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The durability of a hydroxyapatite paste used in decreasing the permeability of hypersensitive dentin

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1. Introduction

Dental problems and its related pain symptoms are mostly related to dental caries, however other dental lesions which are non-cariogenic in origin may lead to similar pain symptoms. These non-caries lesions may lead to loss of the enamel surfaces and exposure of the dentinal tubules to the oral cavity [1] causing pain sensation upon drinking hot or cold drinks. This phenomenon is referred to as "Dentin Hypersensitivity" [1–3]. Many theories were introduced to explain the phenomenon of the dentin hypersensitivity and its concomitant pain symptoms; one of the most accepted theories explaining the dentin hypersensitivity phenomenon is the "Hydrodynamic theory" [4,5]. This theory was strongly supported by many researches which demonstrated that [6,7] any partial reduction of the functional radii of the dentinal tubules will lead to significant reduction in the fluid flow with consequent reduction in dentin hypersensitivity pain symptoms [6–11].

Various aids and materials were employed for treating dentin hypersensitivity, however, the clinical success of these materials is still questionable [1,3]. Other attempts were used to melt some types of low fusing bioglasses on the dentinal surface using considerably low energy produced by CO_2 laser; these results showed that CO_2 laser if used with low output energy will not cause a significant increase of temperature on the pulpal side [12], however, no clinical trial was done to support the use of this technique inside patients' mouths for the treatment of dentin hypersensitivity. Moreover the dentin permeability was not measured after the application of the aforementioned technique.

A technique was recently introduced [11,13–15] during the past few years that included the temporary coverage of a bioactive glass paste by a layer of bonding agent for 24 h and then removal of this temporary bonding agent layer after 24 h. This technique showed plugging of the dentinal tubules by a calcium-phosphate rich layer [11] that was resistant to brushing abrasion challenge [8]. In the current study we used the same previously introduced technique but using the hydroxyapatite paste as a possible dentin desensitizing agent.

The aim of this experiment was to examine the capability of a hydroxyapatite paste to occlude dentinal tubules orifices by a layer of calcium phosphate compounds, moreover, the acid resistance of the formed layer was tested after the application of a low energy Nd:YAG laser.

The hypotheses adopted in this study was that the formed calcium phosphate layer will decrease dentin permeability and that application of Nd:YAG laser will render the formed layer more resistant to acidic-erosion.

2. Materials and methods

2.1. Dentin specimen preparation

40 extracted non-carious third molars were used following the guidelines approved by University Ethical Committee. The enamel on the buccal and the lingual surfaces of theses teeth were cut using a slow speed saw (Isomet, Buehler, Lake Bluff, IL, USA). The superficial dentin surfaces of teeth utilized in the current experiment were ground to obtain 80 dentin discs. All the dentin surfaces were ultrasonicated for 30 s, etched with 0.5% M EDTA (pH 4.7) for 2 min, and then rinsed with air/water for 30 s [8,16]. The specimens were

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Table 1
Summary for the Experimental groups.

Group IV	Group III	Group II	Group I	
+	+	–	–	Hydroxyapatite Application
+	–	+	–	Nd:YAG Laser Application

randomly distributed 4 groups. Specimens of group I served as controls. The Nd:YAG laser was applied on the dentin surfaces of group II. The hydroxyapatite paste was applied on the dentin surfaces in group III, while Nd:YAG laser and hydroxyapatite paste were applied in group IV Table 1, Fig. 1(A–D).

2.2. Hydroxyapatite paste application

One-tenth of a gram of hydroxyapatite powder (011-14882 Apatite HAP, Wako Chemical, Osaka, Japan.) was mixed on a glass slab for 1 min by spatula with 0.2 ml of 25 wt% phosphoric acid that was prepared by the dilution of 85 wt% phosphoric acid (Wako Chemicals, Osaka, Japan) in distilled water to form a paste (pH 3.3) (Table 2). The acidic paste was immediately applied to the dentin surfaces of groups III and IV specimens by microbrush (Microbrush International, Grafton, WI, USA). A layer of bonding agent (Clearfil SE Bond, Kuraray Medical, Tokyo, Japan) was immediately applied over the hydroxyapatite-phosphoric-acid paste and then light-cured (Table 2). After storage in de-ionized water for 24 h in an incubator at 37 °C, the thin layer of the bonding agent was gently removed by means of an excavator, and then rinsed with water spray for 30 s.

2.3. Nd:YAG Laser Application

The Laser system used in this study was Nd:YAG laser system (ULTRA Big Sky Quantel, USA), operating at wavelength in the NIR 1064 nm and delivers Q-switched pulses at of approximately 8 nanosecond durations with repetition rates of 1–20 Hz. and a maximum average power output of 600 mW. The laser was focused onto the dentin surfaces using a 7.5 cm focal length plano convex lens. The distance between the target area and laser beam focusing lens was 50 mm, and the diameter of the Nd:YAG laser beam at this distance was 1 mm. The laser pulse energy used was 30 mJ using a sweeping motion to ensure irradiation of all treated surfaces. The energy density/pulse was 5.1 J/cm^2.

2.4. Dentin permeability analysis

The dentin permeability of all groups ($n = 10$) was measured using an in vitro fluid-transport system before and after the acidic challenge. The system (Fig. 1A) was composed of a reservoir having phosphate-buffered saline (PBS, pH 7.0, Wako Pure Chemical, Osaka, Japan) solution which was placed between a tank of compressed nitrogen gas and a split-chamber device [17]. A pressure of 0.070 MPa [8,18] was used to force the PBS through polyethylene tubing to the split-chamber holding the dentin disk. A 25 µl micropipette connected to the polyethylene tubing was used to insert an air bubble into the tubing. The rate of air bubble movement was monitored using a millimeter ruler and the rate of the air bubble progression through the tubing was recorded every 2 min over a 6 min interval. The rate of dentin permeability was measured for each specimen at baseline and after application of the tested materials. Therefore each disk served as its own control [19]. The obtained values represented 100% permeability, which

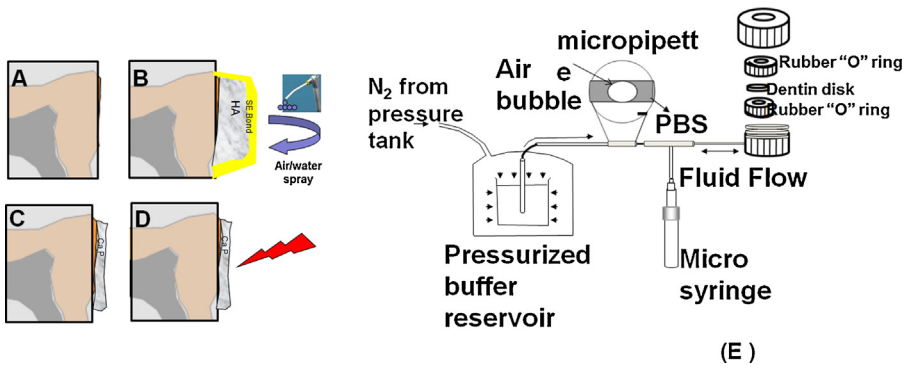

Fig. 1. Summary of procedures and dentin permeability measurement. (A) Obtaining of flat dentin surface and challenging exposed dentin surface by EDTA. (B) Application of hydroxyapatite paste on the exposed dentin surface followed by washing by strong air-water spray after 24 h. (C) The formation of a layer rich in calcium and phosphate. (D) The formed layer is irradiated by Nd:YAG laser (E) Scheme of the apparatus and the split-chamber device used to measure dentin permeability. The movement of the air bubble towards the split chamber represents the rate of fluid filtering across the dentin specimens. (B) Scheme of the erosive citric acid challenge using the magnetic stirrer.

Table 2
Materials used in this study.

Materials		Composition	Procedures
Hydroxyapatite powder (Wako chemicals, Osaka, Japan)	Weight Percent	45%SiO_2, 24.5%Na_2O, 24.4%CaO, 6%P_2O_5	Mix 0.1 gram of hydroxyapatite to 0.2 ml of phosphoric acid
Clearfil SE Bond (Kuraray,Osaka,Japan)	Primer:	MDP,HEMA, Water, PI, accelerators, CA.	Apply self-etching primer(20 s)
	Bond:	MDP, HEMA, MFM, PI, accelerators, CA. microfiller	Apply adhesive, gently air dry, light cure (10 s)

HEMA: 2-Hydroxyethyl methacrylate; MDP: 10-methacryloxydecyl; MFM: Multifunctional methacrylate; PI: Photoinitiator; CA: Catalyst.

represented the baseline permeability for the discs. After application of the tested materials in groups III and IV, the dentin permeability was re-assessed and the percent of dentin permeability reduction was calculated.

2.5. Erosion challenge

The specimens in all groups which had their permeability measured were exposed to 6% citric acid pH 2.1 for 1 min with continuous stirring by a magnetic stirrer at room temperature [20]. The permeability of all specimens was re-measured after the erosion challenge and the percentage of permeability reduction was calculated.

2.6. SEM-EDS surface examination for dentinal orifices closure

Five dentin specimens were selected before/after the acidic challenge in the four groups. All specimens were gradually dehydrated in an ascending ethanol series (50–100%), gold coated and the specimens' surface chemical characterization and morphological features were examined by SEM-EDS (JCM-6000 NeoScope, JEOL, Tokyo, Japan.).

2.7. Cryofracture examination

Five dentin specimens from each group were selected before/after the acidic challenge. A slit was made along the pulpal side of each dentin disk using an Isomet saw under water cooling to facilitate cryofracture of the disk into two halves. The specimens were gold-coated and examined along their fractured edges using SEM/EDS (JCM-6000 NeoScope, JEOL, Tokyo, Japan.).

2.8. FT-IR/ATR analysis

The Infrared spectra of the treated dentin surfaces for all groups were examined before/after the acidic challenge; moreover the layer formed on the dentin surface was gently scraped by a sharp scalpel according to the method described previously [21]. The FTIR spectra were obtained by a FT-IR spectrometer (Nicolet iS5 FT-IR, Thermo Electron Scientific Instruments LLC, Madison, WI USA) equipped with an ATR attachment. The dentin surfaces and the powdered specimens were pressed onto the face of a diamond of the ATR attachment [21]. The spectra were obtained under the following conditions: multiple reflections, 650–4000 cm^{-1} range, 4 cm^{-1} resolution, and entrance angle of 45° [22].

2.9. pH measurements of tested material

The Hydroxyapatite paste was manipulated as was described previously [15]. The hydroxyapatite paste was then placed in a mold of 4 mm diameter and 6 mm height [15,23]. The material was immediately placed in a falcon tube and 5 ml of deionised water was added to the tested material. pH measurements were recorded using a pH meter (Sartorius PB-11, Melsungen, Germany) at 0, 2, 30, 60 and 1440 min after placing the materials in the test tubes.23,24 Bakrycytoxicity). Five separate trials were conducted for the tested material and the means of results were recorded.

2.10. Statistical analysis

The Mann–Whitney test ($p < 0.05$) was used to compare the effects of using the different tested materials on the dentin permeability of all groups before/after the erosion challenge; whenever, there was a significant difference, so the Steel–Dwass test was applied ($p < 0.05$). Wilcoxon Signed-Rank was used to compare the effect of the erosion challenge on the permeability of the treated dentin surfaces ($p < 0.05$).

3. Results

3.1. Dentin permeability

The dentin permeability results are summarized in Fig. 2. The dentin permeability of group III showed significant increase in the dentin permeability after the acidic challenge, however specimens of groups I, II and IV did not show significant changes in dentin permeability after the acidic challenge ($p < 0.05$).

3.2. SEM-EDS top surface examination results

Groups I, II specimens showed patent dentinal tubules with complete absence of any smear layer before conducting the erosion challenge (Fig. 3A, E). Groups III and IV showed complete occlusion of the dentinal tubule orifices by crystal shaped structures (Fig. 3I, M). After conducting the erosion challenge, Groups I, II specimens still showed patent dentinal tubules (Fig. 3C), however, Group III showed few crystal like structures plugging the dentinal tubule orifices (Fig. 3K). Group IV showed the existence of the crystalline layer after the erosion challenge (Fig. 3 O). Chemical characterization by EDS showed that specimens of groups I, II had similar content of phosphorus and calcium Fig. 4. Groups III and IV specimens showed that the newly formed layer was rich in calcium and phosphate Fig. 4. Statistical analysis showed that there was no statistical significant difference for the Ca/P ratio EDS surface analysis before/after the erosion challenge for all groups $p \leq 0.05$.

3.3. Cryofracture examination

Before and after the erosion challenge specimens of groups I and II showed patent dentinal tubule orifices and the dentinal tubules were not obliterated by calcific deposits (Fig. 3B and F). The specimens of Groups III and IV before the erosion challenge showed plugging of the dentinal tubules with calcific deposits (Fig. 3J, N). After the erosion challenge specimens of group III

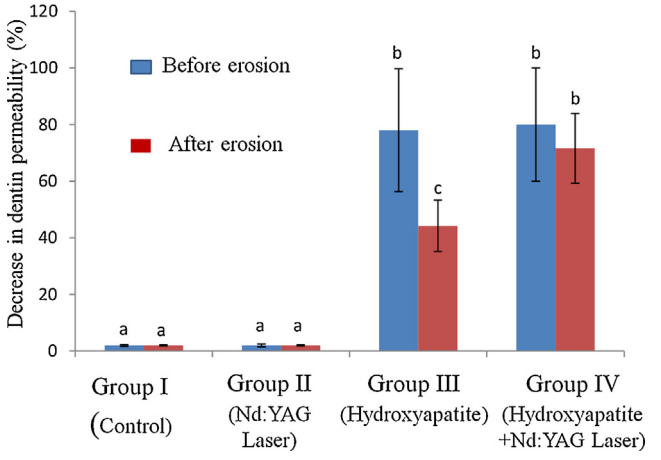

Fig. 2. Results of the dentin permeability test. Same letters are not statistically significant ($p \leq 0.05$).

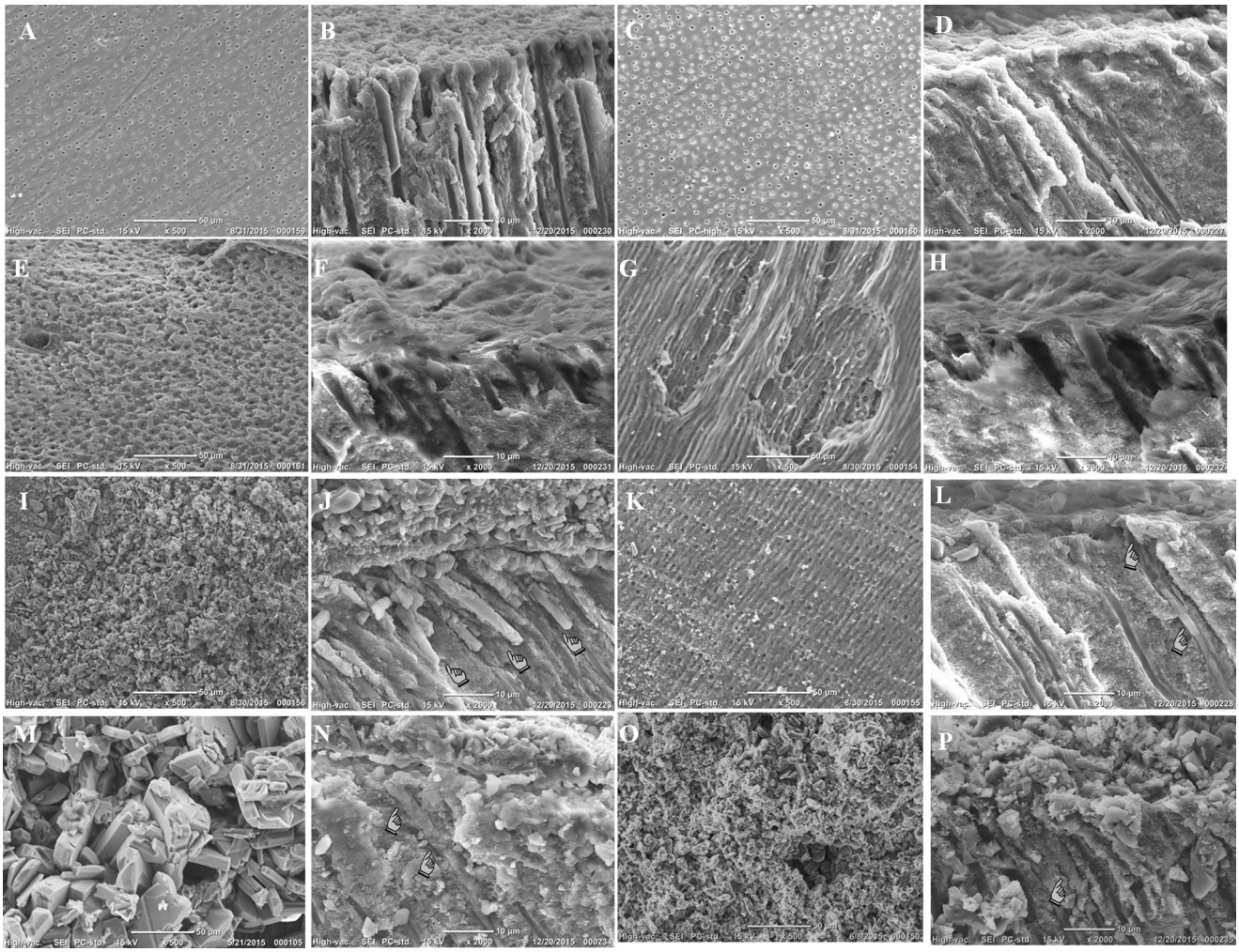

Fig. 3. SEM top surface examination and fractured surface examination. (A, B, E, F, I, J, M, N) represent the 4 groups specimens before erosion. (C, D, G, H, K, L, O, P) represent the 4 groups specimens after erosion. (A and B) Top and fractured surface views for Group I (control) before erosion showing no smear layer and no smear plugs. (C and D) Top surface and fractured surface views for Group I (control) after erosion showing no smear layer and no smear plugs. (E and F) Top and fractured surface views of Group II (Nd:YAG Laser) before erosion showing no cracks or melting of dentin surface with patent dentinal tubules orifice. (G and H) Top and fractured surface views of Group II (Nd:YAG) after erosion showing patent dentinal tubules. (I and J) Top and fractured surface views of Group III (Hydroxyapatite) before erosion showing crystalline structures obliterating the orifices of the dentinal tubules that extend within the dentinal tubules (Finger pointers). (K and L) Top and fractured surface views of Group III (Hydroxyapatite) after erosion showing patent dentinal tubules; however, some crystalline structures within the orifices and the lumens of the dentinal tubules (Finger pointer). (M and N) Top and fractured surface views of Group IV (Hydroxyapatite + Nd:YAG Laser) before erosion showing thick crystalline structures covering the whole dentin surface that extend within the dentinal tubules (white arrow). (O and P) Top and fractured surface views of Group IV (Hydroxyapatite + Nd:YAG laser) after erosion showing crystalline structures covering the whole dentin surface plugging the dentinal tubule orifices with long calcified plugs extending within the lumen of the dentinal-tubules.

showed the dissolution of most of the calcific deposits within the dentinal tubules (Fig. 3L) while, specimens of group IV showed resistance of the calcific deposits within the dentinal tubules to dissolution (Fig. 3P).

3.4. FT-IR/ATR analysis

The examined spectra of the dentin surfaces of groups I and II showed similar composition for normal dentin that was reported previously [21] which confirmed that the Nd:YAG parameters used in the current study was not enough to induce any detectable changes in the dentin composition Fig. 5. The FTIR spectra for the dentin specimens of groups III and IV showed that their surfaces were covered with calcium phosphate compounds giving spectral patterns similar to that of dicalcium phosphate dehydrate (Brushite) and hydroxyapatite [22] Fig. 5.

It is suggested that the bands observed for the layer formed on the dentin surface and that of the powder scraped from these surfaces at 1645 cm^{-1}, 793 cm^{-1} correspond to vibrational mode of PO_4 in hydroxyapatite and brushite. The bands at 1550 cm^{-1} and 873 cm^{-1} may be attributed to the bending mode of Carbonate group in brushite [22] Fig. 5.

3.5. pH measurements of tested material

The initial pH measurements of the hydroxyapatite paste were initially acidic *i.e.* 3.2, however there was a slightly steady increase in the pH along the period of observation till it reached 4 after 24 h.

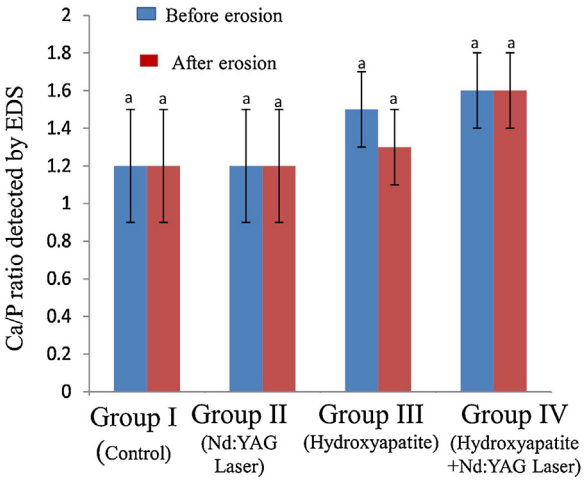

Fig. 4. Bar graph showing the atomic ratios of calcium to phosphorus for the top dentin surfaces of all groups detected by SEM-EDS. Data are represented by mean ± SD; Same letters indicates no statistical significance ($P < 0.05$.).

4. Discussion

The hypotheses adopted in this study were accepted. The key factors that judge the success of the dentin desensitizing agents may include their durability against brushing [8] and acidic challenges [20] after their application intraorally. The method adopted in the current showed the possibility of occluding the dentinal tubules by calcium phosphate deposits that were capable of significantly decreasing the permeability of dentin. Moreover, the resistance of the formed calcium phosphate rich layer to acidic challenge upon the application of Nd:YAG laser was confirmed.

The nature of the layer formed upon mixing the hydroxyapatite paste on the dentin surface was detected by the SEM/EDS which showed that it was rich in calcium and phosphate compounds. FTIR/ATR analysis showed that there is a strong evidence that brushite compounds were formed on top of dentin. Examination of the formed calcium phosphate layer by x-ray diffraction technique is needed to confirm the formation of brushite crystals.

It is suggested that the mechanism of calcium-phosphate layer formation onto the dentin surface using the technique adopted in the current experiment may be as follows; The hydroxyapatite powder upon being mixed with phosphoric acid leached high amounts of calcium and phosphate ions that were protected from being washed away or diluted in water by the temporary coverage of the bonding agent [8,11,14]. It is expected that the acidic paste mobilized some of the calcium and phosphate compounds from the underlying dentin to combine with the products of the hydroxyapatite paste leading to the rapid increase of its pH. These acidic calcium phosphate salts precipitated onto the dentin surface with the smaller crystals penetrating the dentinal tubules.

The pH of the hydroxyapatite paste was detected to examine the safety of applying the hydroxyapatite paste on the hypersensitive dentin surfaces. The pH of the paste was 3.2 and it increased rapidly to be 4 after 2 min. Previous report [15] showed that commercially available dentin desensitizing agent had a pH value of 2 even after 24 h of storage [15]. It should be emphasized that the pH of hydroxyapatite paste was measured in the current study while it was not in contact with the surface of dentin and thus it may be suggested that its pH will increase rapidly when it will be in contact with dentin, due to the excellent buffering capacity of dentin [15,24].

The use of Nd:YAG laser with the low output energy adopted in this study did not cause any detectable chemical damage to the dentin surface when examined by the FTIR/ATR or SEM examinations, moreover, the low energy of Nd:YAG laser used in the current experiment was not enough to cause any melting of the superficial dentin surface as detected by SEM and thus no significant decrease in dentin permeability was noticed for the Nd:YAG lased dentin.

The formed calcium phosphate layer showed complete coverage of the dentin surface and caused significant decrease in the permeability of dentin in group III, however, upon challenging this layer by the acidic challenge a significant increase in dentin permeability was recorded, which may be attributed to the storage conditions adopted in this experiment which was in deionized water for 24 h. It is suggested that storing the samples of the current experiment for longer period in saliva may lead to the transformation of brushite layer to the apatite insoluble form as was demonstrated previously [14]. Moreover, it is strongly suggested that it is of prime importance to improve the oral hygiene of patients treated by the current technique and advice the patients to avoid erosive drinks especially following the application of the hydroxyapatite paste.

It is suggested that Nd:YAG laser irradiation to the calcium phosphate rich layer formed onto the dentin surface in group IV improved the physical properties of this layer and improved its resistance to erosion due to the evaporation of the interstitial water molecules of this layer. Previous research showed that irradiating a calcium phosphate rich layer formed onto dentin surface by CO_2 laser [11] evaporated the interstitial water molecules of this layer [25] and improved its physical properties[11].

The use of the white hydroxyapatite paste on the hypersensitive dentin resembles the application of temporary material in conservative dentistry and thus it is expected that there will be no aesthetic problems associated with this technique. In the current experiment the formed calcium phosphate layer was not covered by any artificial pellicle layer which is expected to be formed inside the patients' oral cavities [26] and thus it may be speculated that the resistance of the calcium phosphate rich layer formed by the aforementioned technique will exert high resistance to erosion challenge if clinically applied on hypersensitive dentin areas; however, studies are needed to determine the patients' acceptance for the current technique.

5. Conclusion

Within the limitation of this *in-vitro* study it may be concluded that the application of hydroxyapatite paste to dentin was able to occlude patent dentinal tubule orifices with a layer of calcium phosphate crystal like structures that was capable of decreasing the dentin permeability. The application of Nd:YAG laser on the formed calcium-phosphate layer enabled the aforementioned layer to resist erosion.

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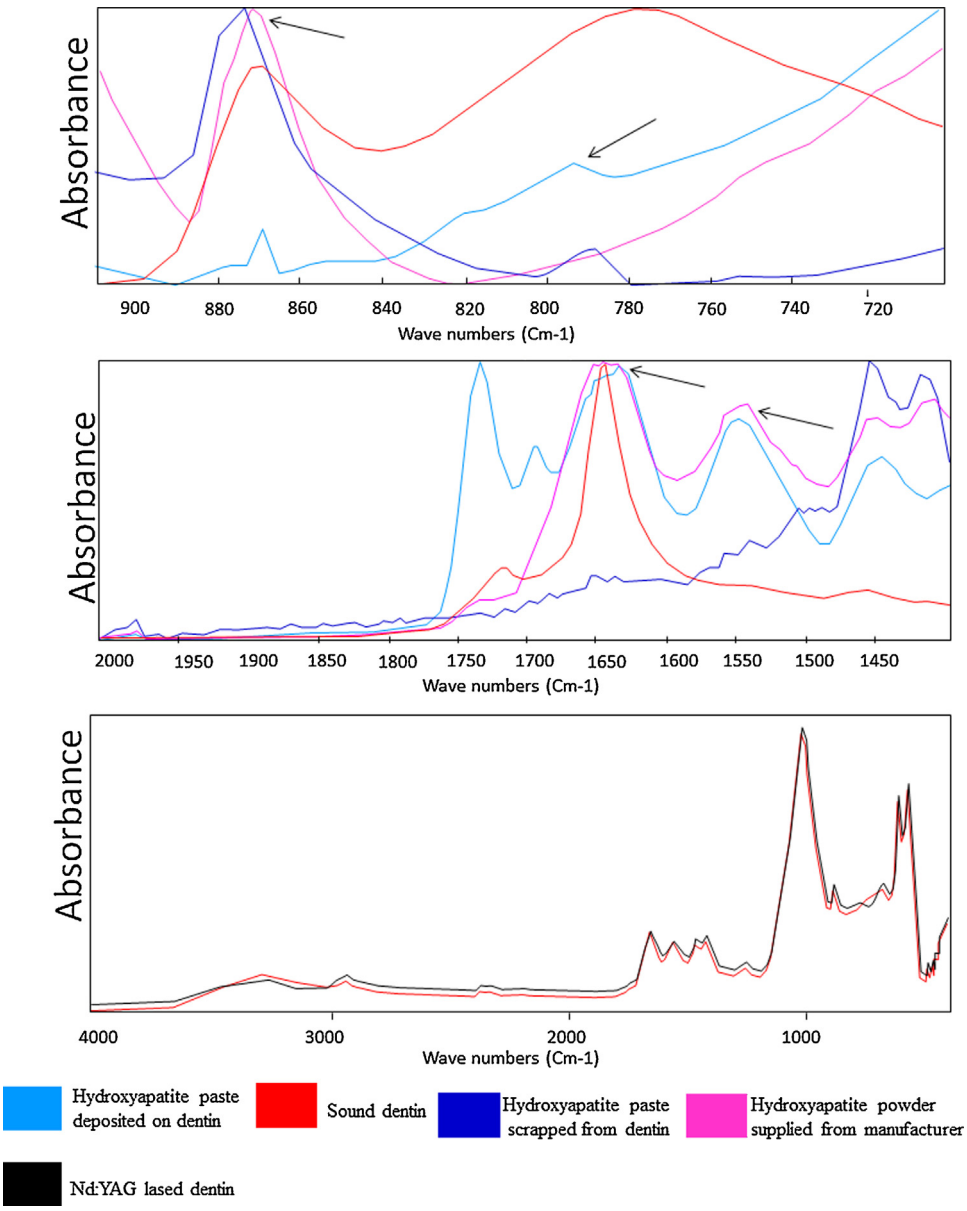

Fig. 5. FTIR-ATR spectra of the layer formed on dentin surface after the application of hydroxyapatite paste. Arrows pointing to bands at 1645 cm^{-1}, 793 cm^{-1} correspond to vibrational mode of PO_4 in hydroxyapatite and brushite. Arrows pointing to bands at 1550 cm^{-1} and 873 cm^{-1} attributed to the bending mode of Carbonate group in brushite. The spectra of the sound dentin surface and the hydroxyapatite powder supplied from the manufacturer are supplied as a reference.

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