Changes in enamel surface after use of nanoencapsulated fluoride for dental caries remineralization: an in vitro study

Alterações na superfície do esmalte após uso de flúor nanoencapsulado para remineralização da cárie dentária: um estudo in vitro

Cambios en la superficie del esmalte después del uso de flúor nanoencapsulado para la remineralización de caries dental: un estudio in vitro

Abstract

Aim: To evaluate through an in vitro study surface changes on decayed enamel after the use of fluoridated dentifrices involving nanoencapsulated technology (NanoF). Methods: Forty blocks of human enamel were distributed among four groups (n = 10): 50% NanoF + 50% NaF (50% nF), 100% NanoF (100% nF), 100% NaF as the positive control (PC) and without fluoride as the negative control (NC). The specimens were subjected to a pH cycling model for 7 days. Surface microhardness (SMH), linear surface roughness (Ra) and area surface roughness (Sa) were measured before and after the carious lesion formation and at the end of the treatment. Percentages of surface microhardness recovery (%SMHR), altered Ra (%RaC) and altered Sa (%SaC) were calculated. The data were analyzed using the ANOVA test, repeated-measures ANOVA and Pearson's correlation test (p<0.05). Results: No significant differences among groups were found for Ra, Sa, %RaC or %SaC (p>0.05). Ra and Sa increased after treatment. A significant correlation was found between Ra and Sa. The PC dentifrice had the highest %SMHR, followed by 100% nF (p<0.05). In contrast, no surface remineralization was found in the 50% nF and NC groups. Conclusion: Enamel surface changes were found after the remineralizing treatment with 100% nF group. The majority of dentifrices with...
fluoride provided an increase in the roughness and surface microhardness. Nanotechnology is an innovative, promising method for the controlled release of fluoride and the remineralization of teeth with dental caries.

**Keywords:** Dental caries; Toothpastes; Fluoride; Tooth remineralization; Nanotechnology.

**Resumo**
Objetivo: Avaliar por meio de um estudo in vitro as mudanças superficiais no esmalte cariado após o uso de dentífricos fluoretados envolvendo tecnologia nanoencapsulada (NanoF). Métodos: Quarenta blocos de esmalte humano foram distribuídos em quatro grupos (n = 10): 50% NanoF + 50% NaF (50% nF), 100% NanoF (100% nF), 100% NaF como controle positivo (CP) e sem flúor como controle negativo (CN). As amostras foram submetidas a um modelo de ciclagem de pH durante 7 dias. A microdureza superficial (SMH), a rugosidade linear da superfície (Ra) e a rugosidade da área superficial (Sa) foram medidas antes e depois da formação da lesão cariosa e ao final do tratamento. Foram calculadas as porcentagens de recuperação da microdureza superficial (%SMHR), Ra (%RaC) alterada e Sa (%SaC) alterada. Os dados foram analisados utilizando o teste ANOVA, ANOVA de medidas repetidas e o teste de correlação de Pearson (p<0,05). Resultados: Não foram encontradas diferenças significativas entre os grupos para Ra, Sa, %RaC ou %SaC (p>0,05). Ra e Sa aumentaram após o tratamento. Foi encontrada uma correlação significativa entre Ra e Sa. O CP teve a maior %SMHR, seguido por 100% nF (p<0,05). Em contraste, não foi encontrada remineralização de superfície nos grupos 50% nF e CN. Conclusão: Foram encontradas alterações na superfície do esmalte após o tratamento remineralizante com o grupo 100% nF. A maioria dos dentífricos com flúor proporcionou um aumento na rugosidade e microdureza superficial. A nanotecnologia é um método inovador e promissor para a liberação controlada do flúor e na remineralização dos dentes com cárie dentária.

**Palavras-chave:** Cárie dentária; Cremes dentais; Flúor; Remineralização dentária; Nanotecnologia.

**Resumen**
Objetivo: Evaluar por medio de un estudio in vitro los cambios en la superficie del esmalte cariado tras el uso de dentífricos fluorados con tecnología nanoencapsulada (NanoF). Métodos: Se distribuyeron 40 bloques de esmalte humano en cuatro grupos (n = 10): 50% NanoF + 50% NaF (50% nF), 100% NanoF (100% nF), 100% NaF como control positivo (CP) y sin flúor como control negativo (CN). Las muestras se sometieron a un modelo de ciclos de pH durante 7 días. Se midieron la microdureza superficial (SMH), la rugosidad superficial lineal (Ra) y la rugosidad de área superficial (Sa) antes y después de la formación de la lesión cariosa y al final del tratamiento. Se calcularon los porcentajes de recuperación de la microdureza superficial (%SMHR), Ra alterada (%RaC) y Sa alterada (%SaC). Los datos se analizaron mediante la prueba ANOVA, ANOVA de medidas repetidas y la prueba de correlación de Pearson (p<0,05). Resultados: No se encontraron diferencias significativas entre los grupos para Ra, Sa, %RaC o %SaC (p>0,05). Ra y Sa aumentaron después del tratamiento. Se encontró una correlación significativa entre Ra y Sa. El CP tuvo el mayor %SMHR, seguido del 100% nF (p<0,05). Por el contrario, no se encontró remineralización superficial en los grupos de 50% nF y CN. Conclusión: Se encontraron cambios en la superficie del esmalte tras el tratamiento remineralizador con el grupo de 100% nF. La mayoría de los dentífricos con flúor proporcionaron un aumento de la rugosidad y la microdureza superficial. La nanotecnología es un método innovador y prometedor para la liberación controlada de flúor y en la remineralización de dientes con caries dental.

**Palabras clave:** Caries dental; Pasta dentífrica; Fluor; Remineralización dental; Nanotecnología.

**1. Introduction**

Dental caries is the most prevalent oral disease in the world. Its occurrence is related to an imbalance in the demineralization and remineralization process of hard dental tissues due to acids resulting from bacterial metabolism (Pitts et al., 2017). The multifactor etiology involves social, biological and behavioral aspects (Paula et al., 2017). The presence of an acidic environment with sugar-based substrates favors the tooth mineral loss to an extent that saliva is not able to buffer (Farooq & Bugshan, 2020). Thus, the literature supports the daily use of fluoride dentifrices (Cate & Buzalaf, 2019, Tomaz et al., 2020).

**In vitro** studies are commonly used to test the efficacy of cariostatic formulations and contribute to the understanding of the fluoride incorporation chemistry (Farooq & Bugshan, 2020). Fluoride forms a layer of calcium fluoride on the tooth surface that offers greater resistance to demineralization. Fluoride can also be incorporated into the enamel structure through an association with hydroxyapatite, forming a more stable and resistant particle denominated fluorapatite (Cate & Buzalaf, 2019). Moreover, fluoride favors a higher concentration of ions, which accelerates the remineralization process (González-
To increase the substantivity of this ion in the oral environment, novel compounds have been developed with the aim of improving the action of fluoride in dentifrices.

Novel approaches to the composition of dentifrices involve the addition of ions, remineralizing substances or changes in the fluoride release pattern (Ahmadian et al., 2018, Sampaio et al., 2020). In this context, nanotechnology has been used to improve the remineralizing performance of fluoride by controlled release systems, such as nanoencapsulated fluoride (NanoF) (Nguyen et al., 2017, Alves et al., 2018). In this system, fluoride is surrounded by a double layer of a natural polymer (hydroxypropyl guar), which serves as a carrier for the ion. The use of a polymer with a low molecular weight and small dimensions increases its surface-to-volume ratio and ability to penetrate the biofilm, resulting in a higher concentration of ions on the enamel surface (Nguyen & Hiorth, 2015, Ahmadian et al., 2018, Lavôr et al., 2020). The NanoF is activated when in contact with the salivary enzyme amylase, resulting in the gradual release of fluoride into the oral environment and extending its period of action (Nguyen et al., 2017, Alves et al., 2018, Lavôr et al., 2020, Oliveira et al., 2021).

Tactile and visual examinations are commonly used to evaluate caries activity in the clinic. However, this approach is not effective in the analysis of test specimens. Thus, other methods are preferred, such as the determination of surface roughness and microhardness (Ando et al., 2018; Sleibi et al., 2018). Enamel surface roughness increases and surface strength decreases with the progression of demineralization (Ando et al., 2018; Ma et al., 2019). In vitro and in situ studies evaluating surface roughness and hardness are important to determine the tooth surface remineralization treated with fluoride dentifrices (Buzalaf et al., 2010, Reis et al., 2017, Ando et al., 2018). Therefore, the aim of this in vitro study was to evaluate surface changes promoted by dentifrices with a controlled fluoride release system (NanoF) investigating the surface enamel microhardness (SMH), linear surface roughness (Ra, μm) and area surface roughness (Sa, μm).

2. Methodology

This study was conducted in accordance with principles contained in the Declaration of Helsinki and Resolution 466/12 of the Brazilian National Health Board and received approval from a research ethics committee in Brazil (certificate number: 45917915.6.0000.5188). The donors of the teeth signed a statement of informed consent.

Calculation of sample size and preparation of specimens

Based on a previous study (Vyavhare et al, 2015), assuming a two-tailed alpha of 0.01 and 80% power, the sample size was determined considering remineralizing potential as the primary outcome. A minimum of seven specimens was required per group, which was increased by 20%, totaling 10 specimens in each group. Forty extracted human third molars were cleaned and examined for enamel alterations. Forty human enamel blocks (4 x 4 x 2 mm) were obtained from the dental crown (buccal and lingual surfaces) of selected teeth. The specimens were embedded in self-curing acrylic resin using circular molds (16 x 3 mm). A metallographic polisher was used to flatten the outer enamel (with 400-, 600- and 1200-grit sandpaper disks) under constant irrigation. The enamel surfaces were polished with wet felt and a 1-μm diamond paste (Extec Corporation, Enfield, CT, USA) in a rotating polishing machine (PSK-2V, Skill-Tec Comércio e Manutenção Ltda, São Paulo, SP, Brazil). All specimens were submitted to water sonication for 5 minutes and stored at -20°C until the day of the experiment. Enamel specimens with surface microhardness (SH0) between 390 and 420 VHN were randomly distributed into four groups (n = 10) according to the dentifrice employed: 50% nF (50% NanoF + 50% free NaF), 100% nF (100% NanoF), PC (positive control - 100% NaF) and NC (negative control - no fluoride). The dentifrices were coded by an independent researcher (Table 1).
Table 1. Dentifrices used in study according to the composition and manufacturer*.

<table>
<thead>
<tr>
<th>Group</th>
<th>Composition</th>
<th>Manufacturer **</th>
</tr>
</thead>
<tbody>
<tr>
<td>50% nF</td>
<td>50% NanoF + 50% NaF (1450 ppm fluoride)</td>
<td>SAVOY, Jundiaí, São Paulo, Brazil</td>
</tr>
<tr>
<td>100% nF</td>
<td>100% NanoF (1450 ppm fluoride)</td>
<td>SAVOY, Jundiaí, São Paulo, Brazil</td>
</tr>
<tr>
<td>PC</td>
<td>100% sodium fluoride - NaF (1450 ppm fluoride)</td>
<td>SAVOY, Jundiaí, São Paulo, Brazil</td>
</tr>
<tr>
<td>NC</td>
<td>No fluoride</td>
<td>SAVOY, Jundiaí, São Paulo, Brazil</td>
</tr>
</tbody>
</table>

*All experimental dentifrices included the following ingredients: water, carboxymethyl cellulose (binder), sodium lauryl sulfate (surfactant), hydrated silica (abrasive), 70% sorbitol (humectant), glycerin (humectant) and methylparaben (preservative). **The patent (BR 102018070679-9 A2) belongs to NANOVETORES TECNOLOGIA S.A. Source: Own resources (2021).

Induction of carious lesion

Following the SH0 measurements, the enamel surface of each specimen was divided into three equal parts. One-third of the exposed enamel was covered with a double layer of nail varnish (Risque, Niasi, Taboão da Serra, São Paulo, Brazil) to preserve the control area in each specimen. Subsurface enamel demineralization was induced in the other two-thirds by immersing each specimen in 30 ml of demineralizing solution [1.3 mM/L Ca(NO3)2 .4H2O, 0.78 mM/L NaH2PO4 H2O in 0.05 M/L acetate buffer, 0.03 μgF/mL (NaF), pH 5.0] for 16 h at 37ºC (Vieira et al., 2005).

Collection of human saliva

Stimulated human saliva was collected from 12 healthy male and female individuals 18 to 35 years old with no systemic conditions and residing in a city without fluoridated water. The exclusion criteria were having used drugs that affect salivary flow or fluoride products within the previous four weeks, the use of an orthodontic appliance and the presence of caries and/or erosion lesions and/or periodontal disease. Stimulated human saliva was collected each day of the study. Each volunteer chewed paraffin wax and spat the saliva into a plastic cup. The specimens were stored in a refrigerator at 5 °C until use (Amaechi, 2019).

Remineralizing pH cycling

The remineralization pH cycling model was based on a previous study (Vieira et al., 2005) another third of the surface of enamel specimens was covered with two layers of nail varnish (Risque, Niasi, Taboão da Serra, São Paulo, Brazil) to provide a carious lesion reference area (SH1). Then, specimens were submitted to a five-day pH cycling model at 37ºC and remained in the remineralizing solution for two days. The blocks were individually immersed in a remineralizing solution (1.5 mmol/L Ca, 0.9 mmol/L P, 150 mmol/L KCl, 0.05 mg F/mL in 0.1 mol/L Tris buffer, pH 7.0) for 18 h. The cariogenic challenge was performed using a demineralizing solution (0.05 mol/L acetate buffer, pH 5.0, containing 1.28 mmol/L Ca, 0.74 mmol/L P and 0.03 mg F/mL) for six hours per day. Treatment consisted of immersing the enamel specimens in dentifrice slurries twice a day (1:3 w/w; 2 mL/enamel specimen) for 2 minutes under agitation. Treatments were performed before and after the demineralizing solution. To activate salivary amylase in the NanoF groups, a volume of approximately 10% of human saliva was added to all dentifrice slurries. Specimens were rinsed with deionized water between steps. The demineralizing and remineralizing solutions were refreshed every day. All groups were submitted to the pH cycling. At the end of the seventh day of pH cycling, specimens were washed again with deionized water under sonication for 5 min and stored in a humidity-controlled environment to prevent drying until further analysis. After remineralizing pH cycling, the nail varnish was removed and surface microhardness was again determined (SH2).
Microhardness analysis

Enamel surface microhardness was analyzed with a microhardness tester (Shimadzu HMV - AD Easy Test Version 3.0). Five indentations spaced 100 µm from each other were performed at the center of the enamel surface (Vickers, 100g, 10s). SH0 was determined first. Additional measurements were taken after the formation of the carious lesion (SH1) and after the remineralizing treatment (SH2) using the same method. The values were averaged and the percentage of surface microhardness recovery (%SMHR) was calculated as follows:

\[
\% \text{SMHR} = \frac{(SH2 - SH1)}{(SH0 - SH1)} \times 100
\]

Roughness assessment

Surface roughness data (Ra and Sa, µm) were obtained with the aid of a noncontact 3D optical profilometer (Talysurf CCI MP, Leicester, UK). All measurements were made respecting the following parameters: magnification of 20x; field of view of 0.865 X 0.865 mm (Paula et al., 2017); working distance of 4.7 mm; numerical aperture of 0.4; and ‘Z’ mode with optimized resolution, as described in a previous study (Soares et al., 2019). The prepared template was used to process the data (Soares et al., 2019). Unmeasured points after surface scanning were filled in the average of the peaks and valleys of nearby areas. The leveling function was used to level the scanned specimens. Next, a 0.25-mm standard cut-off filter was adopted and a threshold evaluation was performed to remove outliers (up to 0.05%).

Three random vertical profile traces (Ra data; 0.84 mm length) and one scanned area (Sa data; \( \approx 0.232 \text{ mm}^2 \)) were considered in the left (RaL or SaL for sound), middle (RaC or SaC for carious) and right (RaR or SaR for treated) areas of scanned human enamel specimens. Then, all files containing roughness data were tabulated on an electronic spreadsheet for the determination of Ra and Sa in each specimen area. The arithmetic mean of the three profiles was obtained to determine the mean Ra per area. Finally, the percentage change in roughness was calculated for Ra (%RaC=(RaT-RaC)/(RaS-RaC) x 100) and Sa (%SaC=(SaT-SaC)/(SaS - SaC) x 100).

Statistical analysis

Statistical procedures were performed with the SPSS software (SPSS, Inc., Chicago, IL, USA). Assumptions of normal distribution were checked for all the variables with the Shapiro-Wilk test, and the data exhibited Gaussian distribution. Multiple comparisons were performed using ANOVA followed by Tukey’s post-hoc test for the intergroup comparisons of all variables (SH0, Ra, Sa, %SMHR, %RaC and %SaC). Repeated-measures ANOVA followed by the Bonferroni post-hoc test was used for the intra-group analyses of SMH, Ra and Sa at the different evaluation times. Pearson’s rank correlation was used to correlate all variables. The level of significance was set at 5% (p<0.05).

3. Results

Table 2 displays the mean and standard deviation values of surface roughness (Ra, Sa) and SMH at the three evaluation times. The Ra results were similar to those found for Sa. In the treated area, the highest values for Ra and Sa were found in the 100% nF group and the lowest were found in the 50% nF group. No statistically significant differences were found among the groups for Ra and Sa, except for SaT. The %SaC increased in all groups, with a significant difference only between 50% nF and 100% nF (p<0.05). An increase in the %RaC was also found in all groups, with no significant differences among groups. Comparing each group individually at the three evaluation times, no significant differences were found among the sound, carious and treated areas (p<0.05) when testing Ra and Sa variables. The results indicate greater roughness after treatment with the dentifrices.
For SMH, no differences were found between groups for SH0 and SH1 (p>0.05). However, significant differences were found regarding SH2 (p<0.05), with the highest SH2 in the PC group, followed by the 100% nF. No surface recovery occurred with 50% nF or NC treatments. In the pairwise comparisons, no significant difference in SH2 was found between 50% nF and 100% nF (p>0.05), whereas NC and PC had significantly different SH2 values compared to other groups (p<0.05).

### Table 2. Mean (±SD) of Sa, Ra and SMH variables before and after treatments*.

<table>
<thead>
<tr>
<th>Group</th>
<th>Ra</th>
<th>Sa</th>
<th>SMH</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>S (±SD)</td>
<td>C (±SD)</td>
<td>T (±SD)</td>
</tr>
<tr>
<td>50% nF</td>
<td>0.24 (0.1)A,a</td>
<td>0.31 (0.1)A,b</td>
<td>1.02 (0.3)A,c</td>
</tr>
<tr>
<td>100% nF</td>
<td>0.22 (0.0)A,a</td>
<td>0.32 (0.1)A,b</td>
<td>1.57 (0.5)A,c</td>
</tr>
<tr>
<td>PC</td>
<td>0.24 (0.1)A,a</td>
<td>0.34 (0.1)A,b</td>
<td>1.11 (0.6)A,c</td>
</tr>
<tr>
<td>NC</td>
<td>0.32 (0.2)A,a</td>
<td>0.33 (0.1)A,b</td>
<td>1.15 (0.3)A,b</td>
</tr>
</tbody>
</table>

*Different uppercase letters denote significant differences between groups (columns) for the same variable. *Different lowercase letters denote significant differences within the group (line) for the same variable.

Source: Own resources (2021).

The comparison of %SMHR, %RaC and %SaC can be seen in Figure 1. %RaC was highest in the NC and %SaC was highest in the 100% nF group. However, no significant differences among groups were found regarding %RaC or %SaC. The analysis of %SMHR revealed an increase in remineralization in 100% nF and PC groups. %SMHR differed significantly in the PC compared to all groups, except for 100% nF. Moreover, no significant difference was found between 50% nF and 100% nF group (p>0.05). Demineralization was found in the NC, with significant differences compared to other groups (p<0.05), except the 50% nF.
Figure 1. Mean surface changes for %SMHR, %RaC* and %SaC* among all tested groups**

* %RaC and %SaC values were divided by -10 for better presentation in figure. **Different lowercase letters denote significant differences between groups for the same variable (ANOVA, p<0.05). Source: Own resources (2021).

Finally, a significant correlation was found between Ra and Sa (r = 0.869, p<0.01) and between SH2 and %SMHR (r = 0.886, p = 0.000).

4. Discussion

Nanotechnology has emerged as a possible way to improve the availability of fluoride by means of a controlled drug delivery (Nguyen & Hiorth, 2015, Ahmadian et al., 2018,). The NanoF used in the experimental dentifrices is a new technology, recently patented, which has already shown satisfactory results in the remineralization of erosive lesions (Nguyen & Hiorth, 2015, Ahmadian et al., 2018). NanoF is not dependent on pH but on enzymatic triggers following a mechanism of stimulus-controlled release, in which some factors, such as treatment time and human saliva, should be considered when evaluating its remineralization potential (Lavôr et al., 2020, Oliveira et al., 2021).

A previous clinical trial using the NanoF dentifrices found that fluoride release in saliva was delayed in the first 60 min after brushing (Moreira et al., 2017). In the same study, the incorporation of fluoride ions into the dental biofilm was prolonged when compared to regular NaF dentifrices. Therefore, remineralizing therapy with nanoencapsulated fluoride is an innovative way to increase the substantivity of fluoride in the oral environment, which may harm the initial carious lesion formation (Kanduti et al, 2016, Piñón-Segundo et al., 2019, Chapter 23, p.570). The present study evaluated the performance of dentifrices containing NanoF using a pH cycling system, with the analysis changes on the enamel surface.

The Ra and Sa analyses revealed no statistically significant differences among the groups for sound and carious areas due to the sample standardization, which shows the accuracy of this study. An increase in surface roughness was found from the sound to the decayed area, which is compatible with findings described by Ando et al. (2018b). Roughness was also greater after the remineralization period. The tooth mineral loss due to the action of cariogenic acids along with the increase in the size and quantity of enamel pores are some of the factors that could explain the increase of surface roughness in the carious area (Pitts et al., 2017). Regarding the roughness analysis method, a significant correlation was found between Ra and Sa...
The main distinction between these two aspects is the geometric dimension of the analysis, as linear analysis is two-dimensional and volumetric analysis is three-dimensional.

The expectation was that the enamel surface would be smoother and more homogeneous in the treated areas, however, this was not observed. Ando et al. (2018a) also found a significant increase in roughness of the areas after dentifrices use. The 50% nF dentifrice led to the lowest surface roughness in the treated areas, while the 100% nF group had the highest surface roughness. According to Sleibi et al. (2018), treatment time can exert an influence on surface roughness, as the change from a rough surface to a smooth surface may not occur quickly. Therefore, the treatment time in the present study could be considered insufficient to lead to surface homogeneity. Moreover, a modification in the structure of the polymer around the fluoride (NanoF) could be a way to improve dentifrices performance. The deposition of calcium fluoride likely occurred in a disorganized manner, resulting in a thicker, more irregular layer, as reported in previous studies (Ando et al., 2018a; Oliveira et al., 2021). This is also demonstrated by the lower %SaC in the NC group.

Regarding the %SMHR, only the PC and 100% nF groups exhibited an increase in surface microhardness after treatment, as reported in a previous study (Oliveira et al., 2021). The 50% nF was similar to the NC group and did not show promises in the treatment of enamel caries, as it did not promote the hardness recovery of the enamel carious lesion. The released fluoride may have undergone solvation and complexation with conventional NaF, preventing the gradually released NanoF from acting as a remineralizing agent. However, 100% nF dentifrice was able to increase the hardness of the enamel surface, although to a lesser degree when compared to the conventional NaF. These findings agree with data from Oliveira et al. (2021) which observed an increase of about 75% in %SMHR for the five-minute treatment, indicating that the double molecular polymer of the NanoF requires a longer treatment time to release fluoride ions more effectively. Another hypothesis is that the NanoF used in a neutral pH environment may have compromised its efficacy. The fluoride-coating polymer in the experimental dentifrices is more effective at more acidic pH than that used in cariogenic challenge (Cardoso et al., 2014, Oliveira et al., 2021).

Despite the attempt to simulate the conditions of the oral cavity, it is not possible to analyze all variables involved in the microbiology of this environment (Tenuta & Cury 2013, Amaechi, 2019, p.391). Important factors for the bioavailability and action of fluoride, such as the biofilm, were not evaluated. However, controlled drug release systems have been increasingly explored and greater efficacy of fluoride is expected when combined with boosting compounds for the treatment of dental caries (Ahmadian et al., 2018). It is important to conduct in situ and in vivo studies to gain a better understanding of the role of nanotechnology in dentistry.

5. Conclusion

Enamel surface changes were found after the remineralizing treatment. Increased Ra and Sa were associated with the probable irregular deposition of calcium phosphate on the treated surface. Nanotechnology dentifrices constitute an innovative, promising method for controlled fluoride release and the remineralization of teeth with dental caries.

Acknowledgments

The authors gratefully acknowledge the assistance of the Morphology Department at the University of Paraíba. This work was financed by Brazilian National Council for Scientific and Technological Development (CNPq) by a scientific initiation scholarship (Grant number: 145677/2019-2)


