Photonic Conversion of Hydroxyapapite to Fluorapatite: A Possible Mechanism for Laser-activated Fluoride Therapy

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Purpose: This laboratory study aimed to determine whether laser activated fluoride (LAF) therapy of sensitive dentine causes conversion of apatite mineral to fluorapatite ("photonic conversion") and thereby reduces the risk of dental erosion.

Materials and Methods: X-ray photoelectron spectroscopy was used to quantify changes in the atomic concentration of the elements fluorine, calcium, oxygen, phosphorus, carbon, nitrogen, and silicon, at five depths up to 52 nm in human dentine slabs with patent dentine tubules, which were either treated with neutral sodium fluoride gel (12,300 ppm) only, untreated, or subjected to laser-activated fluorid therapy (LAF) over a 12-week period.

Results: Fluorine to calcium ratios were increased from zero (control) to 0.27 and 0.28 after fluoride gel and LAF, respectively. However, the chemical composition of dentine was affected by LAF, with the altered fluorine binding energy of 684.6 eV corresponding to formation of fluorapatite. No conversion to fluorapatite occurred when fluoride gel was applied under identical conditions but without lasing.

Conclusion: Partial conversion of apatite minerals with LAF would contribute to the documented protective effects of LAF against dental erosion, and explains the benefits gained clinically when this treatment is applied to sensitive cervical root surfaces.

Keywords: apatite, fluoride, transformation, erosion, dentine.

Dental erosion is the irreversible loss of dental hard tissues from dissolution under acidic conditions, and is a major cause of the exposure of dentinal tubules, which leads to cervical dentinal hypersensitivity. Because of its increasing frequency in many adult populations, dental erosion has been identified as a significant clinical concern.[1-5] Because loss of mineral by acid dissolution is the fundamental event in this condition, there has been interest in the use of laser-acti- vated fluoride (LAF) therapy to increase the resistance of tooth structure to acid dissolution. Recent studies have shown that LAF with neutral sodium fluoride and a range of visible light wavelengths can prevent softening of enamel from exposure to hydrochloric acid in an in vitro model which simulates the conditions of gastric reflux.[6] Clinical studies by the present authors have also shown protective effects of LAF on root surface dentine in xerostomic patients,[7] as well as evidence of

caries arrest and reversal for incipient lesions on cervical surfaces.[8] The current study builds on a considerable literature on LAF by Powell and colleagues, which has shown protective effects of LAF using argon (488 nm) laser energy combined with acidulated phosphate fluoride, in a range of laboratory caries models and in clinical trial settings.[9-14]

A range of possible mechanisms may contribute to the LAF protective effect in a nonexclusive manner. Physicochemical changes, such as deposition of calcium fluoride,[12] formation of microspaces in the dental hard tissue,[15,16] formation of tri-calcium phosphate,[17] and phase transformation of hydroxyapatite to fluorapatite[18] have all been proposed, with tangible evidence of each event being shown, according to the wavelength used and irradiation parameters selected.

The concept of surface alterations by LAF, such as blockage of dentinal tubules, has now been demonstrated formally in vitro by environmental SEM studies of LAF therapy applied at the dentine-enamel junction using a variety of fluoride vehicles combined with KTP or carbon dioxide lasers.[19] Of note, fluoride treatments with stannous fluoride gel, amino fluoride solution, or fluoride varnish did not cause closure of dentine tubules, whilst LAF treatment caused partial or total occlusion, providing an explanation for the desensitizing effect of LAF when used clinically.

In addition to such physical effects, the intriguing possibility also exists that LAF may induce chemical changes in tooth structure, such as partial conversion of various forms of carbonated apatite or hydroxyapatite to fluorapatite, thereby conferring the increased resistance to acid dissolution which has been documented in both caries and erosion models.[6,20] The present study explores this so-called photonic conversion effect, using the surface analytical technique of x-ray photoelectron spectroscopy (XPS), in which a monochromatic source of x-rays is focused on the surface of the sample, under high vacuum conditions, thereby liberating electrons. By measuring the kinetic energy of the emitted electrons, their binding energy can be determine and from this, the chemical structure of the material can be deduced.[22,23] XPS can provide both quantitative and qualitative data regarding atomic composition and chemical structure. Moreover, its shallow depth and broad area of analysis ensure that an accurate representation is obtained of the sample under study. If LAF does in fact cause incorporation of fluoride into apatite, as opposed to physical trapping of fluoride (in the form of unreacted sodium fluoride), this should be readily detectable using the XPS approach.

MATERIALS AND METHODS

Specimen Preparation

Ten slabs were prepared from sound extracted human third molars, which had been obtained with the approval of the University of Queensland Medical Research Ethics Committee. The teeth had been removed for orthodontic reasons from patients who resided in a region without community water fluoridation. After debridement of gingival soft tissue remnants and prophylaxis with a fluoride-free paste, buccal and lingual surfaces were sectioned to create slabs using a diamond saw, and polished with 1200-grit silicon carbide paper. The slabs were then immersed in 1.0 M hydrochloric acid (HCl) for 10 h. Pilot studies had shown that this treatment was sufficient to cause dissolution of any remaining enamel, resulting in complete exposure of the underlying dentine with patent tubules (as confirmed by SEM, Fig 1), thereby replicating the physical properties of sensitive exposed dentine. Following the erosive step, the slabs were gently rinsed with deionized water and then kept moist until treated. After this, and when not undergoing treatment, the slabs were stored in a humidor.

Laser Treatment

A total of 8 slabs (a sufficiently large sample size as determined by a power analysis) were treated with LAF therapy, which was then repeated at 1, 2, 3, 6, and 12 weeks. This protocol was based on a clinical trial of LAF and other laser and nonlaser therapies for cervical lesions.[8] LAF therapy entailed topical application of 100 µl of 1.23% neutral sodium fluoride gel (Colgate NeutraFluor, Sydney, Australia; 12,300 ppm F ion) to the exposed dentine surface, followed immediately by laser treatment (635 nm, energy density 15 J/cm^2, spot size 5 mm, power 50 mW, exposure time 60 s) using an InGaAsP visible red diode laser system (SaveDent, Denfotex; Inverkeithing, Scotland). The wavelength was chosen because previous work examining the effect of wavelength on LAF-induced protection against erosion had shown an optimal response in the visible spectrum in the band between visible green and visible red.[6] An energy density of 15 J/cm^2 was used for each treatment, since this was shown to be the optimal value in previous studies,[9-14] and had been shown in previous studies to be effective for the 635 nm laser wavelength.[6,20]

All 8 LAF-treated slabs underwent a wide survey scan analysis using a Kratos Axis ULTRA X-ray Photo-electron Spectrometer (Kratos Analytical; Manchester, UK), which measured the atomic concentration of seven different elements [carbon (C 1s), oxygen (O 1s), nitrogen (N 1s), calcium (Ca 2p), phosphorous (P 2p), fluorine (F 1s) and silicon (Si 1s)] at depths of 0 nm (surface), 10.4 nm, 20.8 nm, 31.2 nm, 41.6 nm, and 52 nm.

The two remaining slabs were used as controls, and were subjected to high resolution scan analysis. The first of these was a "fluoride only" control, and was treated with 100 μl of topical neutral sodium fluoride gel in an identical manner to the LAF group, but was not lased. This slab was then used for analysis of the binding energy of all elements and determination of chemical bond configurations. The second control slab served as an "untreated baseline" control and did not undergo fluoride or laser treatment. Use of only small numbers of samples is required for XPS analysis because of the inherent compositional consistency of dental hard tissue samples, as shown in previous studies using dentine or enamel.[2,19,23-25]

XPS Analysis

The XPS system at the Brisbane Surface Analysis Facility of the University of Queensland was utilized. The Kratos Axis ULTRA X-ray Photoelectron Spectrometer equipped with a 165-mm hemispherical electron energy analyzer and a monochromatic aluminium filtered x-ray incident radiation source (Kratos) (1486.6 eV) at 150 W (15 kV, 10 mA) was used. Dentine samples were fixed in position on a stainless steel sample holder with double-sided adhesive tape. Base pressure in the analysis chamber was 1.0×10^{-9} torr, while during sample analysis the pressure was 1.0×10^{-8} torr. Survey (wide) scans were taken using an analyser pass energy of 160 eV, while multiplex (narrow) high resolution scans were performed at 20 eV. Survey scans were carried out over a 0- to 1200-eV binding energy range, with 1.0-eV steps and a dwell time of 100 ms. Narrow high-resolution scans were run with 0.05 eV steps and a 250 ms dwell time.

Depth profiling was performed using a differentially-pumped Kratos Minibeam III argon ion gun (Kratos). Argon ions (4 keV) were used at an ion source extractor current of 630 nA, rastered over an area of 3 mm x 3 mm and stabilized by a Pfeiffer RVG 050C controller in conjunction with a Pfeiffer UDV 140 leak valve (Pfeiffer Vacuum; Nashua, NH, USA). The etch
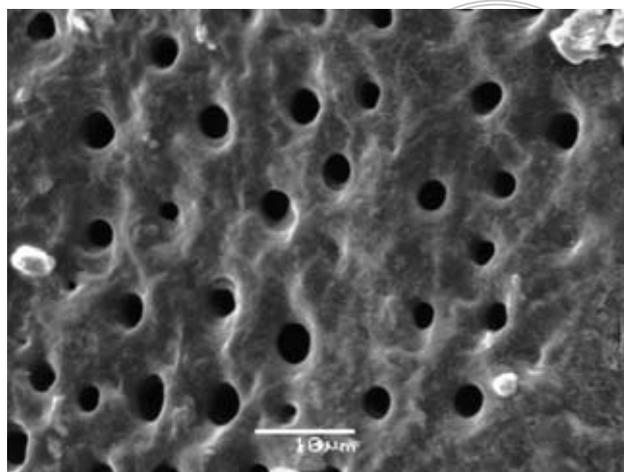

Fig 1 SEM view of the surface of a dentine sample prepared using 1.0 M hydrochloric acid treatment of molar slabs for 10 h. 2000X final magnification. Note the open orifices of the dentinal tubules.

rate was 2.6 nm/min, with wide scans recorded after each 4-min etching cycle. During profiling, the sample analysis chamber (SAC) pressure was increased to 5.0×10^{-8} torr.

High resolution scan analysis, allowing determination of binding energy positions for each element, was conducted on three samples at the final depth of 52 nm. In line with standard XPS procedures for surface analysis,[22,26] the surface (ie, a depth of 0 nm) was not used for detailed analysis because of the possibility of surface contaminants, such as hydrocarbon components from the vacuum pump system. The selected final depth of 52 nm could be demonstrated by its chemical composition to be completely free of any such contaminants, as shown by reduced carbon and increased calcium levels (Table 1). Calcium 2p 3/2 was charge corrected at a binding energy of 347.4 eV with calcium 2p $^1/_2$ at 350.9 eV.[25]

The data obtained from the wide scan analyses were analysed using repeated measures Analysis of Variance (RMANOVA), with post-hoc Tukey-Kramer multiple tests. The p-value used was 0.05. Statistical analysis was undertaken using NCSS 2004 software (NCSS; Kaysville, UT, USA).[27]

RESULTS

Wide Scan Survey Data of LAF Samples

RMANOVA analysis of the eight LAF-treated dentine slabs showed a statistically significant change with
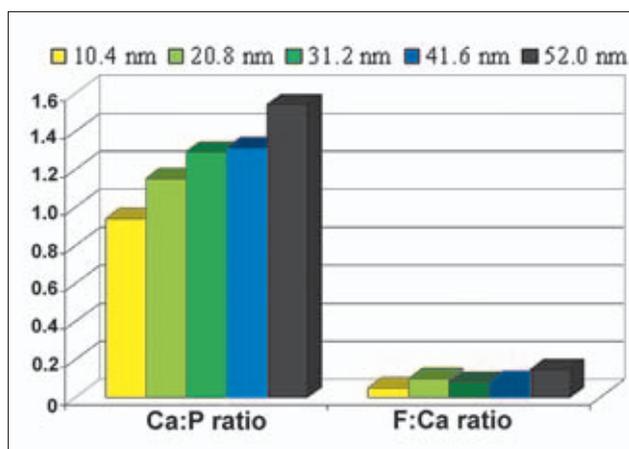
Table 1 Changes in the concentration of the seven analysed elements, with increasing depth

Element	p-value	Change
Carbon	0.0009 *	Decreases
Oxygen	0.0002 *	Increases
Calcium	0.0053 *	Increases
Phosphorous	0.3751	Remains stable
Silicon	0.5533	Remains stable
Nitrogen	0.3861	Remains stable
Fluorine	0.7501	Remains stable

Table 2 Selected compounds and their properties, with reference to the three experimental groups

Material/group	Formula	F:Ca ratio	F binding energy	Ca:P ratio
Hydroxyapatite	$Ca_{10}(PO_4)_6(OH)_2$	NA	NA	1.67
Fluorapatite	$Ca_{10}(PO_4)_6F_2$	0.25	684.6 eV	1.67
Sodium fluoride	NaF	NA	684.5 eV	NA
Calcium fluoride	CaF_2	2.0	684.8 eV	NA
Unlased dentine control	Predominantly hydroxyapatite	0	NA	1.69
Fluoride gel only control	Predominantly sodium fluoride, trapped in dentine tubules	0.28	684.5 eV	1.72
LAF-treated dentine	Predominantly fluorapatite	0.27	684.6 eV	1.49

NA, not applicable.

Fig 2 Changes in ratios of Ca 2p:P 2p, and F 1s:Ca 2p, with depth, in LAF treated samples.

depth in the atomic concentration for three out of the seven elements, namely, carbon, oxygen, and calcium (Table 1). The Ca 2p: P 2p and F 1s: Ca 2p ratios were determined for each sample at each depth. The mean ratios are shown in Fig 2. Calcium to phosphate ratios increased with depth, whilst fluoride-calcium ratios were more constant with depth. Data for ratios between elements are summarized in Fig 4 and Table 2; the latter includes pure compounds as well as the three experimental groups (and their deduced compositions).

High Resolution (narrow) Scan data

Control (untreated) dentine
Summarized XPS data for untreated dentine are shown in the upper panels of Fig 3. Phosphorus (P 2p) at 133.3 eV can be identified as the phosphate species
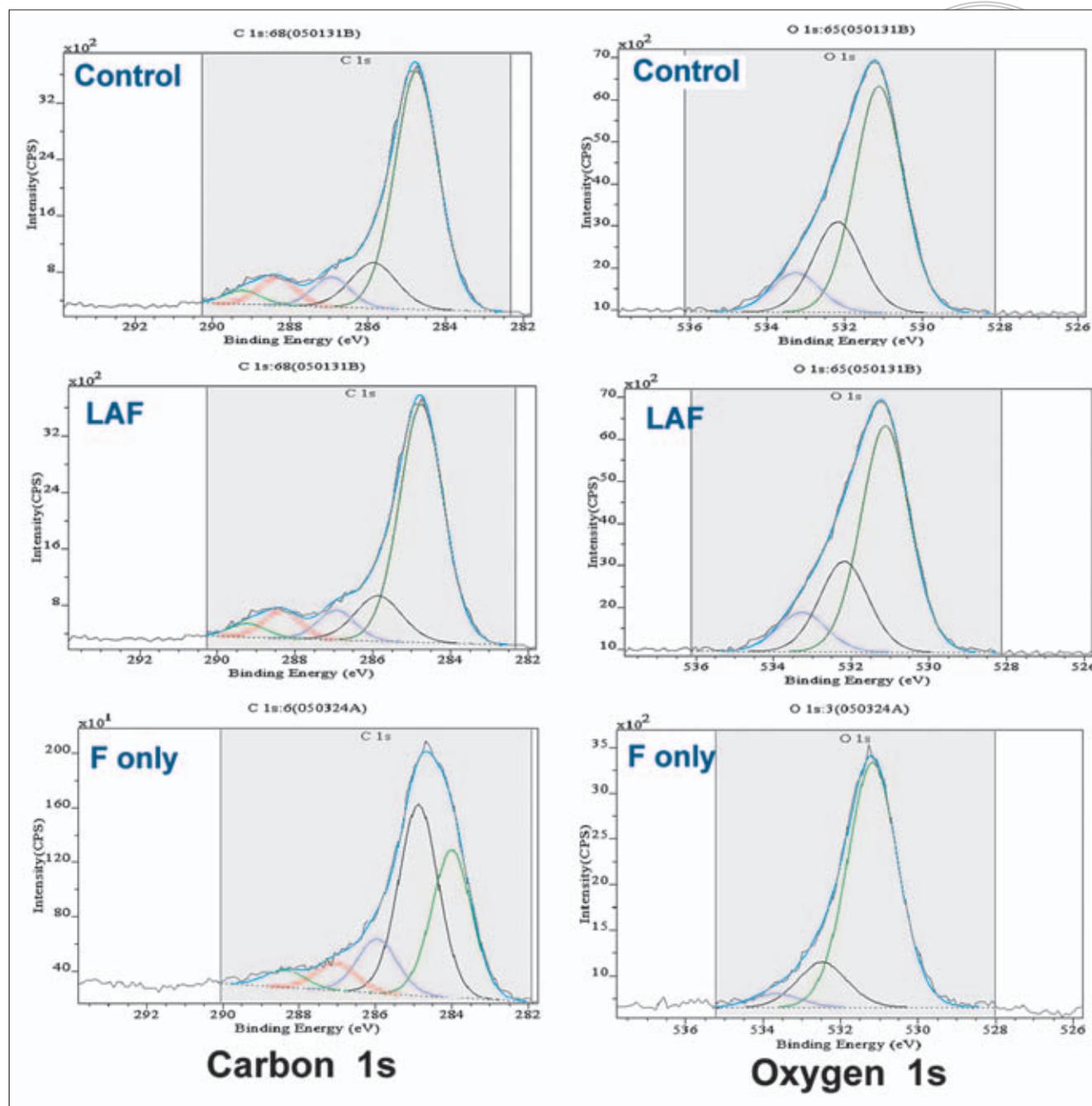
Fig 3 Summary of XPS data, with plots of binding energy (X axis) versus intensity (Y) for each of the elements of interest. The data curves (irregular line) were deconvoluted by software modelling to their constituent component curves. The fitted curve derived from the modelling is presented in blue, and aligns closely with the actual data curve for the element in question. The carbon data are on the left and the oxygen on the right for each of the three experimental groups.

PO_4^{2-}, on the basis of earlier work.[25] N 1s was detected at 399.6 eV, which is in agreement with the peak binding energy for nitrogen of the peptide bond (N-C=O) present in organic matter (collagen) of dentine.[24] The O 1s region was deconvoluted into three peaks with binding energies 531.1, 532.2, and 533.3 eV, relating to PO_4^{2-}, OH-, and C-O=C bonds, respectively.[26] The asymmetric C 1s region was deconvoluted to give 5 peaks. The binding energy positions of 284.8, 285.9, 287, 288.3, and 289.2 eV relate to hydrocar-
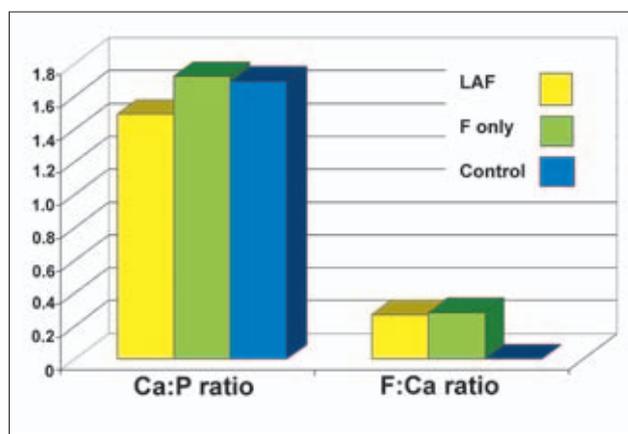
Fig 4 The calcium:phosphate (Ca 2p:P 2p) and fluoride:calcium (F 1s:Ca 2p) ratios in the three experimental groups, measured at a depth of 52 nm.

bons [C-H, C-C]; C-N, C-O-C, N-C=O, and O-C=O bonds, respectively.[26,28]

Dentine treated with LAF

Phosphorus (P 2p) was detected at 133.3 eV (PO_4^{2-}), nitrogen (N 1s) at 399.3 eV (the peptide bond of collagen), and fluorine (F 1s) at 684.6 eV. The O 1s region was deconvoluted to give 4 peaks with 530.2, 531.4, 532.7, and 533.8 eV binding energies relating to H_2O, PO_4^{2-}, OH-, and C-O-C=O, respectively (Fig 3, middle panels). The asymmetric C 1s region was deconvoluted to yield 4 peaks with binding energy positions at 284.4, 285.7, 287.1, and 288.6 eV, relating to C-C, C-H; C-N, C-F, and C-O-C=O bonds, respectively.

Dentine Treated with Topical Fluoride Gel Only

Phosphorus (P 2p), nitrogen (N 1s), and fluorine (F 1s) were detected at binding energies of 133.4, 399.3, and 684.5 eV, respectively. The O 1s region was deconvoluted into three peaks with binding energies 531.1, 532.5 and 533.7 eV relating to PO_4^{2-}, OH-, and C-O-C=O bonds, respectively.[25,26] The asymmetric C 1s region was deconvoluted to yield 5 peaks. The binding energy positions 284.0, 284.9, 286.0, 287.1, and 288.4 eV relate to hydrocarbons [C-H, C-C]; C-N, F-C, and N-C=O bonds, respectively. Hydrocarbons are often represented as two carbon atoms with slightly different binding energies,[23] as seen in this case, with the binding energies being 284.0 and 284.9 eV.

DISCUSSION

In order to consider fully the XPS results, the composition of tooth structure must be borne in mind. Enamel, by weight, is > 96% percent inorganic mineral, mostly hydroxyapatite ($Ca_{10}(PO_4)6(OH)_2$), while the remaining 4% is composed of organic matter, proteins such as enamelin, and water.[31] In contrast, by weight, dentine is 70% hydroxyapatite, 20% organic matter (primarily collagen), and 10% water. This difference in the overall percentage of organic and inorganic matter is reflected in the atomic concentration of elements, with higher carbon levels and lower oxygen, calcium, and phosphorous levels in dentine compared to enamel. In addition, while the presence of nitrogen in enamel is attributed to contamination,[25] its presence in dentine is due to organic matter (collagen).[24] Analysis of the wide scan data confirmed that the samples used were in fact dentine. This analysis also found a decrease in the concentration of carbon and an increase in the concentration of the oxygen and calcium with increasing depth. For the remaining measured elements, their concentration levels did not change significantly with depth.

Although studies have shown that LAF treatment increases the resistance of tooth structure to acid dissolution by organic acids present in caries and erosion, the exact mechanism of action is yet to be elucidated. The first consideration is what the passive effects of fluoride preparations may be, independent of the action of lasing. Previous studies have noted that treatment of tooth structure with fluoride preparations does not affect calcium concentrations, but can affect the phosphorous concentration,[32,33] the latter being largely dependant on the pH of the fluoride delivery vehicle. Acidulated fluoride causes a decrease in the phosphorous concentration, and a high concentration of fluorine at the surface with an F 1s:Ca 2p ratio of 2:1, indicating CaF_2 deposition on the surface.[32,33] The presence of CaF_2 is also indicated by detection of fluorine at a binding energy of 684.8 eV.[34,35] Treatment with stannous fluoride does not affect calcium or phosphorous concentrations.[32] In the present study, in the unlased fluoride control, the fluorine detected had peak binding energy positions of 684.5 eV. This binding energy is known to be associated with sodium fluoride,[35] a result which is expected in the current study since neutral sodium fluoride was the formulation applied to the samples. In fact, the very low fluorine to calcium ratio of 0.27 for fluoride-treated unlased dentine specifically excludes the presence of CaF_2.

Most importantly, in the present study, there is evidence of a dramatically different effect when neutral

sodium fluoride gel application is followed by lasing. There is evidence on several fronts that fluorapatite is present in the LAF samples. Firstly, there is an absence of fluorine at the binding energy of 684.8 eV, which is characteristic of CaF_2, and also at the binding energy of 684.5 eV, which is associated with unreacted sodium fluoride (Table 2). Secondly, there is a very low fluorine to calcium ratio of 0.28, which once again excludes the presence of CaF_2, but approximates that of fluorapatite. Thirdly, the fluorine binding energy of 684.6 eV seen in the LAF samples is known to correspond to fluorapatite.[25,36] Fourthly, the presence of fluorapatite in LAF-treated dentine is further confirmed by the high (11:1) ratio for oxygen (O 1s, as in PO_4) to fluorine (F 1s). Of note, the levels of the element fluorine (and hence fluorapatite), were found to be constant across the 52-nm depth range measured in the LAF samples.

Taken together, the above findings support the transformation of hydroxyapatite to fluorapatite following laser exposure of neutral sodium fluoride gel on dentine. The presence of fluorapatite within the tooth structure would reduce its solubility under acidic challenges, and increase its resistance to acid dissolution. In contrast, topical application of sodium fluoride gel to dentine, under identical conditions but in the absence of laser irradiation, did not result in transformation.

The fact that the samples used were normal dentine with normal compositions of mineral is important in terms of the application of these findings to everyday clinical practice. In examining the high resolution (narrow) scan data, the Ca 2p: P 2p ratio reflects the nature of the apatitic minerals present within tooth structure (Table 2). Theoretically, pure hydroxyapatite $[Ca_{10}(PO_4)6(OH)_2]$ will have a Ca 2p:P 2p ratio of 1.67. However, many studies have reported ratios ranging from 1.04 to 1.59.[28-20,37-39] This variation occurs because apatite mineral in teeth is not pure hydroxyapatite but a mixture of apatites, such as the anion carbonate, as well as various cations (sodium, chloride, magnesium, potassium, etc).[40] In the present study, the Ca 2p:P 2p ratios for untreated dentine, LAF-treated dentine, and fluoride-treated dentine, were all very close to the theoretical value for hydroxyapatite, and well within the range of values reported by other studies.[32,33,35]

CONCLUSION

This laboratory study indicates that the purported mechanism of LAF treatment, a "photonic conversion"

which results in transformation to fluorapatite, can in fact occur in human dentine. Moreover, this event is critically linked to the action of (laser) light, since it did not occur in the absence of laser irradiation under the conditions used in this study. The effect is not merely better bonding of fluoride in the outermost 1 nm of the surface, as has been suggested recently,[19] but rather a deeper effect which extends at least 50 nm into the surface of the dentine.

ACKNOWLEDGMENTS

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