Study Regarding the Resistance of Enamel and Dentine Affected by Dental Fluorosis to Demineralization Challenge

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The aim of the study was to assess the susceptibility of fluorotic enamel and dentine to acid challenge. 10 healthy teeth and 40 teeth having different degree of dental fluorosis according to Thylstrup-Fejerskov classification splited in 4 groups were used in this study. The surface roughness of the enamel and dentine before and after the demineralization was calculated using AFM evaluation. The values were expressed by relative variation of roughness. The relative roughness for enamel and dentine increased with the increase of dental fluorosis severity. The results were analysed using ANOVA and post-hoc Bonferroni statistical tests. Significant statistical results were obtained when comparing the enamel roughness of the teeth having TF2, 3 and 4 and the teeth having TF0 and 1 and when comparing the dentine roughness indices for all study groups. The enamel of healthy and TF1 teeth was significantly more resistant to acid challenge when compared with the enamel of the teeth having superior severity of dental fluorosis. The susceptibility of the dentine to acid challenge increased with the increase of fluorosis severity.

Keywords: enamel, dentine, acid challenge, dental fluorosis, AFM

The use of fluoride in the prevention of carious dental lesions was one of the most remarkable successes of the public health programs. The control of exposure to fluoride in childhood is very important to maintain the fluoridation efficiency and to reduce the risk of dental fluorosis. Nowadays there is no common opinion regarding relation between fluorosis and cariogenic risk [1].

The carious process is initiated by bacterial biofilm [2, 3] produced on any hard surface exposed to adequate quantities of water and nutrients. The bacteria responsible of primary colonisation and the secondary microorganisms generate an extracellular matrix of polymers related to biofilm growing. The biofilm bacteria have an active metabolism causing pH variations. These fluctuations can cause mineral loss when pH decreases and mineral gain when pH increases [4, 5]. The cumulative result of these demineralization and remineralisation processes can be the mineral loss resulting in dissolution of hard dental tissues and carious lesions.

The introduction of artificial carious models contributed significantly to the understanding of carious process kinetics. The development of subsurface lesion concept allowed the clarification of interactions between de- and remineralization processes. The atomic force microscopy is a valuable method for the study of demineralization processes and the effects of diverse solutions or oral environment factors on hard dental tissues.

The aim of this study was to determine the resistance to demineralization of enamel and dentine affected by different degrees of dental fluorosis.

Experimental part

10 healthy freshly extracted teeth and 40 teeth having different degrees of dental fluorosis splited in 4 groups according to Thylstrup-Fejerskov classification (TF 1-4) were used in this study [6]. The teeth were sectioned in two halves to obtain the enamel and dentine samples. The samples were finished using silicon carbide discs (100, 600, 800, 1000, 1200, and 4000) under water cooling, and immersed in ultrasound bath for 4 minutes. The enamel and dentine samples were analyzed using atomic force microscopy (AFM) to determine surface roughness. A chemical model was used to induce structural changes similar with those induced by carious process. The enamel and dentine samples were immersed in acetic acid 0.05M (pH 5) for 16 h. The surface roughness of the enamel and dentine was calculated using AFM evaluation. The values were expressed by relative variation of roughness, using the formula ΔR= root mean squared roughness after demineralization - root mean squared roughness at baseline/root mean squared roughness at baseline.

Results and discussions

The values of relative roughness indices for the enamel samples varied between 6.18 and 6.37 for teeth with fluorosis TF 0; between 6.27 and 6.43 for teeth with fluorosis TF 1; between 6.32 and 6.48 for teeth with fluorosis TF 2; between 6.42 and 6.62 for teeth with fluorosis TF 3, and between 6.95 and 7.26 for teeth with fluorosis TF 4. The relative roughness for enamel increased with the increase of dental fluorosis severity.

The values of relative roughness indices for dentine samples varied between 2.78 and 3 for teeth with fluorosis TF 0; between 2.96 and 3.21 for teeth with TF 1; between 3.18 and 3.26 for teeth with TF 2; between 3.32 and 3.38 for teeth with TF 3, and between 3.61 and 3.67 for teeth with TF 4. The relative roughness for dentine increased with the increase of dental fluorosis severity.

The appearance of enamel before demineralization is presented in figures 1 (2 μm section) and 2 (10 μm section). In these figures can be observed the surface roughness and polynuclear crystals. The profile of 2 μm section proves

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the presence of homogenous roughness related to dimension, with a variation interval of 50-200 nm (fig. 3).

After demineralization the aspect of enamel sections can be seen in figures 4 and 5 (10 μm sections). The profile of 10μm section proves the presence of heterogeneous roughness related to dimensions (fig. 6).

The aspect of dentine before demineralization for teeth with fluorosis degree 3 can be seen in figures 7 (10 μm section) and 8 (5 μm section). The roughness values were lower comparing with values recorded for enamel samples, with a variation interval of 40-150 nm (fig. 9).

The aspect of dentine after demineralization can be seen in figures 10 (10 μm section) and 11 (5 μm section). The roughness values were lower comparing with values recorded for enamel samples, with a variation interval of 100-250 nm (fig. 12).

ANOVA and post-hoc Bonferroni statistical tests were used to compare the differences between enamel and dentine roughness values. Significant statistical results were obtained when comparing the enamel roughness of the teeth having TF2, 3 and 4 and the teeth having TF0 and
 Significant statistical results were obtained when comparing the dentine roughness of the teeth for all study groups.

Our study has shown differences of response to acid challenge related to fluorosis severity, for both enamel and dentin. The teeth affected by fluorosis with degree 1 were more resistant to the acid demineralization comparing with teeth affected by fluorosis with degrees 2, 3, and 4. The storage of fluoride ions in apatite crystals take place through accumulation or ionic substitution and associated with an increase of structural and chemical stability of the resulted apatite crystals [7]. The lower energetic level of smaller crystals is due to the replacement of the asymmetrical and more reactive hydroxyl ion with fluoride ions. That replacement explains lower solubility of fluoridated tissues comparing with non-fluoridated tissues as well as the lower reactivity of fluorapatite crystals.

The minerals can acquire extrinsic components that can have a high influence on the chemical behaviour of enamel when exposed to acid environment. Increased concentrations of magnesium and carbonate were reported in the enamel during the incipient stages of amelogenesis [8]. These inclusions are related to the lower resistance of the apatite against the acid challenge.

The chemistry of crystals in the initial stages of odontogenesis is extremely important, with further implications on the formation of the central bulk of crystals in the mature dental tissues. As crystals grow, magnesium and carbonate phases are recrystallized to the external surface of the crystals. This explains why mineral dissolution of crystals in matured enamel during caries progression is characterized by a preferential removal of magnesium and phosphate ions from crystals surface.

The studies of atomic force microscopy have proved that surface morphology of small crystals can be influenced by increased concentrations of fluoride in the mineralization environment. The fluorotic enamel produced in vitro did not present a decreased roughness during development stages and the roughness during all formation phases was higher when comparing with healthy teeth [9]. This increased roughness associated with the changes in the chemistry of surface crystals, explain the increased quantities of magnesium in the fluorotic enamel [10]. The increase of the crystals surface because of higher roughness can favour the proteins bonding and retention [11]. In the presence of fluoride ions there is an increased bonding between hydrogen and phosphate ions [12]. The roughness related to fluorotic teeth can influence the interaction and ionic exchanges between oral fluids and crystals surface.

The removal of matrix proteins can be influenced by the pH changes caused by fluoride during apatite crystals formation. In the case of unavailable or saturated buffer system of amelogenins, the decrease of pH induced by fluoride can alter the amelogenins structure and functions [13].

High concentrations of fluoride ions do not seem to influence type I collagen, which is a major component of dentine. However the non-collagenic components are modified in a specific way. In vitro studies performed on
animals showed a decrease of molecular weight of dentine phospho-proteins that was associated with a lower content of phosphate ions [14]. Similar studies showed also smaller and more anionic glycosaminoglycans because of the additional presence of dermatan sulphates [15]. The interaction between sulphates and collagen is affected, causing a reduction of mineralization initiation and minerals deposition. These explain the higher susceptibility of dentine to acid action for teeth affected by dental fluorosis and the correlation between dentine susceptibility and fluorosis severity.

Conclusions
The enamel of teeth affected by fluorosis having lower degree of severity is much more resistant to acid attacks comparing with enamel of teeth with higher severity fluorosis. The susceptibility of the dentine to acid challenge increases with fluorosis severity.

References

Table 2
RESULTS OF TEST BONFERRONI FOR MULTIPLE COMPARISONS BETWEEN GROUPS

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<th>(J) GrdF1</th>
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<th>Std. Error</th>
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<th>Lower Bound</th>
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* The mean difference is significant at the 0.05 level.

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