An In Vitro Study of Enamel Surface Microhardness Following Argon Laser Irradiation and Acidulated Phosphate Fluoride Treatment

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Abstract

Purpose: The purpose of this in vitro study was to evaluate the effect of low-fluence argon laser (AL) irradiation and acidulated phosphate fluoride (APF) gel treatment on enamel microhardness.

Methods: Twelve mandibular permanent molars were selected for this study. The teeth were sectioned generating 4 flat enamel surfaces per tooth. The flattened enamel tooth surfaces were randomly assigned to 1 of 4 treatment groups: (1) no treatment (control); (2) the enamel surface was exposed to a 4-minute, 1.23% APF gel treatment; (3) the enamel surface was exposed to AL irradiation of 11.5 J/cm^2 (0.231-W, 5-mm beam size, 10 seconds); and (4) the enamel surface was exposed to the same AL irradiation followed by an APF gel treatment. Using a Buehler Micromet II Digital Microhardness Tester, Knoop hardness was determined using a 1,000-gram load and a dwell time of 12 seconds. Five hardness values were recorded for each enamel surface. Data were analyzed using ANOVA and Fisher's least significant difference post-hoc test.

Results: Mean surface hardness values (±SD) were 298±37 Knoop hardness (HK) for the no treatment (control), 270±70 HK for the APF-only group, 316±25 HK for the AL-only group, and 317±25 HK for the AL-before-APF group. The AL-only and AL-before-APF groups had significantly higher (P<.05) surface hardness values vs the APF-only group.

Conclusions: Enamel surface microhardness is higher when exposed to low AL irradiation only or AL before APF vs a no treatment (control) enamel surface. (*Pediatr Dent*. 2003;25:497-500)

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Enamel surface microhardness refers to a tooth's resistance to scratching, abrasion, and indentation. The physical-mechanical effects of Nd:YAG laser irradiation on sound enamel has been reported to increase surface microhardness and modification of the membrane permselectivity of the enamel.[1] These results are suggested to be connected with the fusion of the enamel surface. In another study, the microhardness of a Nd:YAG laser-irradiated enamel surface was shown to decrease significantly when subjected to higher energy irradiation settings.[2]

Previous in vitro[3-8] and in vivo[9-11] investigations have shown that the surface of enamel, when exposed to low-fluence argon laser (AL) irradiation for a short period of time, exhibits a surface that has been enhanced in caries resistance. An even more protective effect of enhancing the resistance of sound enamel to an in vitro cariogenic challenge has been shown when topical fluoride agents have been added to the protocol.[12-15]

It has been stated that the ionic loss of enamel during demineralization may be interfered with when a tooth is restored with fluoride-releasing dental materials.[16] Two in vitro studies, where AL polymerization of a fluoride-releasing pit and fissure sealant, provided a greater degree of protection against an artificial cariogenic challenge and resulted in significant reductions in primary surface lesion depth and frequency of wall lesions when compared with visible light polymerization.[17,18]

	Surface hardness (Mean ± SD)
APF only	270±70 HK
No treatment (control)	298±37 HK
AL only	316±25 HK*
AL before APF	317±25 HK*

Figure 1. Mean enamel surface microhardness values.
* Significant difference ($P<.05$, ANOVA, Fisher's least significant difference post-hoc test) for APF only vs AL only and AL before APF.

The purpose of this in vitro laboratory study was to evaluate the effect of low-fluence AL irradiation and acidulated phosphate fluoride (APF) gel treatment on enamel surface microhardness.

Methods

Twelve extracted human mandibular permanent molars with macroscopically sound enamel surfaces were selected for this in vitro study. The teeth were first sectioned occlusal-gingivally, using a Silverstone-Taylor Hard Tissue Macrotome to establish flattened and parallel buccal and lingual enamel surfaces. A second occlusal-gingival cut through the middle of the tooth in a buccal to lingual direction divided the specimens into tooth halves. The flattened, lingual enamel tooth surface halves from each specimen were smoothed with a fine polishing disk and then randomly assigned to 1 of 4 treatment groups:

1. No treatment–one tooth-half served as the control.
2. APF only–the no-treatment tooth half surface was then exposed to a 4-minute, 1.23% APF gel (Oral-B Laboratories, Belmont, Calif) treatment.
3. AL only–the other tooth-half served as the surface exposed with low-fluence AL irradiation of 11.5 joules/cm^2 (0.231-W, 5-mm beam size, 10 seconds).
4. AL followed by APF–the AL-only tooth half surface was then exposed to a 4-minute, 1.23% APF gel treatment.

All tooth specimens were stored in deionized-distilled water prior to treatment.

A Buehler Micromet II Digital Microhardness Tester (Buehler Ltd, Lake Bluff, Ill) was the microhardness tester used in this laboratory study. This instrument is designed for rapid microhardness tests of all types and shapes of metallic and nonmetallic materials. A diamond indentor provides diagonal measurements of the indentations and resultant hardness values. A HGM, Inc. (Model 8, Medical Laser Systems, Salt Lake City, Utah) AL with a stationary bare fiber and a 5-mm beam spot size delivered the irradiation to the enamel surfaces.

The flattened buccal surface of the specimen was placed on the microhardness tester's stage so that the lingual enamel surface of the tooth-half to be tested was perpendicular to the diamond indentor. Each lingual enamel surface of the 12 specimens for the 4 treatment groups was subjected to hardness indentations made with the Knoop hardness tester using a 1,000-gram load and a dwell time of 12 seconds. The fluoride gel (APF only) treated and indented surface hardness values were completed directly after the no treatment (control) surface hardness values were recorded. In a similar manner, the fluoride gel treated and previously AL (AL followed by APF) irradiated and indented surface hardness values were completed directly after the argon laser (AL only) irradiated surface hardness values were recorded. All indented surface hardness values were recorded by the same investigator.

For each specimen, 5 Knoop hardness values (hardness values in HK) were recorded. Mean hardness values were than calculated for each of the lingual enamel surfaces. Because of the research design, the surface microhardness values for the no treatment (control) and experimental groups were subjected to analysis of variance (ANOVA) and Fisher's least significant difference post-hoc test, thereby limiting tooth-to-tooth variation in the statistical evaluation. A significance alpha level of $P<.05$ was considered to be acceptable for discriminating differences between groups.

Results

The trend for enamel surface microhardness was lower after exposure to a topical fluoride (APF) treatment (270 HK) when compared to the no treatment (control) group (298 HK; Figure 1). Enamel surface exposed to a low-fluence argon laser (AL) irradiation alone (316 HK) or in combination with a topical fluoride (APF) treatment (317 HK) resulted in higher microhardness when compared to the no treatment (control) group (298 HK). The enamel surface microhardness is significantly higher ($P<.05$) when exposed to low-fluence argon laser (AL) irradiation alone or in combination with a topical fluoride (APF) treatment vs the topical fluoride (APF) treatment alone.

The enamel surface microhardness decreased by 9% for the APF-only treated group when compared to the no treatment (control) group (Figure 1). A 15% increase in hardness occurred for both the AL-only and AL-before-APF groups when compared to the APF-only treated group. In a similar result, the AL-only and AL-before-APF groups had a 6% increase in hardness vs the no treatment (control) group. There was a 0% increase in hardness between the AL-only and AL-before-APF group.

Discussion

The results of increased enamel surface hardness, when exposed to low-fluence AL irradiation alone or in combination with APF gel treatment, are encouraging and may contribute to the prevention of dental caries. Several in vitro[3-8,10,12-14]

and in vivo[9-11] studies have consistently demonstrated the ability of low-fluence AL irradiation to effectively reduce caries initiation and progression. Several possible mechanisms have been proposed for irradiation-induced caries resistance.[6,12,17-24] These include:

1. reduction in hydroxyapatite lattice strain and decreased solubility due to alteration in carbonate, water, and organic content of tooth mineral phases;
2. creation of a microseive or micropore system with the mineral substance of enamel, dentin, and cementum, allowing for reprecipitation or entrapment of mobilized calcium, phosphate, and fluoride during demineralization;
3. reduction in mineral structure permeability secondary to protein denaturation and protein swelling, leading to reduction in microporosites;
4. increased uptake to fluoride, calcium, and phosphate from exogenous sources;
5. creation of surface coatings acting as reservoirs for calcium, phosphate, and fluoride;
6. bacteriostatic or bacteriocidal effect on plaque microorganisms (only at high levels).

It is highly unlikely that the results from this study have much bearing on the clinical situation with respect to the effect of APF softening of enamel following an APF gel treatment. In the clinical situation, there is rapid surface enamel remineralization and incorporation of fluoride and calcium phosphate from the surface coating created by the APF gel treatment. Hence, a limitation to this study is the fact that a synthetic saliva rinsing of the specimens was not performed. This may have assisted to determine if the differences in microhardness were due to the APF gel alone, AL irradiation alone, or the AL irradiation in combination with the APF gel.

The real message of this study is that AL irradiation results in a significant increase in enamel microhardness in the absence of an APF gel treatment. The fact that the addition of APF gel treatment after AL irradiation did not result in a decrease in microhardness attests to the durability of laser-induced hardening of the enamel surface. One might expect that, with APF gel treatment after AL irradiation, the enamel surface would undergo softening due to the acidic nature of the APF gel, especially with an exposure time of 4 minutes. There was effectively no change in the microhardness with the AL irradiation alone or argon laser irradiation followed by the APF gel treatment. This implies that AL irradiation is responsible for microhardness whereas APF gel treatment improves resistance to caries by providing calcium, phosphate, and fluoride coatings that may be incorporated into enamel surfaces prior to or during initiation or progression of in vitro or in vivo caries.

Conclusions

Enamel surface microhardness is higher when exposed to low-fluence AL irradiation alone or in combination with APF gel treatment vs a no treatment (control) enamel surface.

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Abstract of the Scientific Literature

The Effect of Fluoridated Milk on Enamel Demineralization

The aim of this in vitro study is to compare the effect of milk and low-dose fluoridated milk on enamel demineralization combining polarization light microscopy, scanning electron microscopy, and EDX element analysis to detect morphological features and the element content of experimental caries-like lesions after medium-term exposure to whole milk and fluoridated milk. Twelve extracted impacted human third molars were covered in wax, leaving two 3 mm × 3 mm windows on the buccal and lingual surfaces and then incubated alternating in demineralizing solution and in milk, F-milk, saline, and remineralizing solution, respectively. Serial ground sections were cut and analyzed by polarization light microscopy and SEM using EDX element analysis. The results showed increased thickness of the superficial layer in the F-milk samples, and quantitative element analysis revealed a significant increase in the fluoride content of the superficial layer and of the body of the lesion in the F-milk group, which was less demineralized than in the other groups. The investigators conclude that the combination of analysis techniques described in this study is a powerful method to assess caries-like lesion formation. They further conclude that fluoridated milk may have protective properties in inhibiting demineralization.

Comments: This article presents a scientific perspective which suggests that milk can be utilized as an alternative vehicle for fluoride delivery. BB

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