In vitro Evaluation of Enamel Surface Treated with Fluoride After Bleaching and Etching Erosive Processes

ROXANA IONELA VASLUIANU[1], NORINA CONSUELA FORNA[1], ELENA RALUCA BACIU[1]*, MIRELA ZALTARIOV[2], LAVINIA VASILIU[2], ALICE MURARIU[1]

[1]Grigore T. Popa University of Medicine and Pharmacy Iasi, 16 Universitatii Str., 700115, Iasi, Romania

[2]Petru Poni Institute of Macromolecular Chemistry, 41A Grigore Ghica-Voda Alley, 700487, Iasi, Romania

The anti-erosion effect of fluoride on the enamel surface was investigated by ATR-FTIR, SEM and EDX techniques. Four extracted teeth (two incisors and two premolars) were initially bleached with carabamide peroxide and etched with ortho-phosphoric acid then fluoride treatment was applied. Significant differences in enamel composition and morphology were observed providing the effect of fluoride application in remineralization of teeth. Infrared spectroscopy was employed to probe the changes in enamel structure. Scanning electron microscopy/energy dispersive X-ray spectroscopy analysis revealed higher content in F of teeth enamel. Morphology changes revealed a re-mineralization of enamel surface after the treatment with fluoride gel.

Keywords: EDX, SEM, tooth bleaching, orto-phosphoric acid, fluoride gel treatment

Tooth bleaching is a popular method used fin dental practice for aesthetics reason with high concentration of peroxide carbamide that involved some risks: tooth sensitivity, gingival irritation and adverse effects on enamel and restorative materials [1-3]. The investigation of carbamide peroxide bleaching agent showed that surface morphology, structure of the enamel and dentine are affected, causing enamel erosion and lost in inorganic and organic matrix of the tissues [4, 5].

Topical fluoride (dentifrices, mouth rinses, gels and varnish) is used to provide fluoride to exposed surfaces of the dentition at elevated concentrations, for a local protective effect [6].

The effect of fluoride treatment on enamel structure was seriously investigated, being accepted two concepts: first of one its role on the erosion of enamel, and secondly its role in the inhibition of dental caries [7]. Different topical fluoride applications such as sodium fluoride (NaF), acidulated phosphate fluoride, and stannous fluoride are widely used in promoting enamel remineralization [8]. Therefore, the answer to the question regarding the destructive or beneficial effect of fluoride on enamel becomes quite necessary [9, 10].

Enamel structure consists of calcium phosphate and other ions: HPO_4^{2-}, CO_3^{2-}, K^+, Mg^{2+}, Na^+, Cl^- and HO^- [11]. HO^- anions are gradually substituted by Cl^- and F^- anions, so that hydroxyapatite converts in chloroapatite and fluoroapatite, respectively. Due to its small ionic radius, F^- has a higher affinity than $Cl^-$and HO^-, so that fluoroapatite is more stable than hydroxyapatite and chloroapatite. This contributes significantly in decreasing the enamel erosion. On the other hand, the application of fluoride in the treatment of caries is well known in clinical practice [12], although some studies highlighted its destructive effect on enamel structure [13, 14]. Other studies revealed that fluoride alone is not enough to protect the enamel erosion, but the presence of calcium and phosphate ions is also essential for maintaining the structural integrity of enamel [15]. Mostly, topical and systemic fluorides are used. Systemic fluorides include a diet based on fluoride supplements, while topical fluorides, such us fluoride toothpaste and mouth rinses, fluoride gels, foam or varnish are applied directly to the tooth enamel. Topical formulations have more fluoride than water or toothpaste and only take a few minutes to apply. The remineralization effect of fluoride formulations on the surface of enamel and/or different restoration materials has been analyzed [16]. To date, only e few studies reported on the effects of fluorine-containing bleaching agents in the fluoride-containing adhesive restoration [17] but no study has reported on the fluoride effect after the bleaching (with carbamide peroxide) and etching (with *ortho*-phosphoric acid) [4] treatment of the enamel surface. Therefore, this study aimed to evaluate the structural and morphological aspects of fluoride gel application on enamel subjected to 10, 16 and 35% carbamide peroxide for 90 min and then to 37% *ortho*-phosphoric acid solution for 15 seconds. The role of fluoride gel in remineralization and preservation of enamel surface against bleaching-etching induced deterioration was evaluated by ATR-FTIR spectroscopy, SEM imaging and EDX .

Experimental part

We used maxillary incisors and premolars extracted for orthodontic and periodontal reasons, with a completely preserved structure of enamel. The teeth were disinfected, cleaned andstored in ethanol 35%. Before all experiments the teeth were analyzed by Attenuated Total Reflectance spectroscopy and Scanning Electronic Microscopy in order to establish the structural and morphological characteristics. The composition of teeth before and after application of the fluoride gel was evaluated by Energy-Dispersive X-ray Spectroscopy. The commercial fluoride gel (*Fluor Protector*) was applied on enamel surface for 1-2 min. The teeth were left at room temperature for 30 min, and then washed with distilled water, dried in vacuum at 37 ^oC and studied by the above mentioned methods.

Measurements

Fourier transform infrared (FTIR) spectra were recorded using a Bruker Vertex 70 FTIR spectrometer equipped with a ZnSe crystal, in ATR (Attenuated Total Reflectance) mode in the range 600-4000 cm^{-1} at room temperature with a resolution of 4 cm^{-1} and accumulation of 32 scans.

* email: raluca_baciu2002@yahoo.com

For both SEM and EDX analyses, all data were collected on a FEI Quanta 200 (Eindhoven, the Netherlands) operating at 20 kV in low-vacuum mode for secondary electron imaging. The Quanta 200 SEM was equipped with an EDX system for qualitative and quantitative analyses and elemental mapping. To acquire information about elemental content of the enamel, all the obtained spectra across it were analyzed using EDAX Inc. Genesis Spectrum SEM Quanta ZAF Software (version 6.10). Through EDX analysis, the relative amounts of C, N, O,F, Na, P, Cl, Ca were calculated before and after fluoride gel application.

Results and discussions

The main target of this work was the evaluation of fluoride gel application on enamel surface after the bleaching and etching procedures. SEM method has been used to estimate the morphology of the enamel, while the structural changes have been evaluated by ATR-FTIR and EDX techniques.

SEM and EDX analyses

SEM images of the incisors and premolar enamel before bleaching, etching and fluoride treatments showed homogeneous and regular surface, mostly smooth with scattered sediments from mastication, with several defects: irregular scratched pattern or isolated enamel loss (fig. 1a) [18]. After bleaching and etching procedures we found demineralized areas in all teeth enamel, with a rough surface and revealing the dentine underneath (fig. 1b) [19]. Periodontal disease is among the most commonly occurring infections in human, with potentially profound effects on organism health [20-22]. It is known that after whitening teeth appear more tooth decay and complications in young patients [23-26]. The most severe microstructural changes in teeth enamel were found after the etching treatment using *ortho*-phosphoric acid, when a total demineralization was observed (fig. 1c) [4].

After fluoride gel application one can observe the appearance of some crystals, which are fluoroapatite or fluorohysroxyapatite gradually connect, growing and forming a mineral structure and filling the microscopic defects and pores from demineralization of the enamel surface (fig .1d). The presence of fluoride decreases non-apatite impurities observed initially by stimulation of the crystal growth. In vitro model mimics the conditions of the oral environment for the deciduous teeth enamel, revealing a simple and facile method for remineralization of the enamel structure.

The same process was observed in the case of premolar enamel (fig. 2). The SEM images indicated that the application of fluoride gel significantly protected the enamel and decreased the erosion process when compared to the untreated teeth. The fluoride application reduced the lesions after etching procedure leading to an increasing of mineral density.

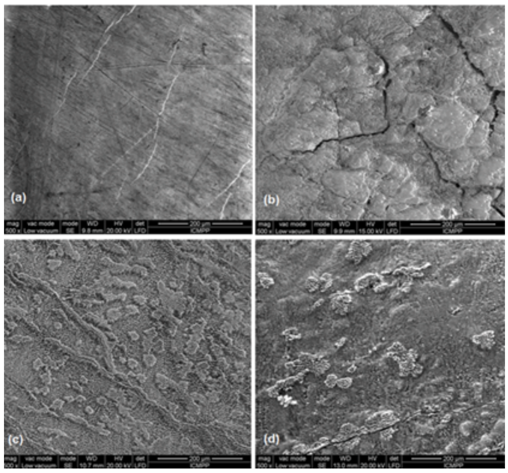

Fig. 1. SEM images of incisor: a. initially, b. after bleaching, c. after etching, d. after fluoride procedures

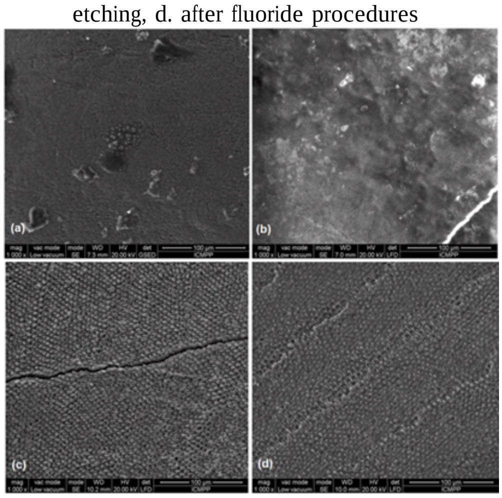

Fig. 2. SEM images of premolar:
a.initially, b. after bleaching, c. after etching, d. after fluoride procedures

The higher mineral density observed after fluoride treatment could be assigned to the formation of calcium fluoride adsorbed on the enamel surface and of the role of fluoride anion as an inhibitor for enamel loss. One can observe that fluoride ions occupies the free spaces remained by demineralization with *ortho*-phosphoric acid and renders the fluorinated crystals better observed in the figure 1d. Thus, SEM analysis demonstrated the protective role of the fluoride in restoring the enamel structure.

EDX analysis

Energy dispersive spectroscopy (EDX) is a micro-analytical technique conventionally used in scanning electron microscopy (SEM) for the local determination of chemical elements in solid samples [27, 28]. In our research, we evaluated the mineral change in the enamel

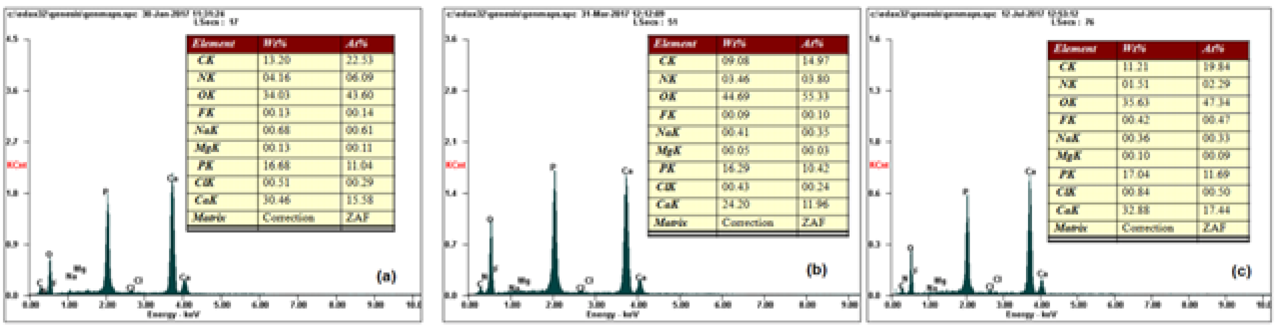

Fig. 3. EDX composition of incisor: a. initially, b. after etching, c. after fluoride procedures

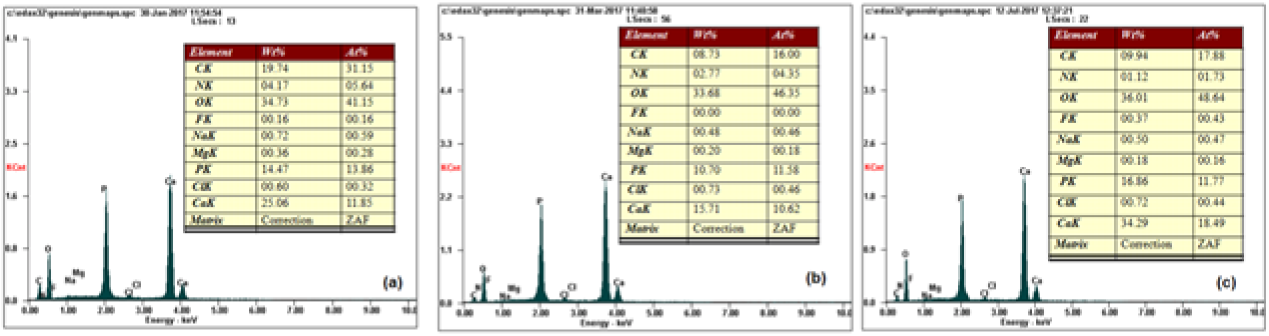

Fig. 4. EDX composition of premolar: a. initially, b. after etching, c. after fluoride procedures

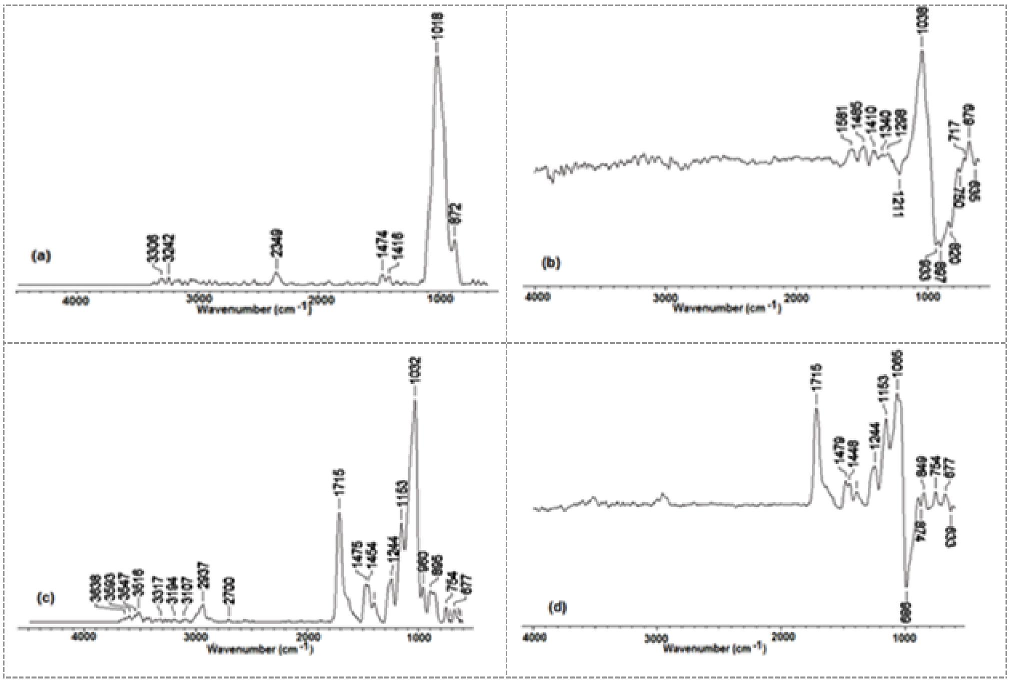

Fig. 5. ATR-FTIR spectra: a. incisor, b. IR subtracted spectra of the incisor after fluoride treatment and etching procedure, c. premolar, d. IR subtracted spectra of the premolar after fluoride treatment and etching procedure

structure before and after the etching procedure and fluoride treatment.

According to our EDX analysis one can observe a decrease in cation content by etching process and an increase in anion content, especially the F^- and Cl^- anions (figs. 3 and 4). Moreover, increased levels of fluoride after application of fluoride gel indicated the remineralization process. Also, the Ca/P ratios after fluoride treatment are similar with those of untreated teeth proving that calcium phosphate was a greater part of enamel inorganic content, and the fluoride application procedure on the enamel surface contributes to a complete remineralization process.

These findings show that the fluoride treatment significantly protect enamel and prevent the loss of its structural integrity.

ATR-IR spectroscopy

In figure 5 the ATR-IR and the subtracted spectra of the incisors and premolars after etching procedure and after fluoride treatment are shown. It can be seen that the composition of the enamel after the etching procedure characterized by a significant lose of specific bands assigned to inorganic matrix at 600-700 cm^{-1} (PO_4^{3-} tetrahedral), 833-845 cm^{-1} (bending mode of CO_3^{2-}) and 900-1200 cm^{-1} (stretching modes of PO_4^{3-}) is changed by the presence of the specific bands of fluoride ions. These can be observed at 717-679 cm^{-1} partially overlap with those of PO_4^{3-} anions. Beside these, the IR subtracted spectra revealed a major remineralization process involving the enamel surface. The presence of the specific bands of inorganic carbonates in the structure of enamel at 1581-1485 cm^{-1} and 1340-1298 cm^{-1} in the IR spectra of incisor (fig. 5a and b) and premolar (Figure 5c and d), respectively clear indicates the remineralization process by fluoride treatment.

Conclusions

The application of fluoride gel after bleaching and etching procedures increased the enamel bond strengths and the remineralization process. SEM images and EDX composition revealed a higher mineral density in the enamel structure. The ATR-IR subtracted spectra highlighted the differences between the enamel composition after demineralization process and fluoride application. Fluoride treatment is effective in preventing enamel erosion/demineralization in enamel surface and improving the remineralization process.

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