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In vitro evaluation of Biosilicate® dissolution on dentin surface. A SEM analysis

Avaliação in vitro da dissolução do Biosilicato® sobre a superfície dentinária. Análise por meio da microscopia eletrônica de varredura

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Resumo

Introdução: Biomateriais, tais como vidros bioativos e vidros cerâmicos têm sido propostos para o tratamento da hipersensibilidade dentinária. **Objetivo:** Avaliar por microscopia eletrônica de varredura (SEM), a dissolução de uma nova vitrocerâmica bioativa (Biosilicato®, partículas de 1-20 µm) na superfície de amostras de dentina, com diferentes métodos de aplicação e de meio de diluição diferente, usado para aplicação de Biosilicato®. **Material e método:** 280 amostras de dentina foram divididas aleatoriamente em quatro grupos: (1) Biosilicato® mais flúor gel aplicado com escova de Robinson, (2) Biosilicato® mais flúor gel aplicado com microbrush, (3) Biosilicato® mais água destilada aplicado com escova de Robinson; (4) Biosilicato® mais água destilada aplicado com microbrush. Após o tratamento, as amostras foram imersas em saliva, em diferentes períodos (0, 30 e 15 minutos, 1, 2, 12 e 24 horas). Duas fotomicrografias foram obtidas a partir de cada amostra e foram analisadas por um examinador calibrado cego de acordo com um "Índice de Dissolução de Partículas", criado para este estudo. **Resultado:** Os dados foram analisados usando o teste de Mann-Whitney, Kruskal-Wallis e Dunn. Não houve diferença estatística entre os graus de dissolução entre os quatro grupos em qualquer período. **Conclusão:** Biosilicato® pode ser incorporado em ambas às substâncias, sem diferenças no grau de dissolução das partículas em qualquer dos períodos de avaliação e a aplicação sobre da dentina pode ser realizada com os dois métodos avaliados.

Descritores: Materiais biocompatíveis; sensibilidade da dentina; flúor.

Abstract

Introduction: Biomaterials such as bioactive glasses and glass-ceramics have been proposed for the treatment of dentinal hypersensitivity. **Objective**: to evaluate by scanning electron microscopy (SEM), the dissolution of a novel bioactive glass-ceramic (Biosilicate® 1-20 µm particles) on dentin surface samples, with different application methods and different dilution medium used for applying Biosilicate®. **Material and method**: 280 dentin samples were randomly divided into four groups: (1) Biosilicate® plus fluoride gel applied with Robinson brush; (2) Biosilicate® plus fluoride gel applied with microbrush; (3) Biosilicate® plus distilled water applied with Robinson brush; (4) Biosilicate® plus distilled water applied with microbrush. After treatment, the samples were immersed in saliva at different periods (0, 15 and 30 minutes, 1, 2, 12 and 24 hours). Two photomicrographs were obtained from each sample and were further analyzed by a blind calibrated examiner according to a "Particle Dissolution Index" created for this study. **Result**: The data were analyzed using the Mann-Whitney, Kruskal-Wallis and Dunn's tests. There was no statistical difference among the degrees of dissolution between the 4 groups in any period. **Conclusion**: Biosilicate® can be incorporated in both substances without differences in the degree of dissolution of the particles in any of the evaluated periods and the application of dentine can be performed with both methods evaluated.

Descriptors: Biocompatible materials; dentin sensitivity; fluorine.

INTRODUCTION

Cervical Dentin Hypersensitivity (DH) is a symptom frequently encountered in the population and its prevalence is estimated to range from 4 to 57%. This percentage tends to increase with a higher awareness of the population about the importance of maintaining healthy teeth in the oral cavity, allowing dental elements to be more susceptible to factors that could cause dentin exposures[1].

DH is clinically defined as an acute, transient and well located pain which occurs in exposed dentin in response to tactile, thermal, evaporative, osmotic or chemical stimuli and which cannot be attributed to any other dental pathology. This pain does not occur spontaneously and does not persist after the stimulus removal[2]. According to the hydrodynamic theory proposed by Brännström, this pain occurs as a result of sensory nerve fibers activation in the pulp which is caused by the movement of fluids within the dentinal tubules. These sensory nerve fibers are activated by a hydrodynamic stimulus applied on the dentin exposed to the oral environment[3].

Given the importance of dentin exposure and opened dentinal tubules, the relationship between conductivity of dentin and the hypersensitivity in the etiology of DH, it is certain that the treatment for such symptoms depends on the obliteration of dentinal tubules. Thus, most researches on treatment for DH, focus on the capability of the available products to promote regression of the pain caused by exposure of these tubules by changing the diameter of the tubules, obliterating them or preventing the sensitization of the sensory nerve fibers[4,5].

The proposed therapy includes periodontal surgery, restorative treatment, laser application and use of chemical substances for tubule obliteration. Numerous treatments are proposed but only those that eliminate the soreness and effectively prevent the recurrence of pain can be considered definitive[6].

Some studies search for a material that obliterates the dentinal tubules in an effective and lasting way. Some authors suggest the use of bioactive glass particles for the DH treatment[7,8].

The elimination of DH is obtained with the dissolution of bioactive glass particles and subsequent precipitation of calcium and phosphate ions at the opening of the dentinal tubules in the form of amorphous calcium phosphate, which subsequently becomes a hydroxycarbonateapatite (HCA) layer[8-10]. The bioactive glasses present in products for DH treatment are constituted of glassy particles with sharp edges. These edges are produced from the grinding of glass material and may cause injury to the gingival margin during the application by friction[11].

A novel particulate bioactive material free of glassy phase in an attempt has been developed for use in dentinal hypersensitivity. This material, commercially called Biosilicate® was developed by the Vitreous Materials Laboratory of the Federal University of São Carlos, Brazil and consists in a crystalline powder of bioactive quaternary P_2O_5-Na_2O-CaO-SiO_2 glass ceramic with the same advantages of the bioactive glasses[12,13]. Biosilicate® consist in a novel fully-crystallized glass-ceramic produced by modifying the structure and concentration of the components in the initial bioglass through a thermal treatment, which results in formation of polycrystalline microstructure having crystals with controlled size and volume fraction[11]. The crystallization process results in particles that can be safely added to any type of formulation to be used in the oral cavity, in addition, the particles can be more easily inserted into the dentinal tubules because there are no edges to deflect away from the orifice[13].

Previous studies evaluated through SEM the surface of dentin samples treated with Biosilicate® mixed with water and Biosilicate® incorporated into a gel and observed obliteration of dentinal tubules and the formation of HCA after immersion in artificial saliva[11]. Because it is a new product, was suggested by laboratory responsible for creating the material, the evaluation of acidulated fluoride as a vehicle for the incorporation of the biomaterial. The acidity of the fluorine could accelerate the dissolution of Biosilicate® and thereby enable a more rapid formation of HCA.

The aim of this in vitro study was to evaluate the dissolution of Biosilicate® particles in artificial saliva using different application methods (microbrush and Robinson brush) and different dilution mediums (fluoride gel and distilled water).

MATERIAL AND METHOD

This study was approved by the Research Ethics Committee of Araraquara Dental School – UNESP – Brazil (protocol # 12/07). A total of 140 third molars were obtained from the Human Tooth Bank of the institution.

1. *Dentin sample preparation*

Samples were prepared by making two parallel grooves on the most regular root surface of the teeth. Two grooves of approximately 0.5 mm in depth were made using a high speed cylindrical bur (4219, KG Sorensen, Barueri, SP, Brazil) under copious water irrigation. The first groove was made horizontally at the cementoenamel junction and the second groove was made approximately 4 mm distant from the first in the apical direction. The same bur was used to remove the surface layer of the root between the two grooves. Two samples were obtained from each tooth and all of them were stored in containers with saline solution (0.9% sodium chloride) at room temperature.

Two hundred and eighty dentin samples were obtained and randomly divided into four experimental groups (n = 70 for each group): 1) 0.6 g of Biosilicate® powder (Vitrovita, São Carlos, Brazil), plus 1 mL of fluoride gel (acidulated phosphate fluoride 1.23%, DFL, Jacarepaguá, Brazil) plus distilled water applied with Robinson brush; 2) 0.6 g of Biosilicate® powder, plus 1 mL of fluoride gel plus distilled water (2 mL) applied with microbrush; 3) Biosilicate® powder (0.6 g) plus distilled water (2 mL) applied with Robinson brush; 4) Biosilicate® powder plus distilled water (2 mL) applied with microbrush. These four groups were further divided into seven subgroups (10 samples each) according to the period that the samples were immersed in artificial saliva after Biosilicate® application: 15 and 30 minutes, 1, 2, 12, and 24 hours.

Prior to the application of Biosilicate®, the samples were etched with 35% phosphoric acid for 15 seconds and rinsed with 10 mL of distilled water, in order to remove the smear layer and open the dentinal tubules.

The Biosilicate® was applied in the dentin samples for 15 seconds and remained on the samples for 5 minutes. After that, the samples were rinsed with 10 mL of artificial saliva and were stored in plastic containers with 10 mL of artificial saliva. The samples were placed in a chamber at constant temperature (37 °C) for 15 and 30 minutes, 1, 2, 12, and 24 hours.

2. *SEM Analysis*

All samples were then mounted on metallic stubs (Senai, Sao Paulo, SP, Brazil) and dried overnight in a dehydration jar (Corning, Sao Paulo, SP, Brazil). After that, the samples were sputter-coated with a thin 25 nm layer of 99.99% pure gold.

Two photomicrographs were obtained from the center area of each sample with 1,500X and 3,500X magnifications, using a scanning electron microscope operated at an accelerating voltage of 20 kV (Jeol T330 A, Jeol Ltd., Peabody, MA, USA). The photomicrographs were evaluated according to a Particle Dissolution Index (Figure 1). This index was created to assess, after the scanning electron microscopy, if the particles were Biosilicate® dissolved, partially-dissolved or non-dissolved particles on the surface of the dentin samples.

Three evaluations at 15 day intervals were performed by a previously calibrated and experienced examiner. Good reproducibility was achieved with a weighted kappa score of 0.85.

3. *Statistical Analysis*

The data were analyzed using statistical software (BioEstat Software, Belem/PA, Brazil). The level of significance was set at 5% for all tests. The data were explored for normality using the Shapiro-Wilk normality test. Once the data did not show normal distribution, the Mann-Whitney nonparametric test was used to determine the difference among the groups within each application method and within each dilution medium. Kruskal-Wallis and Dunn's post hoc tests were used to determine the difference among groups within each different saliva immersion period.

RESULT

Table 1 shows score comparison between the application methods for Biosilicate® dilution in distilled water at different saliva immersion periods. The results presented no statistically significant differences between the application methods when the distilled water was used for dilution. After 2 hours in saliva immersion, there was a tendency of the microbrush application to be less efficient than Robinson Brush application.

Table 2 shows comparison between the application methods for Biosilicate® dilution in fluoride gel at different saliva immersion periods. The results presented no statistically significant differences between the application methods when fluoride gel was used for dilution at the evaluated periods.

Table 3 shows score comparison between the dilution mediums in relation to the use of Robinson brush at different saliva immersion periods. The results presented no statistically significant differences between the application methods when the fluoride gel was used for dilution at the evaluated periods. No statistically significant differences were observed between the dilution mediums of the product when Robinson brush was used for application.

Table 4 shows the comparison between dilution mediums when microbrush is used for application at each saliva immersion period. After two hours of saliva immersion, there was a tendency of microbrush application to present lower particle dissolution in distilled water in comparison to fluoride gel dissolution ($p < 0.0233$). After 24 hours of saliva immersion, the results kept showing lower particle dissolution in distilled water in comparison to fluoride gel dissolution ($p < 0,0028$).

Figure 2 compares the saliva immersion periods according to the median of particle dissolution evaluation scores. There were no statistical significant differences among the groups. There were statistically significant differences between 15 minutes and 24 hours for the Microbrush plus fluoride gel group and between the 2 and 24 hour subgroups for the Robinson brush plus fluoride gel group.

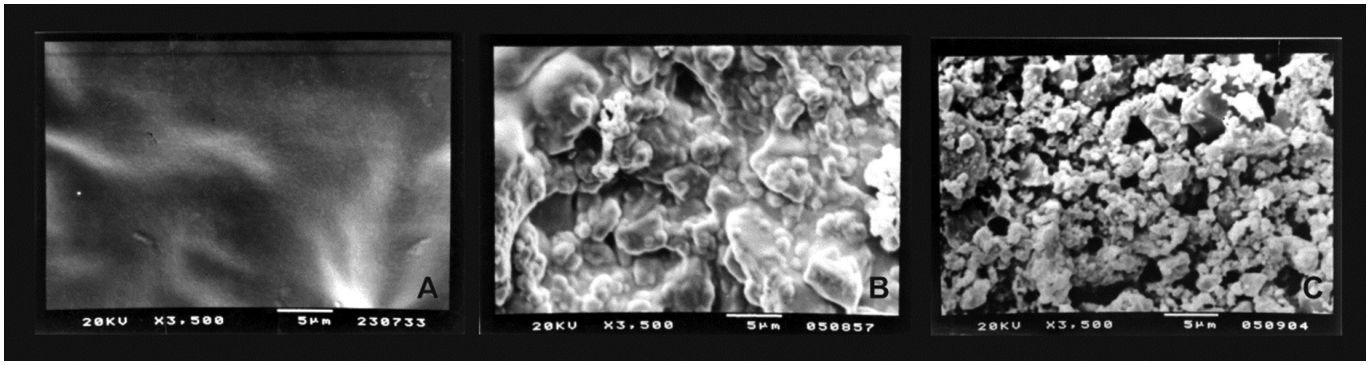

Figure 1. Evaluation criteria used in the Particle Dissolution Index. A) Score 1. Complete Biosilicate® particle dissolution with a homogeneous layer covering the opening of the dentinal tubules. B) Score 2. Partially particle dissolution with a non homogeneous layer and presenting traces of non-dissolved Biosilicate® particles. C) Score 3. No Biosilicate® particle dissolution observed.

DISCUSSION

Biosilicate® is a novel bioactive vitroceramic developed in the Laboratory of Vitreous Materials of São Carlos Federal University. Some studies have shown the effectiveness of this new material to form HCA when it is in contact with body fluids[12]. Thus, this material can be used as bone substitute in areas such as dentistry and medicine[12]. This property permits the use of Biosilicate® in the DH treatment, once HCA has a structure similar to dentin surface. Hydroxycarbonateapatite binds to dentin, bringing a permanent solution to avoid pain[12,14,15]. However, the best form to apply this material on dentin surface has not been established yet.

Table 1. Score comparison between application methods for the dilution in distilled water at different saliva immersion periods (n = 10)

Distilled water				
Periods	**Parameters**	**Robinson Brush**	**Microbrush**	***p****
0 min	Median	2.0	2.0	*0,7055*
	Mean ± SD	2.0 ± 0.0	2.1 ± 0.3	
15 min	Median	2.5	3.0	*0.4497*
	Mean ± SD	2.5 ± 0.5	2.7 ± 0.5	
30 min	Median	2.0	2.5	*0.1306*
	Mean ± SD	2.1 ± 0.3	2.5 ± 0.5	
1 h	Median	2.0	2.5	*0.1306*
	Mean ± SD	2.1 ± 0.3	2.5 ± 0.5	
2 h	Median	2.0	3.0	*0.0588*
	Mean ± SD	2.3 ± 0.5	2.8 ± 0.4	
12 h	Median	2.0	2.0	*0.1212*
	Mean ± SD	1.7 ± 0.5	2.2 ± 0.6	
24 h	Median	2.0	2.0	*0.1620*
	Mean ± SD	1.9 ± 0.3	2.3 ± 0.5	

*Mann-Whitney test; $p < 0.05$

Table 2. Score comparison between application methods for dilution in fluoride gel at different saliva immersion periods (n = 10)

Fluoride Gel				
Periods	**Parameters**	**Robinson Brush**	**Microbrush**	***p****
0 min	Median	2.0	2.0	*1.0000*
	Mean ± SD	2.3 ± 0.5	2.3 ± 0.5	
15 min	Median	2.0	3.0	*0.2568*
	Mean ± SD	2.4 ± 1.5	2.7 ± 0.5	
30 min	Median	2,0	2.0	*0.7055*
	Mean ± SD	2.3 ± 0.5	2.4 ± 0.5	
1 h	Median	2.5	2.0	*0.4497*
	Mean ± SD	2.5 ± 0.5	2.3 ± 0.5	
2 h	Median	2.0	2.0	*1.0000*
	Mean ± SD	2.2 ± 0.4	2.2 ± 0.4	
12 h	Median	2.0	2.0	*0.0821*
	Mean ± SD	1.6 ± 0.5	2.1 ± 0.3	
24 h	Median	1.5	1.0	*0.4497*
	Mean ± SD	1.5 ± 0.5	1.3 ± 0.5	

*Mann-Whitney test; $p < 0.05$.

This study evaluated two mediums for adding Biosilicate® (fluoride gel and distilled water), two application methods of Biosilicate® on dentin surface (Robinson brush and microbrush), and the necessary period to complete Biosilicate® dissolution on dentin surface. Non-dissolved particles of Biosilicate® cover the dentin surface; however, this layer may possibly be removed easily with toothbrushing, diet, or even with patient salivation.

The method used in this study allowed a morphological evaluation of the Biosilicate® layer formed on dentin. In order to evaluate the photomicrographs, an index was created. The

Table 3. Score comparison between dilution mediums when Robinson brush was used for application at different saliva immersion periods (n = 10)

Robinson Brush				
Periods	**Parameters**	**Distilled water**	**Fluoride gel**	***p****
0 min	Median	2.0	2.0	*0.2568*
	Mean ± SD	2.0 ± 0.0	2.3 ± 0.5	
15 min	Median	2.5	2.0	*0.7055*
	Mean ± SD	2.5 ± 0.5	2.4 ± 1.5	
30 min	Median	2.0	2.0	*0.4497*
	Mean ± SD	2.1 ± 0.3	2.3 ± 0.5	
1 h	Median	2.0	2.5	*0.1306*
	Mean ± SD	2.1 ± 0.3	2.5 ± 0.5	
2 h	Median	2.0	2.0	*0.7055*
	Mean ± SD	2.3 ± 0.5	2.2 ± 0.4	
12 h	Median	2.0	2.0	*0.7055*
	Mean ± SD	1.7 ± 0.5	1.6 ± 0.5	
24 h	Median	2.0	1.5	*0.1306*
	Mean ± SD	1.9 ± 0.3	1.5 ± 0.5	

*Mann-Whitney test; $p < 0.05$.

Table 4. Score comparison between dilution mediums when microbrush was used for application at different saliva immersion periods (n = 10)

Microbrush				
Periods	**Parameters**	**Distilled water**	**Fluoride gel**	***P***
0 min	Median	2.0	2.0	*0.4497*
	Mean ± SD	2.1 ± 0.3	2.3 ± 0.5	
15 min	Median	3.0	3.0	*1.0000*
	Mean ± SD	2.7 ± 0.5	2.7 ± 0.5	
30 min	Median	2.5	2.0	*0.7055*
	Mean ± SD	2.5 ± 0.5	2.4 ± 0.5	
1 h	Median	2.5	2.0	*0.4497*
	Mean ± SD	2.5 ± 0.5	2.3 ± 0.5	
2 h	Median	3.0	2.0	*0.0233 **
	Mean ± SD	2.8 ± 0.4	2.2 ± 0.4	
12 h	Median	2.0	2.0	*0.6776*
	Mean ± SD	2.2 ± 0.6	2.1 ± 0.3	
24 h	Median	2.0	1.0	*0.0028 **
	Mean ± SD	2.3 ± 0.5	1.3 ± 0.5	

*Mann-Whitney test; $p < 0.05$.

Particle Dissolution Index is able to verify the dissolution degree of Biosilicate® particles. It can evaluate the samples with non-dissolved Biosilicate® particles, samples with partially-dissolved Biosilicate® particles, and samples with completely-dissolved Biosilicate®particles.

According to the manufacturer (Vitrovita, São Carlos, Brazil), Biosilicate® reaction starts immediately after contact with fluids, so it's important that the product reviews are mixed at the time of application.

Distilled water and fluoride gel showed similar results in particle dissolution on dentin surface (Tables 1 and 2), and this mixture can be applied either with Robinson brush (Table 3) or with microbrush (Table 4). The results suggested that both substances can be used in order to dilute Biosilicate® and that both application methods can be performed.

Although the results for both substances are similar, the mixture of Biosilicate® with fluoride gel is more consistent and it facilitates the application on dentin surface. This fact may be the reason why the best results were achieved with Biosilicate® plus fluoride gel (Figure 2), and Biosilicate® plus distilled water showed no statistically significant differences. Perhaps, the interaction between Biosilicate® and fluoride gel may have facilitated the dissolution of Biosilicate® due to fluoride gel acidity. Nevertheless, this elucidation was not the objective of the present study.

In clinical practice, both microbrush and Robinson brush can be used to apply the substances on dentin surface. Trying to evaluate if there is any difference between these two application methods, the present study did not find a statistically significant difference between them (Tables 1 and 2). These results suggest using the microbrush in clinical practice instead of Robinson brush because the friction of the Biosilicate® hard particle with a Robinson brush on dentin surface can cause abrasion on the gingival margin and patient discomfort.

Pashley et al.[16] (1986) showed that even the act of rubbing an instrument on dentin may promote the obliteration of dentinal tubules through the formation of smear layer. This fact can explain why both Robinson brush and microbrush were able to form smear layer and obliterate the dentinal tubules. The formation of smear layer produced by rubbing Robinson brush or the microbrush associated with the abrasive power of the Biosilicate® was probably responsible for a 2 median score in the subgroups that were not immersed in saliva (Figure 2). Initially, it was expected to observe no dissolved particles (score 3) in these subgroups, as the surface with smear layer is visually similar to the surface with dissolved particles or partially dissolved particles in photomicrographs. Therefore, possibly the layer formed on the dentin surface in the absence of saliva is different from the layer formed on the dentin surface after 12 and 24 hours of immersing samples in saliva[12].

Although smear layer can obliterate the dentinal tubules as a barrier that prevents the dentinal fluid displacement, it can be easily removed by an acid diet, toothbrushing or the saliva soluble effect[17-19]. Probably, in the 15 minute subgroups (Figure 2), the smear layer may have been removed by the saliva soluble effect. In the other subgroups, the smear layer may have been replaced by

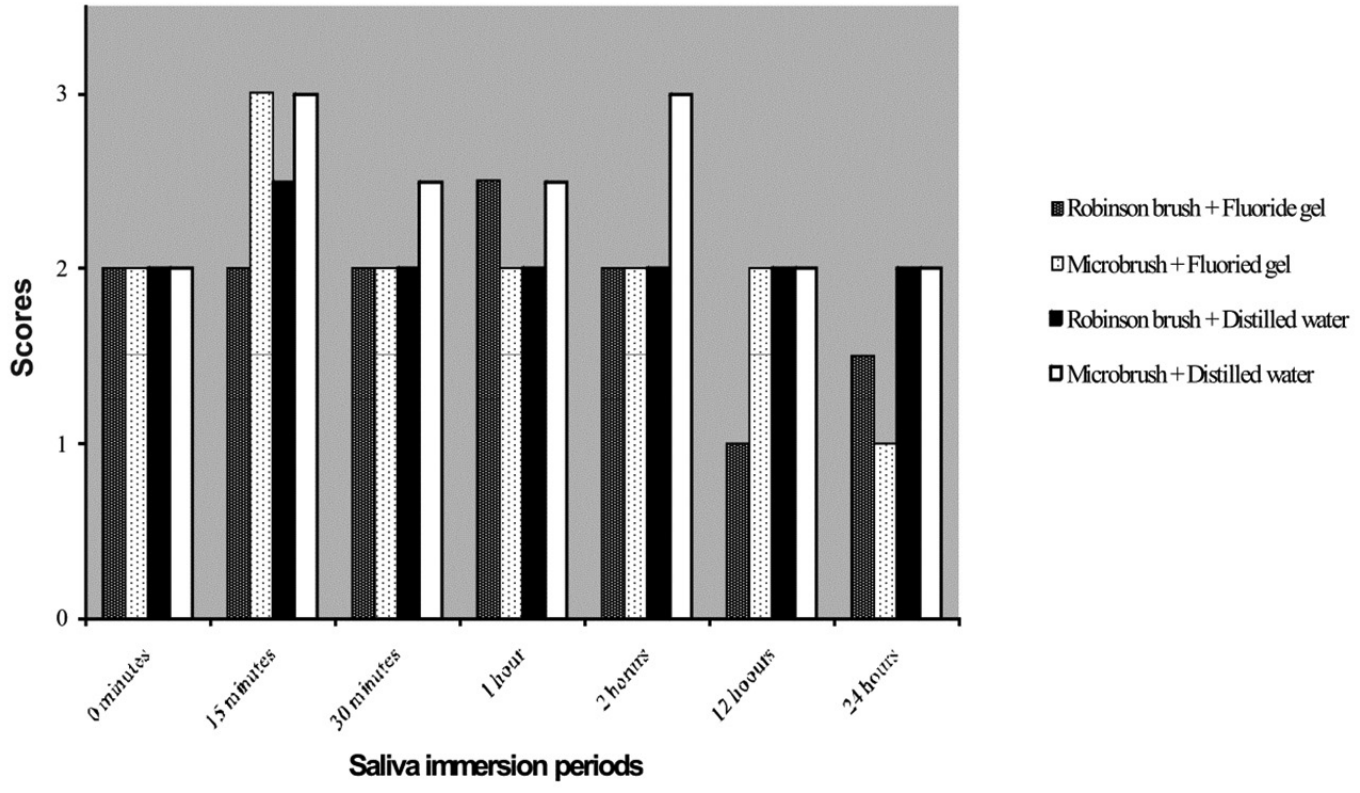

Figure 2. Comparison of median Particle dissolution scores at different saliva immersion periods for the different application methods and dilution mediums.

hydroxycarbonateapatite, forming a more resistant layer to acids. In most groups, the worst scores were found at the 15 minute period. Another possibility for the presence of complete and partially-dissolved particles in samples not immersed in saliva is that the dissolution of Biosilicate® particles may have started in contact with the fluoride and distilled water (time 0), similar to what occurs with other bioglasses when in contact with any fluid containing water[20,21] .

Considering the particle dissolution, there were no statistically significant differences among the groups during the analyzed periods (Figure 2), because the HCA layer is formed according to a process that occurs after 12 hours[11.] This fact may explain the differences between 1 and 24 hour subgroups into the Biosilicate® and fluoride gel group applied with Robinson brush, and between 15 minutes and 24 hour subgroups into Biosilicate® plus fluoride gel group applied with microbrush (Figure 2).

According to the evaluation of the obtained data, it was possible to observe that all application methods were efficient in dentinal tubules obliteration by particle dissolution (Tables 3 and 4). Considering the different dilution mediums, fluoride gel or distilled water, there were no statistically significant differences in relation to obliteration of dentinal tubules (Tables 1 and 2) and among the groups at each saliva immersion period. However, the best results were observed when the Biosilicate® was diluted with fluoride gel and applied with microbrush after 24 hours of saliva immersion (Figure 2). Nevertheless, studies about dentin permeability and acid challenges must be performed in order to evaluate the permanence of the layer formed on dentin surface. Furthermore, clinical researches with Biosilicate® must be performed in order to test clinical efficacy.

CONCLUSION

According to the results obtained and within the limitations of the methodology, it can be concluded that there were no differences between the evaluated application methods. In addition, no statistical differences were observed according to the evaluated dissolution periods.

Both dilution mediums were able to initiate the dissolution of the glass-ceramic particles. Notwithstanding the lack of statistical significance between the dilution mediums, the Biosilicate® plus fluoride gel after 24 hours tended to produce samples with a more homogeneous layer covering the opening of the dentinal tubules.

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CONFLICTS OF INTERESTS

The authors declare no conflicts of interest.

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