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RESEARCH ARTICLE

AN IN VITRO STUDY OF FLUORIDE AND ORTHOPHOSPHORIC ACID EFFECT ON DENTIN HARDNESS

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ABSTRACT

Objectives: To explore the effect of demineralization using orthophosphoric acid and remineralization using different concentrated fluoride solutions on dentine hardness of maxillary premolars.

Methods: Thirty-four maxillary premolars free of caries were included in acrylic resin. The crowns were sliced in coronal and cervical parts and divided into 4 groups: a control group (G1) for teeth treated with physiological serum , a second group (G2) for teeth treated with 3 ppm fluoride solution , a third group (G3) for teeth treated with 750ppm fluoride solution and a fourth group (G4) for teeth treated with 1500ppm a fluoride solution. The four groups underwent 500 thermocycles (5 and 55 degrees C) before being processed with a solution of orthophosphoric acid 37% for 20 seconds and a second thermocycling. The dentin hardness measurements were performed on dentin surfaces using a " D- SHORE HARDNESS DUROMETER ". Three hardness measurements were realized: after the first thermocycling; after acid application and after the second thermocycling. The statistical analysis was performed by SPSS 17.0 and significant results were considered for $p \leq 0.05$

Results: After the first thermocycling, dentin hardness increased significantly according to the fluoride solutions concentration. After acid application, dentin-hardness decreased according to the fluoride solutions concentration.-After reapplication of fluoride, dentin hardness continues to decrease according to the fluoride solution concentration.

Conclusions: The dentin hardness increases when the tooth is subjected to a fluoridated environment. However, acid treatment decreases dentin hardness of teeth initially treated with fluoride, even when processed to refluoridation.

INTRODUCTION

The relationship between dentin hardness and its mineral contents is complex since dentin is composed of inorganic hydroxyapatite associated to hydrated organic component and the hardness of dentin reflects the average of a heterogenous substrate such as inter- and peri-tubular dentin and tubules[16]. Dentin demineralization is initially described at the border between the peri and intertubular dentin, subsequently characterized by centripetal mineral loss starting at the peritubular areas, followed by intertubular demineralization, while the organic compounds remain at the surface. Therefore, a determination of tissue loss in dentin is complicated regarding histological changes and stages during erosion and several reliable techniques used in the literature revealed biases[8].

Since the organic covering surface is not removed, neither by enzymatic action nor by abrasion, it will preserve subjacent mineralized tissues and promote superficial remineralisation[9]. Such remineralisation could be enhanced by fluoride.

In fact, dentin treatment with fluoride induce its mineralization by the dentin tubule occlusion and the formation of a layer of hydroxylapatite-like covering the dentin surface and hardening it[13,6].

Different methods have been used to evaluate the degree of demineralization of dentin in the literature[18]. Hardness testing

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consisting of the measurement of a substrate to the penetration of an indenter is the method of choice for detecting changes in the consistency of the surface. Microhardness either with Knoop or a Vickers diamond indenter, or nanohardness are so sensitive that they are usually used to detect small changes on the surface. Their indenters are small enough to be placed on peri- or intertubular dentine or even a dentin tubule, and all these have distinct properties[10]. The use a less sensitive indenter could be appreciable to avoid such inconvenient.
The aim of this study was to evaluate the hardness of dentin using fluoridated solutions with different concentrations, and to explore the effect of alternating application of acid and fluoride on dentin hardness. Aging effects are produced by using thermal cycling.

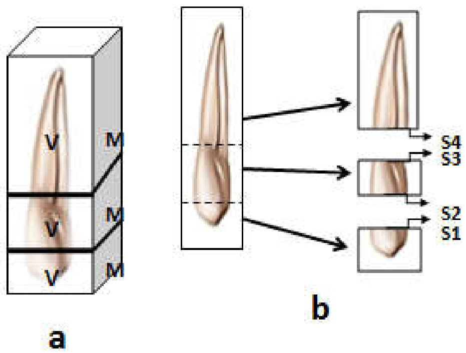
MATERIALS AND METHODS

Sample collection

Thirty four maxillary premolars, extracted for orthodontic reasons, were collected. The collected teeth were free of fluorosis, non-carious and un-abraded. They were conserved in physiological serum immediately after their extraction and kept in the refrigerator at +4°C.

Teeth treatment

Teeth inclusion: Each tooth was included in the acrylic resin (major.repair, 100074, Moncalier, Italy). The coronal and cervical cutting levels and the tooth surfaces were indicated on every resin's bloc. **(Fig1a).**



a: Teeth inclusion **b:** Teeth cutting

Figure1 Teeth treatment

Teeth cutting: Each included tooth was cut, at the two indicated levels, into three slices using the ISOMET TM low speed saw (Buehler, Illinois, USA). Four dentin surfaces were obtained: S1 for down coronal surface, S2 for upper coronal surface, S3 for down cervical surface and S4 for upper cervical surface (**Fig1b**). Each dentin surface was used to measure dentin hardness.

Distribution of the sliced teeth: The teeth were distributed into 4 groups: the Control group (G1) contained 9 teeth conserved in physiological serum; the second group (G2) contained 8 teeth conserved in 3ppm of sodium fluoride solution; the third group (G3) contained 9 teeth conserved in 750ppm of sodium fluoride solution and the fourth group (G4) contained9 teeth conserved in 1500ppm of sodium fluoride solution.

Thermo-cycling

The four groups were treated as follow: 500 thermocycles were performed with 20 seconds at 55°C followed by 20 seconds at 5°C. After acidic application of 20 seconds with orthophosphoric acid solution, all the teeth were rinsed and then 500 thermocycles were performed again with 20 seconds at 55°C followed by 20 seconds at 5°C.

Hardness measurements

The dentin hardness was measured, by the SHORE-D-HARDNESS DUROMETE SHR-D-Gold 15717, at four points at the midway of each dentine surface: Buccal measurement (B), Lingual measurement (L), Mesial measurement (M) and Distal measurement (D) **(Fig 2)**. Each measurement was defined by its side and the surface where it was applied. Three hardness measurements were realized: after the first pH cycling; after acid application and after the second pH cycling.

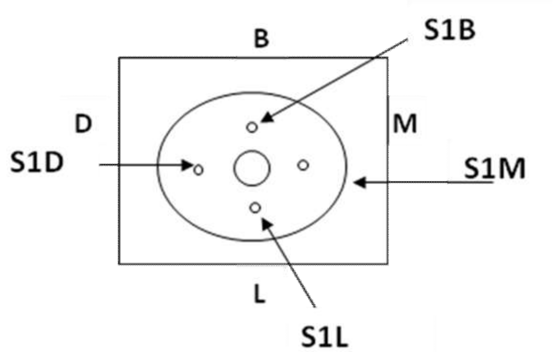


Figure2 Hardness measurements

Statistical analysis

Data were analyzed with Statistical Package for Social Sciences for Windows 17.0 (SPSS Inc., Chicago, IL, USA). Regression analysis was used as statistical test to compare two quantitative variables. Statistically significant difference was performed for P values of < 0.05.

RESULTS

The dentin hardness increases according to the fluoride concentration

The dentin hardness measurements of the four groups were taken after the first 500 thermocyclings.

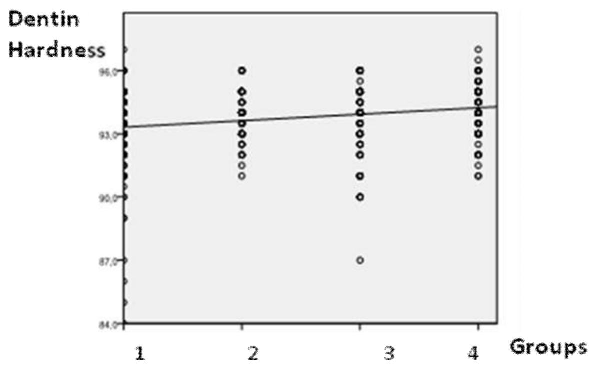


Figure3 Dentin hardness increases according to the fluoride concentration

The highest dentin hardness measurements were found in the G4 (Sodium fluoride concentration= 1500ppm), and the lowest measurements were found in the control group (physiological serum). Regression analyses showed that the dentin hardness increases significantly according to the fluoride concentration from the control group (G1) to Group the fourth group (G4). **(Fig3) p<0.05**

The dentin hardness decreases according to the fluoride concentration after acid application

After acid application, the dentin hardness measurements of the four groups were taken. The highest dentin hardness measurements were found in the control group (physiological serum), and the lowest measurements were found in G4 (Sodium fluoride concentration= 1500ppm). Regression analyses showed that the dentin hardness decreases according to the fluoride concentration from the control group (G1) to the fourth group (G4). **(Fig4)p= 0,05**

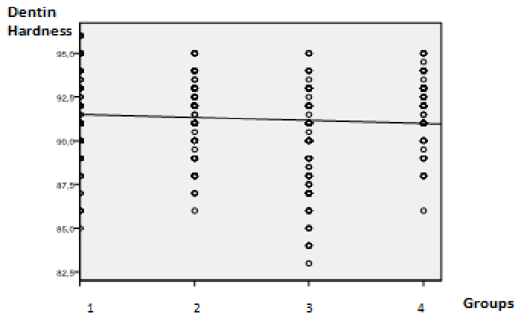


Figure4 After acid application, dentin hardness decreases according to the fluoride concentration

The dentin hardness decreases according to the fluoride concentration, after acid application and reapplication of fluoride

The dentin hardness measurements of the four groups were taken after the second 500 thermocyclings. The dentin hardness decreases according to the fluoride concentration from the control group (G1) to the fourth group (G4) (**Fig5)**. Among the same group (G1, G2, G3 and G4), the dentin hardness decreased from the first 500pH cycling to the acid application to the second 500 pH cycling **(Fig6). p<0.05**

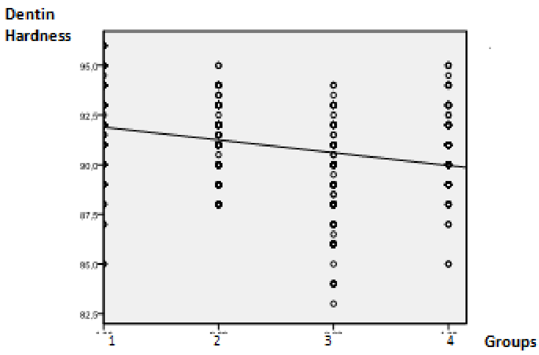


Figure5 The dentin hardness decreases according to the fluoride concentration after acid application and reapplication of fluoride

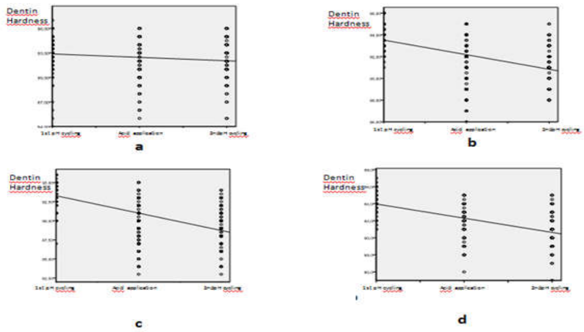


Figure6 Among the same group, dentin hardness decreases from the first pH cycling to the acid application and the second pH cycling

a: Control group
b: Group 2
c: Group 3
d: Group 4

DISCUSSION

This study investigated the in vitro effect of fluoride and acidic applications on dentin hardness in a thermocycling model simulating mineralizing conditions to enhance dentine resistance before acidic conditions and then to evaluate the novel remineralizing conditions. We hypothesized that as dentin is more caries-susceptible than enamel, its demineralization could be more influenced by fluoride.

Hardness testing is a relatively simple and reliable method for the study of the mineral change contents of dental hard tissue[1]. In a first step the increasing of the dentine hardness according to the fluoride concentration of the solutions was demonstrated. Such result was consistent with the microhardness increasing of dentin described under fluoride-releasing adhesive systems subjected to cariogenic challenge and fluoride therapy[11]. It is suggested that an adsorption of fluoride on the surface of the dentine leads to the formation of fluoridated hydroxyapatite that are closely similar to fluoroapatite crystals. These apatites are supposed to be less soluble than hydroxyapatite and to exhibit larger mechanical strength giving to the modified apatite the propriety of protecting the tissue from demineralization[12].

In a second step, when acid was applied, the decrease of surface hardness was noticed suggesting a dentine mineral loss. That was expected as in accordance with previous data concerning the effect of acid on mineralized tissues and particularly on the microhardness of dentin[2]. However, the analysis of hardness values according to the groups revealed that the hardness was the lowest in the groups when fluoride contents of the pre-treating solutions were the highest. These observations could indicate a higher loss of mineralized contents in these groups where dentine was supposed to be protected and more resistant to the acidic effect. It should be noticed that a significant amount of fluoride ion in solution during acidogenic challenge may increase the demineralization rate due to the affinity of fluoride to mineralized tissues enhancing penetration of acids. It is noteworthy that the present result is opposed to that demonstrating less microhardness loss of dentine after acid application, according to the fluoride content[5], but attention should be paid that the maximum of fluoride concentration cited in our study was of 1500ppm, where higher concentrations even up to 5000 ppm were used in the other study. Moreover, in the cited study, no significant difference was found between 2800ppm and 5000ppm fluoride groups. This could be explained by a described logarithmic correlation between dentin relative mineral loss and the fluoride concentration indicating the existence of an optimum concentration for the achievement of maximum uptake of fluoride[7].

In the last step of our experience, dentin hardness decreased significantly according to the concentration of fluoridated solutions after fluoridated, acidic and fluoridated treatment. Two hypotheses are suggested to explain this result. The first one suggests that demineralization continues despite the use of fluoride. In fact it is worth mentioning literature considering that dentin needs a higher fluoride concentration in its surrounding solution than enamel to get an inhibition of demineralization process, reaching 5000ppm for the treatment of initial root caries lesions for example [3]. The second hypothesis suggests that a remineralizing process took place despite the use of lower fluoride concentrations, with calcium fluoride-like material precipitating on the tooth's surface. The calcium could originate from the tooth after the application of the acid solution and fluoride from the prepared solution. In fact, an increased CaF_2 formation is described with a decreasing pH or an increasing fluoride concentration [17] suggesting that precipitation should be more important in group 4 than the other groups. However, the measurement of hardness was inversely proportional to the concentration of fluoride used in the solutions. This could be explained by the structure of CaF_2 appearing as spherical globules instead of crystals compared to fluoridated apatite or hydroxyapatite when observed in scanning electron microscopy[14]. In fact, during the experiment, a white precipitated material was easily observable on the surface of the teeth confirming the hypothesis of CaF_2 formation as being the most important and the only reaction product on the dental hard tissues after local application of fluoridation media[15]. In such conditions, it's better to speak about reprecipitation than remineralization[4]. Such precipitate is considered to be important in the caries-prophylactic effect of fluoride, but similar to the surface of white spots that is based on a fluoride-promoted remineralization (10), the surface is porous and the precipitation doesn't mean that it harden the surface of the mineralized tissue. It seems that hardness testing doesn't only allow the study of mineral contents, but also the evaluation of mineral structure.

Within the limitation of this study, it can be concluded that the use of daily therapy with fluoridated solutions limited to a maximum concentration of 1500 ppm either in tooth paste or mouthwashes could help in hardening the exposed dentin surface during periodontal recession for example, but considering the acidic attack described during tooth erosion or caries process, this amount could be insufficient as dentin is a tissue that demineralizes easily but its remineralisation is hard, needing higher values of fluoride.

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