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In Situ Response of Nanostructured Hybrid Fluoridated Restorative Composites on Enamel Demineralization, Surface Roughness and Ion Release

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Abstract - *Recurrent caries at the tooth-restoration margins is the main reason for composite failure. Fluoride-releasing nanohybrid composite resin may reduce the recurrent caries rates. A fluoride-releasing resin (FCR) and non-fluoride-releasing resin (CR) were tested using an in situ model. Demineralization (ΔS), ion release and surface roughness of composite specimens were determined. The F concentration in the group FCR was higher than the CR group. ΔS (Mean±SD) was 2579±1582 and 1705±1292, respectively, for FCR and CR. Surfaces roughness was altered by biofilm accumulation. The hybrid fluorated restorative composites containing nanoparticles have a slight anticaries action without alteration of surface smoothness of the material.*

KEY WORDS: Dental caries, biofilm, nanotechnology, in situ study, composite resin.

INTRODUCTION

Dental resin based composite (RBC) have become one of the most popular and widely used direct restorative materials due to their esthetic quality and acceptable physical properties. [1] However, failures resulting from bulk fracture and secondary caries around restorations are major challenges to overcome when using this material. [2,3]

There are several strategies involving restorative dental materials to combat dental caries. For example, the incorporation of fluoride compounds in commercially available products has been one tactic to reduce secondary caries.[4] Extensive scientific literature has shown that glass-ionomer cements have the highest cariostatic effect among fluoride-releasing materials. [5-7] However, relatively low mechanical properties suggest that glass ionomer materials are inadequate for stress bearing areas.[5] Fluoride compounds have also been added to RBCs in attempt to achieve a significant anticaries effect while maintaining suitable physical strength. However, current RBCs release only a small amount of fluoride.[8]

Dental composites are constantly changing in an attempt to improve their properties.[9] The properties of RBCs may be greatly influenced by the size of filler particles. Nanotechnology has driven the re-design of dental resin based composites by the incorporation of nano-filler particles.[10] The addition of nano-particles in the resin matrix (organic content) has claimed to combine acceptable mechanical strength with optimal polishing/optical properties.[11,12] The benefit of combining different average sizes of particles led to the fabrication of nano-hybrid resin based composites.[13]

Surface roughness has a great impact on plaque accumulation and may be routinely observed in daily practice.[14] Since the incorporation of nano-particles promotes better polishing features, the characteristics of nanotechnology-based composites may be affected by alteration to the materials surface.

Differences in the anticaries performance of nano-particles-containing fluoridated resin based composite in relation to fluoride release may be observed. The ion-exchange process is quite complex and different factors, such as the particle size fractions of resins, may influence the kinetics release (diffusion rates) of ions immobilized in the polymeric matrix.[15,16] It is possible that the incorporation of nano-particles loading in the hybrid fluoridated dental composite may have significant implications on the fluoride releasing mechanism.

However, little is known with regard to the caries prevention performance of dental hybrid fluoridated composite containing nano-particles.

Therefore, the main objective of the present *in situ* study was to evaluate the anticaries effect of fluoride-releasing nano-hybrid resin based composite using an *in situ* biofilm model. To give support to findings regarding demineralization, the calcium and phosphate ion concentrations in biofilms formed by this model on restored enamel slabs were also determined. The null hypotheses were: (1) there is no difference in the demineralization inhibiting properties of fluoride-releasing nano-hybrid resin based composites assessed by microhardness, and (2) there is no difference in the composition of the biofilm formed over restorations or surface alteration in tested RBCs.

MATERIALS AND METHODS

The study protocol was approved by the research and ethics committee of the local institution (protocol # 027/2011)

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and volunteers signed an informed, written consent according to the code of ethics of the World Medical Association (Declaration of Helsinki) for experiments involving humans. Twenty healthy adults (14 females and 6 males), mean age 26.4 years, satisfied the following criteria: normal salivary flow, good general and oral health, with no active caries or periodontal disease, and the ability to complete the experimental protocol. Volunteers who had used antibiotics in the last three months, as well those under orthodontic treatment were excluded from the study sample.

A single-center, prospective split-mouth *in situ* design was conducted. Twenty volunteers wore palatal devices. The palatal devices contained 4 bovine enamel slabs. Two slabs were restored using F-containing nano-hybrid resin based composite (RBC) and two slabs were restored with non F-containing nano-hybrid resin composite (CR). Two composite resin specimens F-CR and CR, were also placed for surface roughness analysis (Fig.1A).

Following the protocol of previous studies, eighty enamel slabs (5 x 5 x 2 mm) were prepared[17,18]. Size-standardized cavities were prepared in the enamel slabs of approximately 2.4 ± 0.1 mm width, and 1.5 mm depth using a water-cooled high-speed turbine and a # 2294 cylindrical diamond tip (KG Sorensen, São Paulo, SP, Brazil). Slabs were randomly divided according to a computer-generated list into 2 groups of 40 slabs each. The cavities were restored with one of the following materials: RBC (F-CR-Tetric-N-Ceram) / Total etch adhesive system (Single Bond), and RBC (CR-EvoluX/Single Bond) (Table 1), according to the manufacturers' recommendations and guidelines. The RBC was placed in two increments and light activated for 20 s using a Light Emitting Diode (Optilight LD Max, Gnatus, Ribeirão Preto, SP, Brazil) with an output of 600 mW/cm^2 (Fig.1A). All restored slabs were stored in 100% humidity for 24 hours then polished using aluminum oxide particle impregnated discs (Sof-lex disk system, 3M Dental Products, St. Paul, MI, USA). To prepare the RBC specimens in similar dimensions of slabs, a squared silicone index mold was used. The unpolymerized resin was carefully packed inside the mold in two increments; the surface was covered with a polyester matrix tape (TDV Dental, Pomerode, SC, Brazil) and then light-activated for 20s.

Before and after the *in situ* cariogenic challenge, the average surface roughness (Ra-μm) of the composite specimens was measured using a surface profilometer (Hommel Tester T1000, Hommelwerke, GmbH, Germany). The stylus

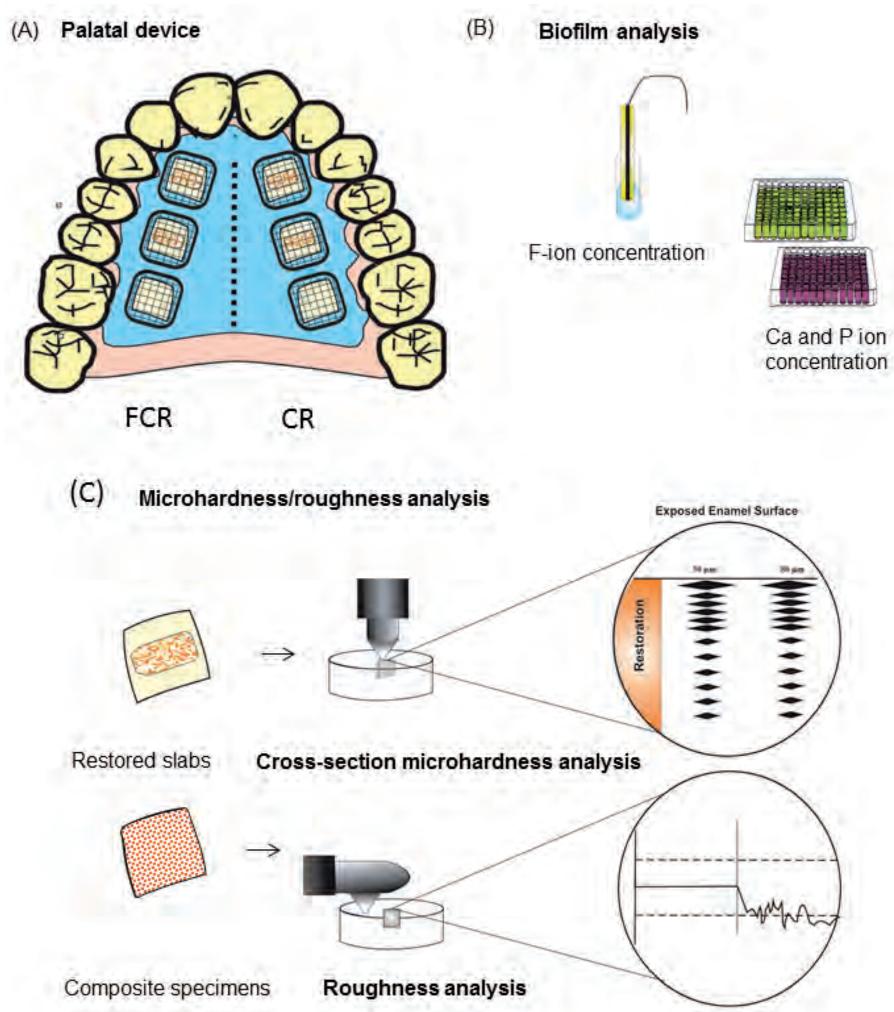

Figure 1. *Illustration of the experimental design and analysis realized in this study. 1A: Specimens set-up in the palatal device- each side has two slabs of restored enamel and one composite specimen. 1B: F, Ca and Pi analyses performed using biofilm samples. 1C: Microhardness analysis performed on restored slabs and roughness analysis performed on composite specimens.*

Table 1. Characteristics of composite resin used in this study.

Restorative material	*Formulation**	*Classification*	*Manufacturer*
F-CR Tetric-N-Ceram, shade A3, lot: L55479	Urethane dimethacrylate, Bis-GMA; Ethoxylated Bis-EMA; Triethyleneglycol dimethacrylate, Barium glass, ytterbium trifluoride, mixed oxide, silicon dioxide, Prepolymers, Additives, stabilizers, catalysts, pigments; filler level: 63.5wt%	Nanohybrid resin based composite; particle sizes in the micro- (< 1 µm) or nano range (< 100 nm, mean 40 nm)	Ivoclar Vivadent, Schaan, Liechtenstein
CR EvoluX, shade A3, lot: L234534B	Urethane dimethacrylate, Bis-GMA; Ethoxylated Bis-EMA; Triethyleneglycol dimethacrylate, barium aluminum borosilicate glass; silica nanofillers; filler: 75-77 wt%	Nanohybrid ; particle sizes in the micro- (0.02 to 3.0 µm) or nano range (4-7 to 10-20 nm)	Dentsply; York, PA, USA

* Based on information provided by the manufacturers.

traversed the surface of the specimen at a constant speed of 0.5 mm/s, force of 4 mN, with a 0.25-mm cutoff value and 1.5-mm tracing length. The difference between the Ra final and Ra initial was calculated and expressed as delta Ra (ΔRa) (Fig.1C).

During the 14-day trial, volunteers wore removable acrylic custom-made palatal devices, each containing four restored enamel slabs and two composite-made specimens. The slabs and specimens restored/made with the different materials were positioned on opposite sides of the appliance (Fig.1A). Each slab/specimen was covered with a plastic mesh with 1-mm space to protect the biofilms (18). The slabs with two different treatments on two sides of the same device enabled a similar oral environment without cross-over effect.[20] Seven days prior to and 14 days during the experimental period, volunteers brushed their teeth with non-fluoridated toothpaste. The purpose of this approach was to avoid the interference from fluoridated toothpastes on the performed analysis. Eight times per day at predetermined times, volunteers removed the appliance from the mouth and placed one drop of a 20% sucrose solution on each slab/specimen. After a 5-min waiting time, to allow for the standardized diffusion of sucrose in the biofilm, the device was placed back into mouth.[21]

On day 14, biofilms on slabs/specimens were collected for analysis (Fig. 1B). Calcium (Ca), inorganic phosphorus (Pi), and fluoride (F) concentrations were measured in the dental biofilm according to Tenuta et al. [21,22] (Fig. 1B). This analyses was performed because of the Ca /PO_4 ions potential to interfere in the formation of calcium fluoride (CaF_2) salt reservoirs, and the acid buffering effect by $CaPO_4$. Samples were treated with 0.5 M of HCl to extract acid-soluble whole-biofilm calcium and phosphate ions agitated at 30 rpm for 3h and centrifuged. The supernatant was collected for calcium and phosphate ion measurement via a spectrophotometric method. [23] For F measurement, TISAB II solution (containing 20 g NaOH/L) was added to supernatant. The amount of acid-soluble F was determined using an ion-selective electrode Orion 96-09 and an ion analyzer Orion EA-940.[21,22]

The ΔS (integrated demineralization) calculation was performed using a cross-section micro-hardness testing (CSMH) using microhardness tester (Future Tech, FM-ARS, Tokyo, Japan). First, restored enamel slabs were longitudinally sectioned through the center of the restoration. One of the remaining halves was embedded in acrylic resin and subsequently flattened and polished. CSMH was performed using a Knoop indenter with 25 g load for 5s. The values of the Knoop hardness numbers (KHN) at distances 10, 20, 30, 40, 50, 60, 80, 100, 120, 140, 160, and 180 µm from the outer enamel were obtained. Second, KHN was plotted against depth for each slab and the integrated hardness profile of the treated enamel was calculated. The mean depths higher than 120 µm were used as measure of the integrated hardness profile of inner sound enamel and ΔS was calculated, for details see previous publication.[17,24] The ΔS (integrated demineralization) were obtained at 30 and 80 µm from the preparation margin (Fig.1C). Data was expressed in Knoop hardness number to calculate ΔS.

Assumptions of the equality of variances and normal distribution of errors were checked for all the response variables tested. Results of ΔS were transformed using Box-Cox power transformation to the linear equation. [25] Data from F, Ca, Pi, ΔS and Ra were submitted to unpaired t-test and ΔRa data were analyzed by paired t-test. The level of significance of all tests was set at $\alpha = 0.05$. Statistical appraisal was computed with SPSS for Windows XP 17.0 (SPSS Inc., Chicago, IL, USA).

RESULTS

Table 2 shows the results for all variables studied. No significant effects of calcium and phosphate concentration in whole biofilm were found for any of the biofilm location under study. Fluoride concentrations on the biofilm were significantly higher for the fluoride releasing nano-hybrid resin based composite in both locations: over the resin (p=0.033) and over the restored slab (p=0.031).

Regarding mineral loss (ΔS) in enamel, a statistically significant difference between FCR and CR (p=0.039) was found only at 30-µm from the restoration margin; no statistical difference was found at 80 µm (table 2). The micro-hardness data at each depth from the dental surface is illustrated in Fig. 2.

Table 2. Mean ± Standard Deviation of Ca, Pi and F, concentrations in whole plaque like biofilm, demineralization (ΔS) and surface roughness Ra (µm) for studied conditions.

Restorative material	*Biofilm location*	*Ca, µmol/g Wet biofilm*	*Pi, µmol/g Wet biofilm*	*µg F / mg wet weight biofilm*	*Distance from restoration margin (Δ S)*		*Δ Ra*
					30 µm	80 µm	
CR	Over slabs	75 (43.2)^{a}	19.9 (12.2)^{a}	0.3 (0.2)^{a}	2579.8 (1582.4)^{a}	655.6 (392.9)^{a}	--
F-CR	Over slabs	75.3 (50.6)^{a}	19.7 (13.8)^{a}	0.5 (0.2)^{b}	1705.7 (1292.1)^{b}	749.3 (598.1)^{a}	--
CR	Over composite	80.8 (53.7)^{A}	17.9 (12.6)^{A}	0.3 (0.2)^{A}	--	--	0.05 (0.03)^{a}
F-CR	Over composite	77.2 (28.0)^{A}	18.6 (13.2)^{A}	0.5 (0.1)^{B}	--	--	0.04 (0.03)^{a}

Data are expressed as mean ± standard deviation (n = 20). Mean values represented with the small superscript letters within the columns are related to biofilm over the slabs and same small letters are not statistically different (p > 0.05). Superscript capital letter within the columns are related to biofilm over the resin specimens and the same capital letters are not statistically different (p > 0.05)

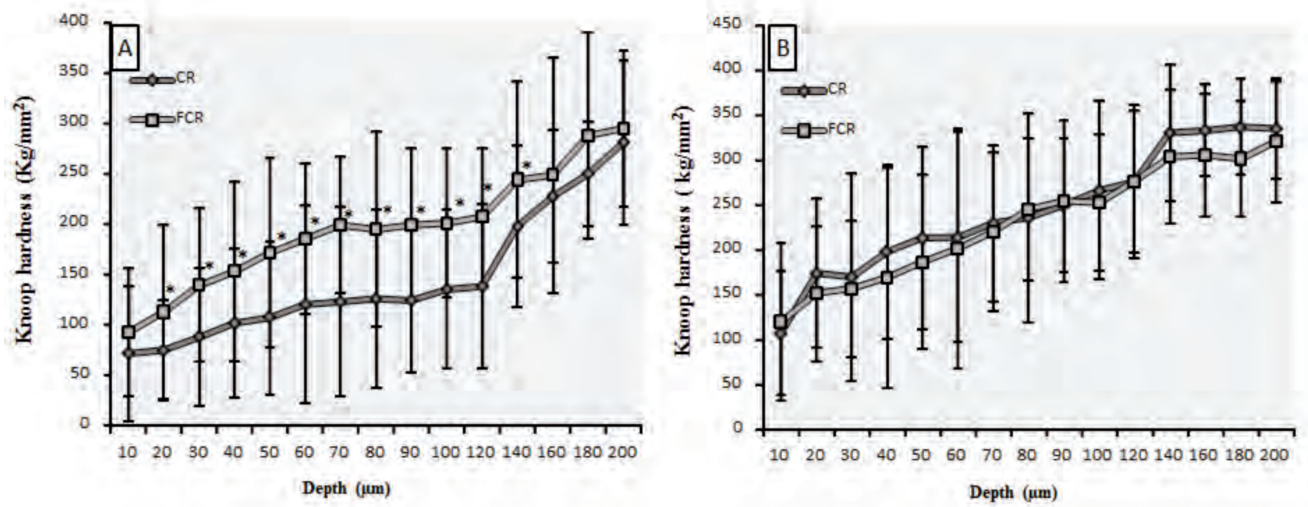

Figure 2. *Graphs showing the depth profile of microhardness from evaluated groups, at the distance 30 and 80 µm. (*) Means values statistically different by unpaired t test.*

The mean Ra values for nano-hybrid resin based composites tested after cariogenic challenges are reported in table 2. The differences of surface roughness for the materials tested before and after cariogenic challenge and ΔRa were analyzed. A significant difference was found between baselines and post challenge surface roughness for both nano-hybrid resin based composites (p=0.030 and 0.016, respectively for CR and FCR). No statistically significant differences were found between the ΔRa of tested materials (p=0.490).

DISCUSSION

The results of this study contributed to understanding the relationship between nano-filler size and fluoride release, as well as other factors associated with caries prevention. Such factors include the demineralization that often occurs around one of the most widely used direct restorative dental materials: nano-hybrid resin based composites.

In attempts to understand better the behavior of materials during fundamental aspects in the caries process, and to observe a more clinically relevant outcome, the specimens were submitted to intraoral exposure. Simulating the realistic clinical condition, under a cariogenic challenge and a short-time/accelerated process, *in situ* models have been shown to adequately study the enamel caries process.[18] Variables related to the environment, such as components of saliva, acquired pellicle, pH, ion concentration and temperature, may be conceived during the complexity of fluoride release process.[26,27]

According to our results, the first null hypothesis stating no difference in caries inhibited demineralization for the tested materials was partially rejected. The fluoridated material was able to show values statistically different from the resin based composite (control group) at 30 µm. Findings from this study demonstrated that even a small amount of fluoride leaching from composites may provide some cariostatic effect. At the 30-µm-distance the fluoride releasing nano-hybrid resin was able to improve enamel resistance to demineralization closer to the restoration margins compared to the control. This result is in accordance with previous studies in the literature, [28] and may suggest that nano-filler incorporation did not impart an improvement on fluoride releasing this material.

This lower demineralization closer to the fluoridated material may be explained by a high fluoride concentration in the biofilm present in this area. Recent studies have suggested that fluoride, even in small amounts, acts to inhibit the fall of pH at the bacteria–material interface.[29] In addition, the second null hypothesis, which stated there is no difference in the composition of the biofilm formed on the slabs restored using the materials, was rejected.

Considering the study by Cenci et al.[30], the effect of the biofilm formed on the restorations is more important than the effect of the micro-leakage. Our results have shown that the fluoride concentration in the biofilm related to the F-CR group was higher than the CR group. This may be more relevant to secondary caries prevention in the presence of marginal defects around composite restorations. The clinical significance of this improvement is yet to be fully confirmed since other factors such as additional sources of fluoride, such as fluoridated water, need to be considered.

Currently, only a few studies with conflicting results have determined the demineralization behavior of enamel around fluoride-releasing composites using an *in situ* model. [19, 30, 31] Kielbasa et al. [32] evaluated the effects of composite containing fluoride on secondary caries formation and showed no caries protective effect on the surrounding enamel. In another study, Dijkman et al.,[33] observed that fluoridated composites were able to reduce the enamel demineralization significantly when a higher fluoride content (26%) was incorporated in the material.

In relation to the composition of the studied material, the tested nano-hybrid fluoride-releasing composite presented similar caries inhibition, as compared to previously studied micro-filled resin-based material.[34] This may suggest that nano-filler incorporation did not interfere with diffusion rate and the anti-caries effect of the material. This could result in pre-clinical (*in situ*) studies where the nano-sized fluoridated particle may be investigated similar to previous in vitro studies.[35,36]

A higher protective effect could be expected from nanotechnology-based material if the type and particle size of leachable fluoridated glass fillers are altered. Weir et al, [35] highlighted that the nano-size of the fluoride source can play an important role in the performance of fluoride-releasing composite. This confirms a previous statement by Xu et al [36], which found a high fluoride release when nano-particles of calcium fluoride (CaF_2) were incorporated in a composite, yielding long-term mechanical properties that were comparable to those of a commercial composite with little F releasing.

Surface roughness is another key property for caries management and is directly related to the plaque accumulation on restorative materials.[37,38] According to previous reports, [39,40] the release of fluoride increases the roughness of the finished materials. Results for the tested nano-hybrid resin based composites showed similar ΔRa for both tested materials. It can be suggested that fluoride release does not interfere significantly in the surface roughness of tested nano-hybrid composite. This is mainly the result of an improvement in surface smoothness expected when the nano-fillers are incorporated to matrix. An explanation of this phenomenon can be related to the exposure of particles, such as silicon dioxide and barium fluorosilicate, which are extremely small and irregular, improving the quality of roughness, and lowering the contact angles for exposure with water. [40] Also the ΔRa values were close to the critical surface roughness (Ra=0.2 μm) [40] previously determined as the limit for sufficient surface smoothness of composite restorations. This suggests a negative effect on the surface roughness mainly due to the exposition of acid production by the bacterial biofilm, during the clinical phase [42.]

Some limitations of this study were the compositional variability of commercially available materials and the absence of occlusal forces on materials. It is known that under cyclic loading, there are some micro-cracks on materials that are potential paths for the fluoride ions diffusion. Future studies should consider using well-known filler composition, filler particle size and size distribution to better understand their influence in the fluoride release process. In addition, long-term evaluation also needs to be addressed since the amount of fluoride released from fluoride-releasing materials decreases over time. This experiment was performed after a 14 day-period, which is considered a short time and coincident with the boost period of fluoride release.

CONCLUSION

In summary, this *in situ* study provides evidence that the fluoride-releasing nano-hybrid resin based composite has a limited anti-caries action with no alterations in superficial roughness. However, this effect may be important to reduce demineralization adjacent to restorations in critical areas of high susceptibility to demineralization due biofilm accumulation.

ADDRESS FOR CORRESPONDENCE

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