INTRODUCTION

Dental caries is a public health problem and the most prevalent adverse oral condition found in the 2010 Global Burden of Disease study regarding the permanent dentition\(^1\). Approximately 2.4 billion people of the world’s population are affected by dental caries, which has a significant impact on quality of life and systemic health\(^2\,3\)。

The control of biofilm and the balance between demineralization and remineralization processes are considered preventive approaches to avoid the recurrence of caries\(^4\). The topical application of fluoride is a non-surgical approach to the treatment of dental caries in the early stages.

Fluoride stabilizes the mineral apatite and favors the remineralization of tooth enamel, which then becomes more resistant to demineralization\(^5\,6\). However, the success of fluoride application depends on the early diagnosis of caries, the control of sugar intake and knowledge on the part of dentists regarding the use of different forms of fluoride\(^7\).

Studies have demonstrated the effectiveness of fluoride for the prevention and control of dental caries in early stages. However, data on the best form of fluoride presentation for the treatment of incipient caries remains scarce\(^4\,5\,8\,9\,10\).

The aim of the present in vitro study was to evaluate the microhardness of teeth with caries created artificially using the Featherstone et al.\(^{10,11}\) model and after different fluoride application protocols. Enamel surfaces were also characterized. The hypothesis was that different fluoride application protocols would not influence microhardness, mineral recovery or the morphology of enamel surfaces.

MATERIALS AND METHODS

Experimental design

The factors were “time” (baseline, induction of caries lesion, after 7 days of treatment, after 14 days of treatment and 1 week after 28 days of treatment) and “topical fluoride protocol” (1.23% acidulated phosphate fluoride gel (APG), 2% neutral fluoride gel (NFG), 1.23% acidulated fluoride mousse (AFM) and fluoride varnish (5% Duraphat, DFV). Knoop microhardness (KHN) was evaluated after 7 and 14 days of treatment as well as 1 week after 28 days of treatment. Electron and confocal microscopy and energy dispersive spectroscopy were performed. KHN data were treated with two-way ANOVA (material×time) and Tukey’s test at a 5% significance level. Differences were found among groups over time (p<0.001). Microhardness varied after 7 and 14 days of treatment and remained stable 1 week after 28 days of treatment. Mineral recovery and enamel topography varied among groups, with the fluoride varnish achieving the most uniform topography.

Keywords: Enamel, Caries, Fluoride, Microhardness

The aim of the present study was to evaluate microhardness, mineral recovery and the enamel surface after the application of topical fluoride to artificial dental caries. Twenty-five bovine enamel blocks were prepared for artificial caries-like lesions and randomly divided into five groups (n=5): untreated (C control), 1.23% acidulated phosphate fluoride gel (APG), 2% neutral fluoride gel (NFG), 1.23% acidulated fluoride mousse (AFM) and fluoride varnish (5% Duraphat, DFV). Knoop microhardness (KHN) was evaluated after 7 and 14 days of treatment as well as 1 week after 28 days of treatment. Electron and confocal microscopy and energy dispersive spectroscopy were performed. KHN data were treated with two-way ANOVA (material×time) and Tukey’s test at a 5% significance level. Differences were found among groups over time (p<0.001). Microhardness varied after 7 and 14 days of treatment and remained stable 1 week after 28 days of treatment. Mineral recovery and enamel topography varied among groups, with the fluoride varnish achieving the most uniform topography.

Microhardness of bovine enamel after different fluoride application protocols

Marcia Regina Cabral OLIVEIRA\(^1\), Pedro Henrique Cabral OLIVEIRA\(^1\), Luiz Henrique Cabral OLIVEIRA\(^1\), Anna Carolina Ratto Tempestini HORLIANA\(^1\), Paulo Francisco CESAR\(^2\), Sandra Kiss MOURA\(^1\) and Sandra Kalil BUSSADORI\(^1\)

\(^1\) Department of Biophotonics Applied to Health Sciences, Universidade Nove de Julho, UNINOVE, Rua Vergueiro 235/249, 01504-001, São Paulo, Brazil

\(^2\) Department of Biomaterials and Oral Biology, Universidade de São Paulo, Faculdade de Odontologia, Av. Prof. Lineu Prestes 2227, 05508-000, São Paulo, Brazil

Corresponding author, Sandra Kalil BUSSADORI; E-mail: sandra.skb@gmail.com

The aim of the present study was to evaluate microhardness, mineral recovery and the enamel surface after the application of topical fluoride to artificial dental caries. Twenty-five bovine enamel blocks were prepared for artificial caries-like lesions and randomly divided into five groups (n=5): untreated (C control), 1.23% acidulated phosphate fluoride gel (APG), 2% neutral fluoride gel (NFG), 1.23% acidulated fluoride mousse (AFM) and fluoride varnish (5% Duraphat, DFV). Knoop microhardness (KHN) was evaluated after 7 and 14 days of treatment as well as 1 week after 28 days of treatment. Electron and confocal microscopy and energy dispersive spectroscopy were performed. KHN data were treated with two-way ANOVA (material×time) and Tukey’s test at a 5% significance level. Differences were found among groups over time (p<0.001). Microhardness varied after 7 and 14 days of treatment and remained stable 1 week after 28 days of treatment. Mineral recovery and enamel topography varied among groups, with the fluoride varnish achieving the most uniform topography.
Table 1 Description of the microsurface hardness values by groups and stages of evaluation and results of the statistical analysis

| Time                        | Group       | Control | NFG | FFN | AFM | Varnish | p group | p moment | p interaction |
|-----------------------------|-------------|---------|-----|-----|-----|---------|---------|----------|--------------|
| Initial                     |             | 306.3±21.6 | 308.0±30.4 | 311.0±27.8 | 322.0±3.4 | 318.8±11 | —       | —         | —            |
| Carious lesion induction    |             | 235.9±22.3 | 244.4±18.6 | 202.8±26.5 | 221.8±18.5 | 206.6±27.9 | —       | —         | —            |
| Week 1 fluoride             |             | 238.2±14.5 | 257.5±18.6 | 246.3±19.9 | 232.6±9.2 | 236.4±6.8 | 0.243   | <0.001    | <0.001        |
| Week 2 fluoride             |             | 231.4±9    | 215.9±10   | 268.7±11.4 | 247.1±20.9 | 252.9±11.8 | —       | —         | —            |
| 5th Week fluoride           |             | 233.1±13.1 | 297.9±9.2  | 272.6±16.8 | 259.4±6.5 | 290.8±22.7 | —       | —         | —            |

NFG: Neutral Fluoride Gel; AFM: Acidulated Fluoride Mousse

use of laboratory animals (NIH Publications No. 8023, revised 1978). Twenty-five recently extracted bovine teeth were maintained in a 0.1% thymol solution at 4°C. The teeth were cleaned and randomly divided into five groups: acidulated phosphate fluoride gel (APG), neutral fluoride gel (NFG), acidulated fluoride mousse (AFM), fluoride varnish and remineralizing solution (control). The vestibular surface of all teeth was sequentially wet sanded with silicon carbide sandpaper with grits of 400, 600 and 1200 (Buehler, São Paulo, Brazil) and polished with felt discs and diamond paste with granulations of 3, 2, 1 and 0.5 μm (FOX Foco Tools, São Caetano do Sul, Brazil) prior to the microhardness assays. Enamel blocks measuring 7×4×4 mm were cut starting from the prepared vestibular surface with double-sided diamond discs (7020, KG Sorensen, Barueri, Brazil) at low speed and with cooling (Kavo, Joinville, Brazil). The sides of the bovine enamel blocks were isolated with nail polish (COLORAMA, L’Oréal, Brazil), leaving only the sanded enamel surface exposed12).

**Formation of artificial caries and pH cycling**

The specimens were stored at 37°C and submitted to pH cycling for 30 days for the induction of artificial caries: 18 h in a demineralizing solution and 6 h in a remineralizing solution10,11). The pH cycling was performed after the induction of artificial caries (6 h in a demineralizing solution and 18 h in a remineralizing) to simulate a patient with a cariogenic diet during and after treatment with fluoride. The test samples were immersed in a 5-mL demineralizing solution for 6 h, followed by rinsing with distilled water and immersed in a remineralizing solution for 18 h at 37°C on a shaking table (MacLab, Jacareí, Brazil) to simulate the clinical conditions of a patient with a high risk of dental caries10,11).

**Fluoride treatment**

The APG, NFG, AFM and varnish groups were submitted to weekly topical applications following the recommendations of the manufacturers (Table 1) for a period of 28 days following the induction of artificial caries. The control group received no treatment but was submitted to pH cycling. Excess fluoride was removed immediately with absorbent paper and the specimens were again placed in the remineralizing solution on the shaking table (MacLab).

**Microhardness evaluation**

Surface microhardness was evaluated using a Knoop indenter with a load of 50 g for 20 s (HMV Micro Hardness Tester, Shimadzu, Kyoto, Japan). The specimens were divided into quadrants. Indentations were made in the center of each quadrant, avoiding areas near the sides of the exposed surface. The fifth reading was performed 500 μm from the first indentation in quadrant 1 to ensure that the same area was not measured. The five indentations were separated by a distance of 100 μm and the mean was used for statistical purposes11). Microhardness was measured in five time periods: 1) with sound enamel immediately after the preparation of the samples; 2) after the induction of caries; 3) after 7 days of treatment; 4) after 14 days of treatment; and 5) 1 week after 28 days of treatment.

**Determination of sub-surface microhardness and mineral recovery (ΔzR)**

After sectioning, the test samples were submitted to sub-surface microhardness assays prior to pH cycling. After the respective treatments, the test samples were submitted to an additional longitudinal cut with double-sided diamond discs (7020, KG Sorensen) at low speed and with cooling (Kavo) for the determination of sub-surface microhardness. The base of the micrometer tester was positioned such that the largest diagonal of the indentation was parallel to the outer surface of the enamel. Three indentations separated from each other by a distance of 20 μm were performed with the tip of the
Knoop indenter with a load of 50 g for 20 s to a depth of 60 μm from the surface (Fig. 1). Sub-surface microhardness was measured to evaluate mineral recovery in all specimens in all groups. The difference between the sound mineral profile and the profile in the remaining groups resulted in delta Z (ΔZ), which is considered the area of mineral loss when studying demineralization. In the present study, the area of mineral recovery (ΔZR) was used as an additional parameter for comparing treatments.

The mean of three microhardness readings obtained at each depth were converted into percentage of mineral volume (%vol). The microhardness in the longitudinal sections of the enamel was determined to evaluate the mineral recovery, since, according to some authors, there is a good relation between enamel microhardness and %vol. Using microradiography of the dental caries, the area below the curve (%vol X μm) was calculated to determine the mineral profile. The calculation of the mineral profile of sound enamel was obtained from the projection of %vol. The mean among depths of 20, 40 and 60 μm of all specimens in each group was calculated.

The difference between the sound mineral profile and the mineral profile of the remaining groups resulted in ΔZR, which was used to compare the treatments.

### Statistical analysis

The data were compiled in Tables and generalized estimation equations were performed. Analyses were followed by multiple Bonferroni. ΔZR values were described for each group and analysis of variance (ANOVA) was used for comparisons among groups, with multiple Bonferroni comparisons based on the ANOVA results. The results of the microscopic analyses were expressed in descriptive form.

### RESULTS

#### Surface microhardness

Statistically significant differences in mean surface microhardness values were found in the groups throughout the different evaluation times (p<0.001, Table 1).

At baseline and after induction of artificial caries all groups presented similar values, however, the NFG group showed lower values and the control group presented similar values after induction of caries for all times.

The APG and control group showed a decrease in values in the second week and stabilized until the 5th week. The AFM group exhibited a significant reduction in microhardness only between the initial evaluation and after the induction of caries (p=0.017). The varnish group exhibited a reduction in microhardness between the initial evaluation and after the induction of caries as well as a significant increase in microhardness 1 week after the end of treatment.

#### Sub-surface microhardness and mineral recovery

Table 2 shows that the mean percentage change in microhardness differed significantly among groups independently of depth (p<0.002) as well as among
Table 2  Results of Multiple comparisons of microhardness over time for each group

| Groups                  | Comparisons                              | Difference average | Error pattern | p      | IC(95%) Less | Superior |
|-------------------------|------------------------------------------|--------------------|---------------|-------|--------------|----------|
| Control                 | Carious lesions with early induction      | 70.45              | 24.27         | >0.999| -24.98       | 165.88   |
|                         | Injury induction decay- Week 1 with fluor | -2.28              | 24.27         | >0.999| -97.81       | 93.15    |
|                         | Injury induction decay- 2nd Week with fluor| 4.54               | 27.38         | >0.999| -103.1       | 112.21   |
|                         | Injury induction decay- 5th Week with fluor| 2.83               | 28.44         | >0.999| -109         | 114.66   |
| AGP                     | Carious lesions with early induction      | 63.76              | 24.27         | >0.999| -31.67       | 159.19   |
|                         | Injury induction decay- Week 1 with fluor | -13.1              | 24.27         | >0.999| -108.5       | 82.33    |
|                         | Injury induction decay- 2nd Week with fluor| 28.45              | 27.38         | >0.999| -79.21       | 136.12   |
|                         | Injury induction decay- 5th Week with fluor| -53.48             | 28.44         | >0.999| -165.3       | 58.34    |
| NFG                     | Carious lesions with early induction      | 109.07             | 24.27         | >0.999| -138.9       | 51.98    |
|                         | Injury induction decay- Week 1 with fluor | -65.84             | 27.38         | >0.999| -173.5       | 41.83    |
|                         | Injury induction decay- 2nd Week with fluor| -69.76             | 28.44         | >0.999| -181.6       | 42.07    |
| AFM                     | Carious lesions with early induction      | 101.5              | 24.27         | >0.999| -102         | 84.68    |
|                         | Injury induction decay- Week 1 with fluor | -25                | 27.38         | >0.999| -133         | 82.37    |
|                         | Injury induction decay- 2nd Week with fluor| -37.74             | 28.44         | >0.999| -149.4       | 74.28    |
| Varnish                 | Carious lesions with early induction      | 112.21             | 24.27         | 0.002 | 16.68        | 207.64   |
|                         | Injury induction decay- Week 1 with fluor | -28.81             | 24.27         | >0.999| -125.2       | 65.62    |
|                         | Injury induction decay- 2nd Week with fluor| -46.28             | 27.38         | >0.999| -154         | 61.39    |
|                         | Injury induction decay- 5th Week with fluor| -84.18             | 28.44         | >0.999| -196         | 27.64    |

AGP: Acidulated Gel fluoride; NFG: Neutral Fluoride Gel; AFM: Acidulated Fluoride Mousse

Table 3  Mean ΔZ_R values and respective standard errors according to group

| Group    | ΔZ_R      | p     |
|----------|-----------|-------|
| Control  | 9.6±2.4   | —     |
| AFG      | 1.2±3.6   | —     |
| NFG      | 5.0±1.3   | <0.001|
| AFM      | 8.1±0.8   | —     |
| Varnish  | 3.5±3.3   | —     |

Table 4  Mean and respective standard errors of percentage change in microhardness according to group and depth

| Depth (μm) | Control | APG     | NFG     | AFM     | Varnish | p group | p depth | p interaction |
|------------|---------|---------|---------|---------|---------|---------|---------|---------------|
| 20         | 5.9±16.3| -13.8±30.8| -15.1±22.4| -6.6±18.1| -16.6±24.4| —       | —       | —             |
| 40         | 25.3±17.7| 13.1±12.1| 3.5±18.8| 8.7±23.4| -3.4±13.9| 0.002   | 0.002   | 0.970         |
| 60         | 30.7±8.4| 5.5±10  | 1.3±15.5| -3.5±30.7| -2.1±22.9| —       | —       | —             |

depths independently of group (p=0.002).

The inter-group comparisons of ΔZ_R demonstrate that the APG and control groups had a mean of 8.39±1.6, which is higher value than that found for the AFM and control groups (-6.86±1.6; p<0.001 and p=0.004, respectively). The varnish and control groups also had a low value: -3.76±1.6 (p=0.011). Table 3 shows these findings. Table 4 show a higher percentage change in microhardness in the control group. In all groups, the change at a depth of 20 μm was less than at other depths.
Fig. 2  (A) Sound enamel—regular surface with slight roughness; arrows indicate zones of loss of continuity. (B) Carious lesion (magnification: 30,000×); yellow arrows indicate eroded areas with considerable mineral loss; blue arrow indicates area with increased roughness and dissolution of minerals in inter-prismatic areas. (C) and (D) respectively present APG and mousse samples; arrows indicate edge of craters still present after treatment; notable difference between morphology and mineral deposition on surface. (E) Absence of craters and uniform surface layer (magnification: 15,000×). (F) Arrow indicates crater from artificial carious lesion and discrete change in surface. (G) 2D confocal microscopy image (magnification: 50×) of sound tooth enamel surface. (H) 3D image of sound tooth enamel—a absence of large surface irregularities, only those caused by technique. (I) 2D image (magnification: 50×) —peaks larger than 30 μm in red and white. (J) 3D of same sample—peaks and valleys characterizing considerable erosion and surface roughness. (K) 2D image (magnification: 50×) of APG group—scratches stemming from sanding of enamel. (L) 3D image (magnification: 50×)—absence of discrepant peaks and values in sample submitted to induction of artificial caries. (M) 2D image of sample from NFG group. (N) 3D image of sample from NFG group—well-distributed peaks and valleys; absence of craters. (O) 2D image of sample from mousse group—well-distributed peaks and valleys; absence of craters. (Q) 2D image of varnish group. (P) 3D imaged of varnish group—well-distributed peaks and valleys and absence of craters, but smaller peaks in comparison to other groups.

| EDS     | F   | Na  | Mg  | Cl  | Ca  | Si  | O  | Total |
|---------|-----|-----|-----|-----|-----|-----|----|-------|
| EDS CO  | 0.81| 15.37| 0.32| 38.54| 44.94| 100 |
| EDS Mousse | 22.48| 1.46| 0.42| 13.03| 32.16| 30.45| 100 |
| EDS APG | 21.02| 1.37| 0.4| 12.65| 0.16| 33.82| 30.58| 100 |
| EDS Varnish | 2.42| 0.75| 0.21| 16.9| 41.1| 38.63| 100 |
| EDS NFG | 3.52| 1.34| 0.27| 11.8| 39.5| 47.57| 100 |
**Scanning electron microscopy**

The morphological changes in the enamel surfaces were accompanied microscopically. The initial sound enamel was free of craters and erosions, demonstrating only slight roughness and the loss of continuity at some points due to the technique (Fig. 2A). After the induction of caries, the enamel surface became irregular, with peaks and valleys as well as the loss of surface minerals (Fig. 2A, magnification: 3,000×). The demineralization of the inter-prismatic zone was similar to the clinical aspect of an incipient carious lesion.

New mineral deposits were found in the APG and mousse groups 1 week after the end of treatment (Figs. 2C and D). However, the AFM group exhibited larger, less uniform agglomerates as well as erosive areas measuring approximately 2 micrometers. A uniform, flat layer of newly deposited mineral was found in the varnish group 1 week after treatment, which can be characterized as remineralization with morphologically distinct areas, but with a dimensional pattern and the absence of erosions stemming from the carious lesion (Fig. 2E).

The image that represents the NFG group (Fig. 2F) still exhibited large erosive areas and surface topographic discrepancies. The mousse and NFG groups (Figs. 2C and D) did not have considerable topographic discrepancies between each other. However, the varnish group (Fig. 2E) exhibited greater uniformity, which suggests less roughness.

**Confocal microscopy**

The same samples selected for SEM were used for confocal microscopy. The sound tooth enamel exhibited a regular surface in the two-dimensional and three-dimensional images, with no erosions, only scratches stemming from the preparation of the sample, which is similar to what was found in the SEM analysis (Fig. 2A). Figure 2B represents the carious lesion with peaks and valleys dispersed throughout the sample and the increase in surface roughness due to the induction of artificial caries.

Surface topographic evenness was found in the APG group in both the two-dimensional and three-dimensional images. The NFG (Figs. 2C and D) and AFM (Figs. 2E and F) groups demonstrated an increase in surface roughness, represented by larger peaks and valleys in size and number and arranged uniformly. The varnish group (Figs. 2G and H) exhibited peaks and valleys with discrete topographic heterogeneity distributed throughout the entire area analyzed.

**Energy dispersive X-ray spectrometry (EDS)**

The mean of five samples was used to express the chemical profile of the surface of the enamel blocks in each group 1 week after the end of treatment. The EDS results demonstrate the absence of fluoride on the surface in the control group. The largest fluoride concentrations were found in the AFM and APG groups. The lowest concentration was found in the NFG group, followed by the varnish group.

**DISCUSSION**

Clinically, the effect of topical fluoride occurs in two ways: first, by maintaining the concentration of fluoride in saliva through the frequent application of a method, such as the use of a fluoridated dentifrice and, secondly, through the enamel-dentin reaction with fluorine, forming calcium fluoride (CaF$_2$), which is deposited in the biofilm and initial carious lesions at concentrations able to avoid the progression of white spots. During topical treatment with fluoride at concentrations higher than 1,000 ppm, the formation of CaF$_2$ occurs rapidly.

CaF$_2$ deposits can be retained for months, releasing trace fluoride at times when the pH drops and maintaining a constant concentration in the oral cavity, while also increasing the resistance of the tooth to acid attacks and potentiating remineralization. Several factors affect the formation of CaF$_2$, such as fluoride concentration during the application, pH of the medium, application time, the mineralized structure, dental eruption time and dental status. The use of products with a high concentration of fluoride is indicated for the treatment of individuals at high risk of dental caries and those with special needs. Fluoride presentations in the form of gel, mousse and varnish are widely employed in pediatric dentistry, but questions remain regarding the application time and best form of presentation as well as the ability to affect the remineralization of initial carious lesions on tooth enamel. In the present study, surface and sub-surface microhardness values were high even 1 week after treatment, thereby rejecting the hypothesis of the study.

The remineralization process was followed up for 4 weeks, which is the time established for the treatment of incipient caries. Moreover, an additional evaluation was performed 1 week after the end of treatment to confirm the validity of *in vitro* remineralization. It has been established that all carious lesions on tooth enamel can be remineralized with the topical application of fluoride. However, some authors suggest that the deposition of minerals in deeper portions of carious lesions is insufficient, leading to only partial remineralization. In the present study, sub-surface microhardness was promising at depths of 20 and 40 micrometers but remained equal to the baseline reading at a depth of 60 micrometers, which is in agreement with data described in the literature.

Both forms of fluoride gel proved beneficial. However, studies report greater remineralization efficiency and the formation of CaF$_2$ when an acidulated phosphate fluoride gel is used. This was also found in the present study, as the APG group, together with the varnish group, had the best microhardness results. The microscopic analysis revealed that the CaF$_2$ layer created by the varnish was more uniform and less rough, which could be an additional benefit when one considers the clinical aspect. However, this product is contraindicated for preschool children and patients with special needs due to the risk of ingestion, which can cause harm to the stomach and increases the risk of toxicity.
The weekly and final results of the AFM and neutral fluoride gel were similar. Thus, the use of fluoride varnish and mousse is a safe, practical manner to arrest the progression of white spots and promote remineralization. Fluoride varnish at a concentration higher than 23,000 ppm of F in weekly applications over 4 consecutive weeks favors the remineralization of carious lesions in both surface and sub-surface regions. However, the results 1 week after treatment were similar to those found in the APG and the microscopic analysis revealed larger zones of surface roughness. The remineralizing effect was high beginning with the first week of treatment due to the concentration of fluoride and high degree of substantivity. Fluoride mousse exhibited discrete remineralizing power in comparison to the other groups, with similar results 1 week after treatment to those found in the control group. Despite being acidulated, this form of fluoride has a low degree of wettability and no substantivity. However, it is capable of promoting remineralization and morpho-structural changes in the surface prismatic zone.

A previous study showed that three applications of acidulated phosphate fluoride at 12,300 ppm in addition to a fluoride dentifrice (1,000 ppm) resulted in a larger reservoir of fluoride for the control of enamel lesions compared to the fluoride dentifrice alone. The significance of the present study is high, since different methods were used to confirm that all forms of fluoride investigated were able to increase microhardness and promote morphological changes in the enamel. However, randomized, controlled, clinical trials are needed to gain a better understanding of the effect of a high concentration of fluoride on the mineral recovery of enamel with carious tissue.

CONCLUSION

All presentations of fluoride studied were able to increase the microhardness of enamel after 7 and 14 days of application and remained stable 1 week after 28 days of treatment. Fluoride varnish demonstrated the greatest stability, whereas the most uniform topography was found in the varnish group.

REFERENCES

1) Marqueses W, Kassemam NJ, Bernabé E, Flaxman A, Naghavi M, Lopez A, Murray CJ. Global burden of oral conditions in 1990-2010: a systematic analysis. J Dent Res 2013; 92: 592-597.
2) Petersen PE. World Health Organization global policy for improvement of oral health —World Health Assembly 2007. Int Dent J 2008; 58: 115-121.
3) Richards D. Fluoride gel effective at reducing caries in children. Evid Based Dent 2015; 16: 108-109.
4) Cury JA, Tenuta LM. Enamel remineralization: controlling the caries disease or treating early caries lesions? Braz Oral Res 2009; 23: 23-30.
5) Marinho VC, Higgins JP, Sheilham A, Logan S. Combinations of topical fluoride (toothpastes, mouthrinses, gels, varnishes) versus single topical fluoride for preventing dental caries in children and adolescents. Cochrane Database Syst Rev 2004; 1:CD002781.
6) Tenuta LM, Zamataro CB, Del Bel Cury AA, Tabchoury CP, Cury JA. Mechanism of fluoride dentifrice effect on enamel demineralization. Caries Res 2009; 43: 278-285.
7) Fejerskov O, Kidd E. Dental caries: the disease and its clinical management 2015, Wiley Blackwell, Oxford.
8) Ten Cate JM. In vitro studies on the effects of fluoride on demineralization. J Dent Res 1990; 69: 614-619.
9) Benson PE, Parkin N, Dyer F, Millett DT, Furness S, Germain P. Fluorides for the prevention of early tooth decay (demineralised white lesions) during fixed brace treatment. Cochrane Database Syst Rev 2013; 43: 12.
10) Featherstone JDB, O’Reilly MM, Shariati M, Brugler S, Leach SA. Enhancement of remineralization in vitro and in vivo. Factors relating to demineralization and remineralization of the teeth 1986. Oxford: IRL Press.
11) Featherstone JDB, Ten Cate JM, Shariati M, Arends J. Comparison of artificial caries-like lesions by quantitative microradiography and microhardness profiles. Caries Res 1983; 17: 385-391.
12) Vieira AE, Delbem AC, Sasaki KT, Rodrigues E, Cury JA, Cunha RF. Fluoride dose response in pH-cycling models using bovine enamel. Caries Res 2005; 39: 514-520.
13) Meyerowitz C, Featherstone JD, Billings RJ, Eisenberg AD, Fu J, Shariati M, Zero DT. Use of an intra-oral model to evaluate 0.05% sodium fluoride mouthrinse in radiation-induced hypoproliferation. J Dent Res 1991; 70: 894-898.
14) White DJ, Featherstone JD. A longitudinal microhardness analysis of fluoride dentifrice effects on lesion progression in vitro. Caries Res 1987; 21: 502-512.
15) Navarro M, Monte Alto LA, Cruz RA, Prazeres J. Calcium fluoride uptake by human enamel after use of fluoridated mouthrinses. Braz Dent J 2001; 12: 178-182.
16) Alexandria AK, Meckelburg NA, Puettet UT, Salles JT, Souza IP, Maia LC. Do pediatric medicines induce topographic changes in dental enamel? Braz Oral Res 2016; 30: 1-8.
17) Park JW, Song CW, Jung JH, Ahn SJ, Ferracane JL. The effects of surface roughness of composite resin on biofilm formation of Streptococcus mutans in the presence of saliva. Oper Dent 2012; 37: 532-539.
18) Maguire A. ADA clinical recommendations on topical fluoride for caries prevention. Evid Based Dent 2014; 15: 38-39.
19) Jardim JJ, Pagot MA, Maltz M. Artificial enamel dental caries treated with different topical fluoride regimens: an in situ study. J Dent 2008; 36: 396-401.