⁹ AV's medicinal effect *in vitro* or on animal models has demonstrated anti-inflammatory, antiarthritic, antibacterial effects, immune-boosting, and hypoglycemic properties.²⁰ Other studies have evaluated AV's effect on systemic disease, cancers, regulation of blood glucose levels, and wound and infection healing.¹⁹

Several published studies have further reported the use of AV in dentistry for various purposes such as its antiseptic effect on *Candida albicans* and aphthous stomatitis treatment,²¹ as well as its use as an ingredient in toothpastes and mouthwashes for the treatment of gingivitis.²²⁻²⁴ However, there are only few other studies about the particular effect of AV in the remineralization of early carious lesions.²⁵

This study intends to evaluate the *in vitro* effects of the local application of AV gel to demineralized teeth and, ultimately, observe the possibility of using AV gel or an AV-based toothpaste as an adjuvant substance to fluoride toothpastes.

Materials and Methods

In this study, 40 sound permanent premolar and molar teeth extracted for diverse purposes were collected from the Department of Oral Surgery, Faculty of Dental Medicine, Saint Joseph University of Beirut. The study was approved by the university ethics committee.

The 40 teeth were cleansed of soft tissue debris and inspected for cracks, hypoplasia, caries, and white spot lesions, then coated with a nail varnish, leaving a narrow 4 mm × 1 mm wide window on the intact surface of the buccal enamel. They were then immersed in the demineralizing solution for 4 days to produce lesions in the window area following the published protocol of Kumar et al.²⁶ The demineralizing solution contained 2.2 mM CaCl₂, 2.2 mM NaH₂PO₄, and 50 mM acetic acid and had a pH adjusted to 4.4 at 37°C with 1 M KOH. Each tooth was then sectioned buccolingually, thus creating two sections from each tooth to measure the lesion depth. Eleven hemisections were very thin and were excluded from the study because of technical

problems arising while using the scanning electron microscope (SEM).

The prepared specimens were then gold sputtered and observed under the SEM at a magnification of 1,000. Energy dispersive X-ray (EDX) analysis was performed to assess the mineral content in the samples. SEM was used for the structural analysis, whereas EDX was used for the elemental analysis.

Test Groups

The sections were randomly assigned to four treatment groups as follows. Group A, a positive control group, included 14 hemisections treated with a 1,450-ppm fluoride toothpaste (Colgate Total, Bangkok, Thailand). Group B, an experimental group, comprised 14 hemisections treated using an AV 1,000-ppm fluoride toothpaste (Dr Organic Aloe vera toothpaste, Holland & Barret). The experimental group C consisted of 15 hemisections treated using an AV nonfluoridated toothpaste (Aloe Dent whitening, Holland and Barret). As to group D, it comprised 14 experimental hemisections coated with an AV leaf gel.

Toothpaste supernatants in groups A, B, and C were prepared by suspending 10 g of the respective toothpaste in 30 mL of deionized water to achieve a ratio of 1:3 (toothpaste:deionized water).²⁶ The suspensions were thoroughly stirred and mechanically shaken for 1 minute.

For group D, a sufficient amount of gel from the AV leaf was deposited in a sterile container, stored at 4°C until application time and changed daily. The gel's chemical analysis demonstrated the presence of 98.5% of water. The remaining parts are solid matter, compounds comprising vitamins, minerals, enzymes, polysaccharides, phenolic compounds, and organic acids in variable proportions (→ Fig. 1).

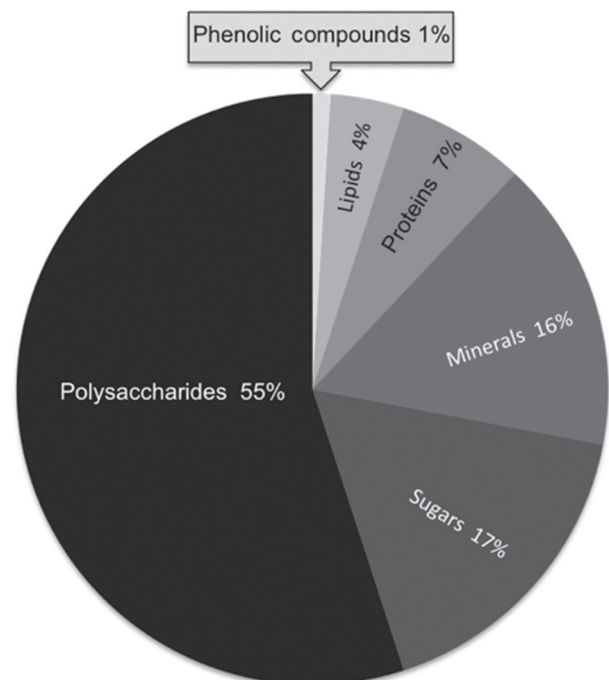


Fig. 1 Composition of the solid matter of the *Aloe vera* gel.

Each group was treated with their, respectively, assigned toothpaste for 10 days, twice daily for 3 minutes, at 10 AM and 8 PM while placed on an orbital shaker. For the remaining time during these 10 days, the sections were immersed in the already prepared remineralizing solution consisting of 1.5 mM of CaCl_2 , 0.9 mM of NaH_2PO_4 , and 0.15 M of KCl with a pH adjusted to 7 at 37°C. SEM-EDX was conducted after the remineralization. The calcium (Ca) and phosphorus (P) mean value and standard deviation as well as the Ca:P ratio were calculated, then compared between the groups.

Statistical Analysis

The Statistical Package Software for the Social Sciences (SPSS for Windows, Version 25.0, Chicago, Illinois, United States) was used to perform statistical data analysis. The α error was set at 0.05. The mean percentage and standard deviation of each group were subsequently calculated.

Kolmogorov–Smirnov’s tests were conducted to verify the distribution normality of quantitative variables. Repeated-measures analyses of variance followed by univariate analyses and Bonferroni’s multiple comparisons tests were performed to compare the Ca:P ratio within time among toothpaste groups.

Results

The mean and standard deviation of the Ca:P ratio for the four toothpastes are presented in ►Table 1, and the diagrams showing the weight percentage of Ca and P ions before and after remineralization are presented in ►Figs. 2 and 3.

Before and After Remineralization

This study showed that the average ratio as well as the Ca percentage significantly increased after mineralization in the groups treated with the AV 1,000-ppm fluoridated toothpaste (p -value = 0.001), 1,450-ppm fluoride toothpaste (p -value < 0.001), and AV gel (p -value = 0.002). Although the average

ratio increased in the fluoride-free AV toothpaste group, the increase was close to the significance (p -value = 0.054).

Comparison between Groups

After demineralization, the difference was not significant between the four groups (p -value = 0.301). However,

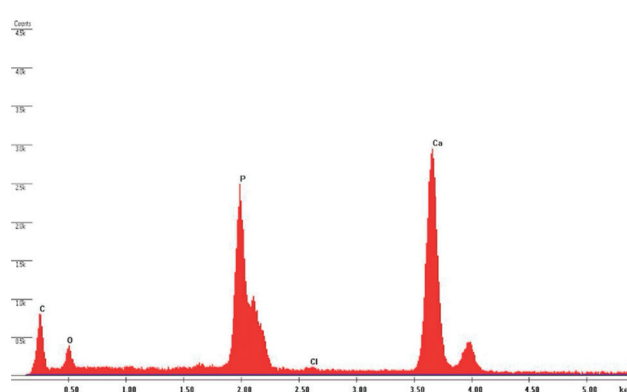


Fig. 2 Energy dispersive X-ray diagram showing wt% of Ca after demineralization.

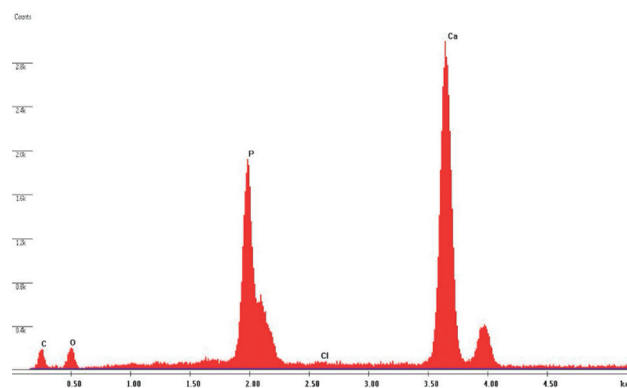


Fig. 3 Energy dispersive X-ray diagram showing wt% of Ca after remineralization.

Table 1 Calcium-to-phosphorus ratio before and after respective treatment

	Groups	Mean	Standard deviation	N	p-Value
Calcium/phosphorus ratio before	<i>Aloe vera</i> and fluoride toothpaste 1,000 ppm	1.800	0.1840	14	
	Nonfluoridated <i>Aloe vera</i> toothpaste	1.687	0.1356	15	
	Fluoridated toothpaste 1,450 ppm	1.700	0.1664	14	
	<i>Aloe vera</i> gel	1.735	0.2039	14	
Calcium/phosphorus ratio after	<i>Aloe vera</i> and fluoride toothpaste 1,000 ppm	2.043	0.1828	14	0.001
	Nonfluoridated <i>Aloe vera</i> toothpaste	1.820	0.2426	15	0.054
	Fluoridated toothpaste 1,450 ppm	2.057	0.2027	14	<0.001
	<i>Aloe vera</i> gel	1.950	0.2226	14	0.002

Note: N, number of elements in the sample.

following remineralization, the mean ratio was significantly different among the groups. It was smaller in the AV fluoride-free group (p -value = 0.026), whereas the difference was not significant between the 1,000-ppm fluoridated AV, AV gel, and 1,450-ppm fluoride toothpaste (p -value > 0.05)

Discussion

AV nonfluoridated toothpaste induces a remineralization lower than that obtained from the 1,450-ppm fluoride toothpaste, but the addition of AV to a 1,000-ppm fluoride toothpaste serves as a remineralization adjuvant. The AV gel offers remineralization similar to that induced by a 1,450-ppm fluoride toothpaste. Even under fluoride-free conditions, the AV gel acts as a dental remineralizing booster.

The protocol of this study was developed to reduce bias: SEM allows the evaluation of morphological variations between treated and untreated surfaces. However, one key drawback inherent in SEM's exclusive use is that it does not assess the treated and untreated surfaces' chemical composition as well as their mineral loss and gain. To overcome this problem, EDX tooth analysis was used in conjunction with SEM analysis to obtain the Ca and P percentage in the teeth before and after remineralization. Shaik et al, Chaudhary et al, and Sriamporn et al also used SEM-EDX in their *in vitro* studies.²⁷⁻²⁹

The study's protocol conditions were most compatible with the oral conditions. The remineralizing solution used is the closest in its composition to mineral ions and in its supersaturation to natural saliva as reported first by ten Cate and Duijsters.³⁰

A 4 mm × 1 mm-wide window was created on the buccal surface of the enamel to standardize the window size in all specimens, produce lesions only in the area of the window, and limit the area of paste application according to Shirahatti et al and Vashisht et al.^{31,32} The teeth were immersed in a demineralization solution for 4 days, and then evaluated for any mineral content loss (% by weight) using SEM-EDX after sectioning them in halves.

A 10-day pH-cycling period was followed, which is sufficient to create changes in lesions without the risk of damaging thin enamel specimens.³³ The solutions used were changed regularly to avoid solution saturation or exhaustion due to the accumulation of the enamel dissolution products.³⁰ Specimens in each group were shaken during the 3-minute application of the respective treatment to prevent the formation of deeper lesions and greater mineral loss compared with unstirred systems.³⁴

Our *in vitro* study shows that remineralization has been achieved across all groups, with the highest rate of remineralization achieved when treatment was performed with the AV gel, followed by the AV 1,000-ppm fluoride toothpaste both at a level similar to that of a 1,450-ppm fluoride toothpaste. In the literature review on natural products used for tooth decay prevention, the role of polyphenols in dental remineralization has systematically been preeminent.³⁵ In fact, the gel alone is composed of 98.5% of water. The remaining components represent a mixture of vitamins, minerals, enzymes, polysaccharides, phenolic compounds, and

organic acids in different proportions. Polyphenols, including anthraquinones, seem to be the essential active chemical component responsible for the AV gel's capacity in inducing remineralization.

On the contrary, the remineralization effect of the AV fluoride-free toothpaste is less than the other three groups, including the AV gel group. This can be explained by the concentration of the active components, present in lesser amounts in the toothpaste while they are more saturated in the gel or due to the industrialization process.

Since the remineralizing effect of the AV toothpaste and AV gel was not previously developed in the literature, the claim can be made that the study results are of a pioneering nature; hence, they are not comparable to those of other studies on AV dental use. Thus, a comparison of the results with other *in vitro* studies experimenting the remineralization action of new nonfluoridated remineralization products will be followed in this discussion, which also used the SEM-EDX technique to measure changes in Ca and P concentrations in the carious lesion.

Casein phosphopeptide–amorphous calcium phosphate, known as CPP-ACP, plays a role in preventing demineralization and improving remineralization.³⁶ Hegde and Moany (2012) aimed to evaluate CPP-ACP potential for remineralization on enamel lesions over time. A pH cycling of 7, 14, 21, 28, and 35 days followed a 5-day demineralization procedure.³⁷ The collected specimens were then analyzed by SEM-EDX and the Ca:P ratio was calculated. The results showed an average Ca:P ratio of 1.5 prior to treatment, which aligns with that obtained in this study. After 14 days of remineralization by CPP-ACP, the Ca:P ratio was 1.7, and it is only after 35 days that the Ca:P ratio reached 1.9, a value reached by treatment with AV gel after 10 days only. In addition, treatment with AV toothpaste and 1,000-ppm fluoride has a remineralizing efficacy (2.0043) greater than that with CPP-ACP even after 35 days of application (1.93). Also, among the natural products that have a role in dental remineralization, we cite the *Galla chinensis* extract (GCE).

Cheng et al³⁸ in their study on the effect of GCE on the remineralization of the enamel surface *in vitro* used bovine teeth to which a 12-day pH cycling was performed to try to remineralize the carious lesions using four different types of products including fluoride, GCE, gallic acid, and demineralized water as a control group. The enamel specimens were analyzed using a SEM-EDX device. The percentage of Ca obtained in the NaF group (30.88%) is lower than that obtained in our study (41.47%) in the similar group; however, the Ca:P ratio of 2.12 is comparable to the ratio obtained in the present study (2.043). These differences can be explained not only by the use of different protocols and demineralization models in the two experiments but also by the different EDX calibration procedures and the number of measurements taken for each sample. In addition, different counting rates, dead times, measurement times, and measurement windows could explain these differences. Taking into account the comparability of the Ca:P ratios, this can be extrapolated to compare the effectiveness of remineralization between AV and GCE: Cheng et al

obtained a ratio of 1.91 after treatment with GCE, whereas in our study, we obtained 1.950 after treatment with AV gel and 2.043 for a toothpaste based on AV fluorinated at 1,000 ppm. This result is favorable to the gel of AV, which, according to the results obtained, has remineralizing properties on the surface better than those found with the extract of GCE.

Also, Zhang et al³⁹ in 2009 performed a comparative study on the remineralizing power *in vitro* of GCE and a fluoride toothpaste. The measurement technique used is similar to that used in our study, SEM-EDX. The percentage by mass of Ca (46.73%) and P (19.14%) after treatment with fluoride toothpaste is consistent with the results obtained in our study with fluoride toothpaste. Although the percentage of Ca in the GCE group increased statistically significantly after treatment, the ratio obtained (1.52) remains lower than that obtained with AV gel (1.950).

Conclusion

Within the limitations of this study, the remineralization induced by 1,000-ppm fluoridated AV toothpaste and AV gel showed a similar rate to that of the 1,450-ppm fluoride toothpaste. This study may be considered a pivotal cornerstone for future studies targeted at explaining the process by which AV gel transports Ca ions to the tooth surface.

However, chemical characterization and standardization of the use of AV, as well as *in vivo* studies using various measurement techniques, are required prior to further implementation in trials and clinical use.

Funding

None.

Conflict of Interest

None declared.

Acknowledgments

This article and the research behind it would not have been possible without the financial and moral support of the Saint Joseph University of Beirut, through its research council. We thank the Lebanese University for providing some of the equipment needed, our colleague Dr. Nada Osta for the statistical guidance, as well as Dr. Roland Habchi, Jamil Ali, and Charbel Mansour for the laboratory assistance.

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