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DOES HYDROXYAPATITE NANOPARTICLES' ADDITION IMPROVE ENAMEL ESTHETICS AFTER BRACKETS' DEBONDING?

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ABSTRACT:

Objectives: *To evaluate the effect of hydroxyapatite nanoparticles' addition and determine the utility role of CO2 laser in improving esthetics of enamel surfaces after debonding.* ***Methods:*** *90 extracted human premolars were used. 10 teeth were used as a control group. Trans bond Plus Self Etching Primer with Trans bond Supreme Low Viscosity Light Cure Adhesive, 3M/Unitek, was used to bond the orthodontic brackets (NU-EDGE.022" ROTH RX.TP Orthodontics) to the labial enamel surfaces. All specimens were stored in artificial saliva for 6 months. Orthodontic brackets were then removed using bracket removing pliers (Ortho Organizers, Carlsbad, USA).Residual composite removed by Sof-Lex finishing discs. The 80 teeth were divided randomly into 4 groups (n=20) according to the protocol of nanoparticles' addition, 1) no nanoparticles were added, 2) nanoparticles suspended in water and rubbed on the dry enamel surface, 3) nanoparticles suspended in ethanol and rubbed on the etched enamel surface, and 4) nanoparticles suspended in bonding agent [Single Bond 2 adhesive (SB)] & rubbed on the etched enamel surface. Each group assigned into two subgroups (n=10) according to exposure to CO2 laser (9.6μm), where enamel of the first subgroup was irradiated at 3W; 20Hz; 4sec and*

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212J/cm2 while the other subgroup was not irradiated. The SE bonding agent was applied to the third group after its treatment. Specimens were tested for color changes using Quanta Environmental Scanning Electron Microscope. Data were statistically analyzed using two-way Analysis of Variance (ANOVA) and Tukey's post-hoc test. ***Results:*** *highest mean color value was found with no particles. There was no significant difference between group 1 and 2. There was no difference between groups 3 and 4; both showed the statistically significant lowest mean color values. Meanwhile, no significant effect was found between laser and non-laser subgroups.* ***Conclusions:*** *the addition of hydroxyapatite nanoparticles improved the enamel esthetics after brackets debonding.*

INTRODUCTION

For many years, orthodontic practice has been counting reliably on direct bonding of brackets, where a strong bond is created between the bonding materials and enamel, but debonding of the brackets and improper finishing and polishing at the end of treatment have the potential to cause iatrogenic enamel damage[1], excessive plaque accumulation, gingival irritation and increased surface staining.[2, 3]

Dental enamel, the hardest and most mineralized tissue in the human body, makes up the outer layer of tooth crowns. Although mature enamel contains 95 wt% carbonated apatite and less than 1–2 % organic material and water, the forming early secretary enamel consists of approximately 30 wt% mineral, 20 wt% organic matrix (protein), and 50 wt% water.[4, 5] The possibility of demineralization of the enamel at the labial surfaces of teeth during and after orthodontic therapy, constitute a major problem in clinical dentistry. It was reported that, enamel demineralization developed in nearly 50% of orthodontic patients and progressed as rapidly as only 4 weeks which is the usual time between orthodontic visits forming the enamel white spot lesions. Overall management of white spot lesions involves consideration of methods of preventing demineralization and also methods of encouraging re-mineralization of existing lesions by using topical fluoride application. This non-operative approach is often successful to arrest lesion progression. However, especially deep lesions tend to re-mineralize only superficially. Consequently, the whitish appearance often persists.[6, 7]

Obtaining good occlusion after the conclusion of any orthodontic case is desired by both the operator and the patient. However, if a damaged enamel surface layer in structure or appearance is produced after removal of bonded orthodontic appliance, it would be of great devastation no matter how perfect the occlusion has become. Previous studies used different methods for cleaning the residual resin after removal of orthodontic attachments.[8,9] One-step polishing systems, including diamond impregnated rubber cups, points and silicon carbide brushes, as well as So flex finishing discs were introduced for finishing and polishing resin composites to a smooth and glossy appearance.[10,11] Information about the effect of different systems that would return the enamel surface as closely as possible to its original state after removal of orthodontic brackets seems to be limited in the literature. The search for the ideal method that returns the enamel surface as closely as possible to its original state is still ongoing.[12]

Nano-hydroxyapatite (Nano-HAP) is considered one of the most biocompatible and bioactive materials. It is widely applied in medicine and dentistry as a bone substitute and for tooth remineralization.[13,14] Evidence has demonstrated that nano-sized particles have similar morphology and crystal structure compared with dental apatite. Recent reports have shown that Nano-HAP has a good potential to remineralize enamel carious lesions,[15,16] but limited information is available regarding the prevention of demineralization and on dentin lesions regression as well. Alternatively, products containing other calcium phosphate salts, with a similar acting mechanism have also been tested. Pastes containing a complex of casein phosphopeptide (CPP) and amorphous calcium phosphate (ACP), with or without fluoride, have shown potential to prevent dental demineralization, increase remineralization in vitro,[17,18] and to repair initial enamel caries lesions in vivo as well.[19,20] It has been suggested that casein phosphopeptides (CPP) have the ability to stabilize calcium phosphate in solution by binding amorphous calcium phosphate (ACP) with their multiple phosphoserine residues, thereby allowing the formation of small CPP-ACP clusters.[21] The use of lasers in the medical field is very old. In dentistry, only during the last two decades have commercially available lasers been used as adjunctive in delivering tissue management conducive to achieving hard or soft tissue procedures. Laser may offer an opportunity to deliver hard and soft tissue treatment that could attempt to make the patient experience somewhat easier than conventional methods. Several studies have shown the possible applications of different types of lasers in dentistry clinics, as for example, in the inhibition of carious lesions, [22] as well as in their removal,[23] in the oral mucous[24] and in gum healing[25].

The scanning electron microscope has shown the different morphological alterations in the enamel and dentin surfaces irradiated with the CO2 laser. The CO2 laser is a gas-active medium laser that is delivered through a hollow tube-like wave guide in continuous or pulse gated mode. The laser has a wavelength of 10,600 nm and is well absorbed by water. It is a rapid soft tissue remover and is especially useful in cutting dense fibrous tissue. It has the highest absorption in hydroxyapatite than any dental laser. It is useful in orthodontics for bracket debonding procedures.[26, 27]

Considering the lack of studies testing the effect of Nano-HAP on the prevention of dental caries lesions and on dentin substrates, the research hypothesis is that experimental Nano-HAP pastes have a better esthetic effect compared with commercial pastes, and both are more effective than the control and placebo paste groups on dental demineralization in vitro.

The purposes of this study were:

To evaluate the effect of hydroxyapatite nanoparticles addition on the esthetics of enamel surfaces.

To determine the utility of CO2 laser in improving esthetics of enamel surfaces after debonding.

METHODS

Ninety human freshly extracted caries-free human premolars were used in the study. Extracted teeth were washed under running water and were stored at 4°C in phosphate-buffered saline containing 0.002%

sodium azide to prevent microbial growth and were used within 1 week after extraction. 10 teeth were used as a control group as no brackets were bonded to this group. Transbond Plus Self Etching Primer with Transbond Supreme Low Viscosity Light Cure Adhesive (3M/Unitek) (table1), were used to bond the orthodontic brackets (NU-EDGE.022" ROTH RX.TP Orthodontics) to the labial enamel surfaces. All specimens were stored in artificial saliva (table 1) for 6 months. The apices were embedded in plaster to facilitate identification and manipulation. Orthodontic brackets were then removed using bracket removing pliers (Ortho Organizers, Carlsbad, USA). Brackets were debonded by hand rather than using a testing machine in shear or tension to simulate clinical procedures. Bond strength was not investigated, as multiple laboratory studies had previously found clinically adequate bond strengths (6–8 MPa or greater). Residual composite removed by Sof-Lex finishing discs(3M, Unitek), where a low speed hand piece was used, rotating at 20,000 RPM with 2.4 atomic air pressure, mounted to a special device to ensure standardization of refinishing pressure, direction and rate to which the samples were subjected. Finishing procedure was performed until the debonded enamel surface appeared smooth to the naked eye.

Then, the 80 teeth were divided into 4 groups (n=20) according to the used protocol of nanoparticles' addition;

1- No nanoparticles were added,

2- Nanoparticles suspended in water and rubbed on the dry enamel surface,

3- Nanoparticles suspended in ethanol and rubbed on the etched enamel surface, and

4- Nanoparticles suspended in bonding agent [Single Bond 2 adhesive (SB)] (table 1) & rubbed on the etched enamel surface.

In group 2, 2 wt% HA-nanoparticles suspended in water were rubbed on the etched surface for 10 sec and excess water was blot- dried, leaving the surface shiny without pooling of water. In group 3, 2 wt% HA-nanoparticles suspended in absolute ethanol were rubbed for 10 sec to the etched surface and excess ethanol was evaporated with a gentle air stream for 10 sec. Whereas, in group 4, the bonding agent with added 2 wt% HA-nanoparticles was applied to the etching enamel surface. Etching of the enamel surfaces in groups 3 and 4 was performed using 35% phosphoric acid (Scotchbond™ Etchant).

Synthesis of HA-nanoparticles:

HA-nanoparticles (table 1) were synthesized by the biomimetic precipitation method (HAn1) by dissolving 0.555 g of calcium chloride (CaCl2), 0.150g of sodium dihydrogen phosphate (NaH2PO4) and 0.073g of sodium bicarbonate (NaHCO3) in 500ml of distilled water. The solution was stirred at 80 rpm at 37˚C for 24h. Then, the precipitate was washed with deionized water and dried in an oven at 110˚C for 2h.[28] the nanoparticles were close to spherical in shape, with an average diameter of 27±5nm. Physical, chemical and morphologic characterization of the synthesized powder were analyzed using Fourrier transform infrared spectroscopy, X-ray powder diffraction and transmission electron microscopy to ensure the elemental composition and high degree of crystallinity of HA-nanoparticles. [28]

HA-nanoparticles were added to distilled water, absolute ethanol or SB at a concentration of 2 wt%[29] and were homogenized using a powerful small digital probe sonicator with 70 W power (Sonoplus HD 2070, Bandelin, Germany) for 2 min. The colloidal stability measurement was performed for 60 h using visible light with 100% intensity. It was also investigated indirectly, based on the knowledge of zeta potential which is the electrical charge of particle surface, using an electrophoretic technique to measure electrophoretic mobility of the dispersed particles, the measurements were converted to zeta potential values using the Helmholtz-Smoluchowski method.[30, 31]

After application of the nanoparticles, each group was assigned into two subgroups (n=10) according to exposure to CO2 laser (9.6µm) [DEKA UltraSpeedTM CO2 (Smart US20 D) Calenzano, FI-Italia] (Fig.1), where debonded enamel was irradiated at 2W; 20Hz; 4sec and 1-1.5J/cm2, or enamel was not irradiated.

Specimens were tested for quantitative color changes using Quanta Environmental Scanning Electron Microscope (QESEM) (Fig.2), and specific computer software. By using XT Document program, the scanning photomicrograph taken by the QESEM (Figure 3, a) was converted to have Quantitative computerized image analysis using digital scanner with a special computer program. This program divides the surface of all specimens image on computer monitor into points (Fig.3, b). Each point has a pixel value at the two coordinates (x, y) as shown in the excel sheet. From the data from the two coordinates, the gray value for each point was calculated.

Figure 1 DEKA UltraSpeed™ CO_2 (Smart US20 D.

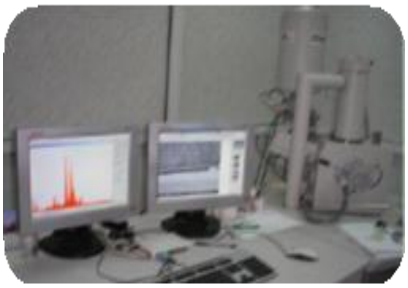

Figure 2 Quanta Environmental Scanning Electron Microscope (QESEM) computer software.

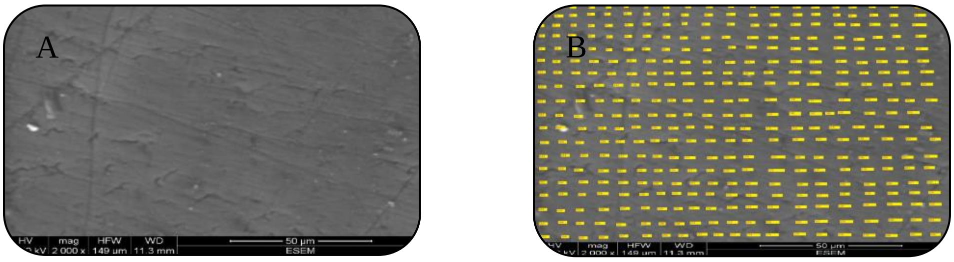

Figure 3: (a) Scanning photomicrograph-representing group 4 that subjected to CO_2 laser. **(b)** Image analysis divided the surface into points.

Table (1): Compositions and manufacturers of the used materials

Material	Main constituents	Manufacturer
Transbond Plus Self Etching Primer (An all-in-one bonding solution)	Propenoic acid, 2-methyl-, phosphinicobis (oxy-2,1-ethandiyl) ester, water, Mono HEMA Phosphate, Tris [2- (methacryloyloxy) ethyl] phosphate, Dl camphor Quinone, N, n-dimethyl benzocaine, Dipotassium hexafluorotitanate.	3M Unitek, USA
Transbond Supreme LV Low Viscosity Light Cure Adhesive (flowable orthodontic adhesive)	The resin consists of the dimethacrylate monomers Bis-GMA, TEGDMA, and Bis-EMA. contains a dimethacrylate Polymer. The fillers (65% by weight) are a combination of agglomerated and non-agglomerated nanoparticles, composed of the following: • 75 nm diameter non-agglomerated/non-aggregated silica Nano filler • 5-10 nm diameter non agglomerated/non-aggregated zirconia Nano filler • Loosely bound agglomerated zirconia/silica nanocluster, consisting of agglomerates of 5-20 nm primary zirconia/silica particles. The cluster particle size range is 0.6 to 1.4 microns.	3M Unitek, USA
Adper Single Bond 2 [SB] (Total etch, visible-light activated dental bonding agent)	10% by weight of 5nm diameter spherical silica filler, BisGMA, HEMA, dimethacrylates, ethanol, water, a novel photo initiator system and a methacrylate functional copolymer of polyacrylic and polyitaconic acids	3M/ESPE, St. Paul, MN, USA.
HA-nanoparticles	hydroxyapatite nanoparticles	Nanotech Egypt Co, (Cairo, Egypt)
Absolute ethanol ≥99.8%	Ethanol	Sigma-Aldrich, GC
artificial saliva	The solution contained (mmol/L): 1.5 mM Ca2+, 0.9 mM phosphate, 150mM KCI and 20 mM TRIS buffer at pH=7	Nanotech Egypt Co, (Cairo, Egypt)

STATISTICAL ANALYSIS

Data were presented as mean and standard deviation (SD) values. Regression model using two-way Analysis of Variance (ANOVA) was used in testing significance for the effect of protocol of nanoparticle addition, laser application and their interactions on color values. Tukey's post-hoc test was used for pair-wise comparison between the mean values when ANOVA test is significant. The significance level was set at $P \leq 0.05$. Statistical analysis was performed with IBM® SPSS® Statistics Version 20 for Windows.

RESULTS

Two-way ANOVA results (Table 2):

The results showed that the protocol of addition and the interaction between the two variables had a statistically significant effect on the mean color value (P-value = 0.003 and 0.043, respectively).

Laser had no statistically significant effect on the mean color value (P-value = 0.075).

The observed power of the study was high (Over 0.8) for all the variables, denoting that the used sample size was accurate.

Table (2): Results of two-way ANOVA for the effect of protocol of nanoparticle addition, Laser application and their interactions on color values

Source of variation	Sum of Squares	df	Mean Square	*F*-value	*P*-value	Observer power
Protocol of addition	1538.9	2	769.4	10.4	0.003*	0.957
Laser	378.6	1	378.6	4.2	0.075	0.834
Protocol x Laser	858.6	2	429.3	4.8	0.043*	0.918

*df: degrees of freedom (n-1), *: Significant at $P \leq 0.05$*

Effect of protocol of addition (Table 3 and Fig. 4):

The mean and standard deviation values of color values were 133± 1.5, 150 ± 2.3, 135.5 ± 17.6, 122.9 ± 8.2 and 120 ± 6.3 for control, no nanoparticles, nanoparticles dissolved in water, nanoparticles dissolved in ethanol and nanoparticles dissolved in bonding agent,

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® SPSS, Inc., an IBM Company.

respectively. The statistically significantly highest mean color value was found when no nanoparticles were added. There was no difference between control and nanoparticles dissolved in water groups; both showed lower values. There was no difference between nanoparticles dissolved in ethanol and nanoparticles dissolved in bonding agent groups; both showed the statistically significant lowest mean color values.

Table (3): The mean, standard deviation (SD) values of the color change values and results of comparison between protocols of addition regardless of Laser.

Control		**No nanoparticles**		**Nanoparticles in water**		**Nanoparticles in ethanol**		**Nanoparticles in bonding agent**		***P*-value**
Mean	**SD**	**Mean**	**SD**	**Mean**	**SD**	**Mean**	**SD**	**Mean**	**SD**	
133 [b]	1.5	150 [a]	2.3	135.5 [b]	17.6	122.9 [c]	8.2	120 [c]	6.3	0.003*

**: Significant at P ≤ 0.05, Different letters are statistically significantly different*

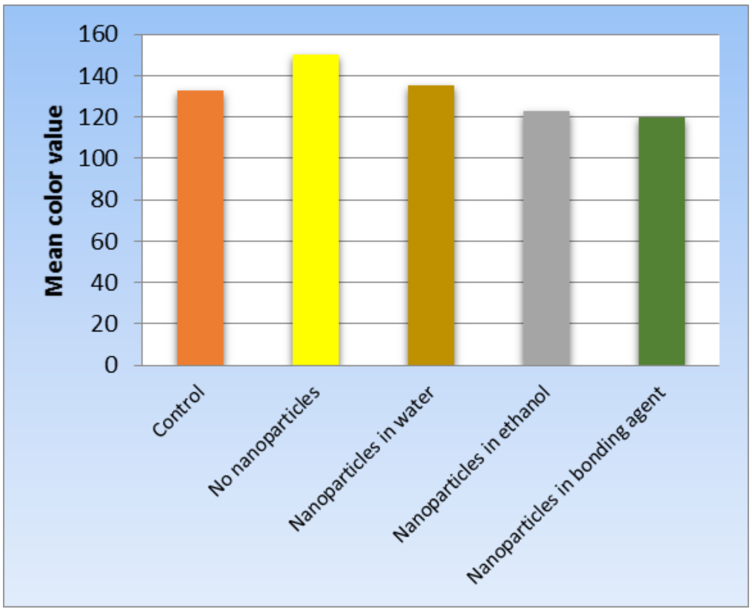

Figure (4): Bar chart representing mean color values with different protocols

Effect of Laser (Table 4 and Fig. 5):

The mean and stan dard deviation values of color values were 120.5 ± 4.4 and 131.7 ± 16.1 with and without Laser, respectively.

There was no statistically significant difference between color values with and without Laser application.

Table (4): The mean, standard deviation (SD) values of color change values and results of comparison between Laser applications regardless of protocol

Laser		No Laser		*P*-value
Mean	SD	Mean	SD	
120.5	4.4	131.7	16.1	0.075

**: Significant at P ≤ 0.05*

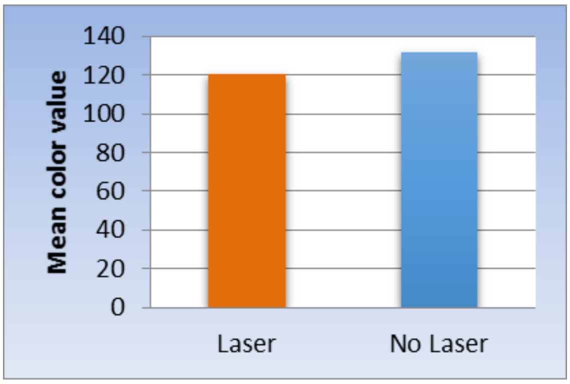

Figure (5): Bar chart representing mean color values with and without Laser

Comparison between different variables interactions (Table 5 and Fig. 6):

There was no statistically significant difference between (No particles with and without laser) and (Nanoparticles in water with no Laser); both showed the statistically significant highest mean color values.

There was no statistically significant difference between (Control), (Nanoparticles in water with Laser), (Nanoparticles in ethanol with no Laser) and (Nanoparticles in bonding agent with Laser); all showed lower mean color values. There was no statistically significant difference between (Nanoparticles in ethanol with Laser) and (Nanoparticles in bonding agent with no Laser); both showed the statistically significant lowest mean color values.

Table (5): The mean, standard deviation (SD) values and results of comparison between the different interactions

Protocol x Laser	**Mean**	**SD**	***P*-value**
Control	133 [b]	1.5	0.043*
No nanoparticles x Laser	150 [a]	2.3	
No nanoparticles x No Laser	150.2 [a]	2.3	
Nanoparticles in water x Laser	120.3 [b]	2.1	
Nanoparticles in water x No Laser	150.7 [a]	1.8	
Nanoparticles in ethanol x Laser	115.9 [c]	3.1	
Nanoparticles in ethanol x No Laser	129.9 [b]	2.8	
Nanoparticles in bonding agent x Laser	125.4 [b]	3	
Nanoparticles in bonding agent x No Laser	114.7 [c]	2.8	

*: *Significant at $P \leq 0.05$, Different letters are statistically significantly different*

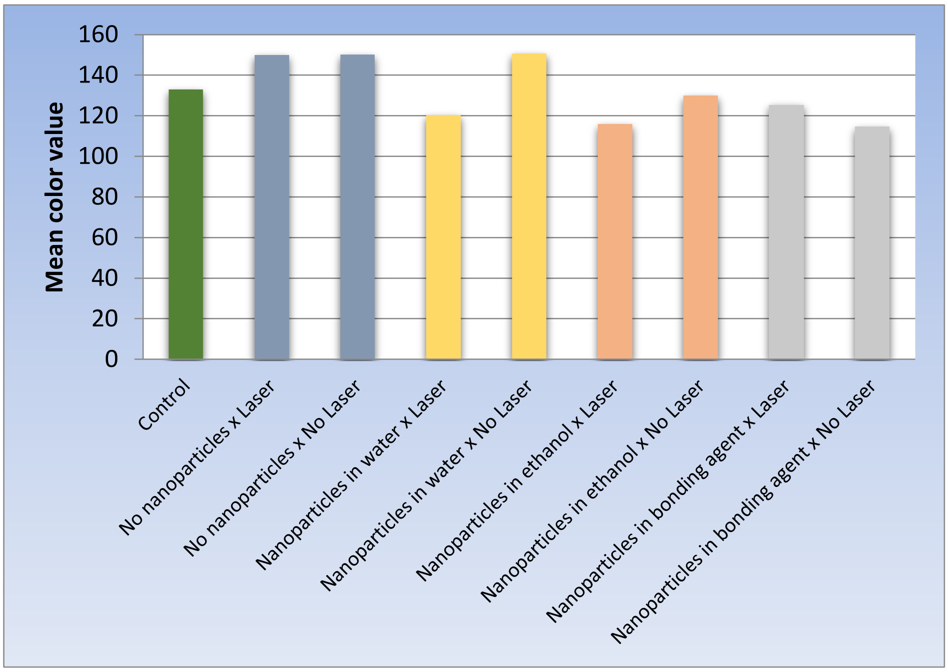

Figure (6): Bar chart representing mean color values with different variables interactions

DISCUSSION

To evaluate the complex surface structure of enamel, visualization at the submicron levels is essential. Scanning electron microscope (SEM) was used in this study to examine the changes on enamel surface after debracketing of orthodontic brackets using different interactive methods. Although SEM provides information that has its limitations,[1] the effectiveness of various methods and instruments on the topography and morphology of the tooth surfaces are best examined under SEM.[12]

The present study analyzed the effect of a new product on the prevention of dental demineralization that in turn leads to color differentiation upon analyzing its effect. Currently, there is lack of knowledge about the remineralizing and desensitizing potential of biomaterials based on nanotechnology, which, in turn, may be an important strategy to be applied in orthodontic patients utilizing bonded brackets or even high-caries risk patients.

It is important to clarify that the design of the present study was not focused on showing the impact of the experimental suspensions on the remineralization of pre-formed enamel, but rather on the prevention of demineralization shown in the form of less enamel color changes examined after debonding. It was expected that the nanoparticles, which presents hydroxyapatite particles with characteristics similar to the dental apatite, would have the ability to provide calcium and phosphate in an adequate concentration and velocity to react with the dental structure[6, 7], repairing or promoting the dental crystal growth.[32] The results showed that protocol of addition of nanoparticles to debonded enamel surfaces regardless of the method of addition had significant effect on mean color value table, indicating the beneficial effect of such addition on the improvement of changes in color (Table 2). Other studies investigated the Impact of Experimental Nano-HAP Pastes on Bovine Enamel and Dentin supported the current study results.[33]

Irradiation of dental hard tissue with lasers of sufficient power leads to a variety of structural and ultra-structural changes of the tissue near the surface. These changes depend on such irradiation parameters as wave length, pulse duration, pulse energy, number of pulses, repetition rate, and beam spot size.[24, 25] Co2 laser used in this study was known to have the highest absorption in hydroxyapatite than any dental laser, that is why it has been chosen among other known types as YSGG and Er: YAG laser, [34] in an attempt to enhance the remineralization effect of Nanoparticles. Though laser technique appears promising regarding finishing of enamel surface after debonding of orthodontic brackets, regarding this study, Laser had no statistically significant effect on mean color value (P-value = 0.075) (Table 4) on one hand, and on another hand, there was no statistically significant difference between color values with and without Laser application regardless whether the protocol for the addition of nanoparticles were used or not (Fig. 5, 6).

Another important aspect is that the experimental nanoparticles do not come handy as commercial preformed materials readily contained within bonding materials, dentifrices or even a solely pre- bonding gel or paste, but rather a crude form that it would be as confusing as first discovering fire and not knowing exactly what you do with it. Therefore, in the present study, nanoparticles manipulation took all different potentially possible protocols to decide which one can squeeze as much as possible every benefit out of the Nano-Hydroxy Apetite Technology, in an attempt to come out with the most beneficial easiest manipulation procedure possible.

In this study, different protocols of nanoparticles' (NP) addition, with or without laser incorporation, reacted differently regarding how close to the mean color values of the control group. Looking at the values found regarding the group of no NP added, the most significant color mean value was found in comparison with controls (Fig. 6). Leaving no doubt that NP addition works well regarding color preservation or even improvement of the color (esthetic) and hence remineralization or even diminishing demineralization process of enamel. But the question to be asked would be, among the given addition protocols, which one is the best within the limitations of this study? Results have shown that the closest protocol to control group would be the rubbing of NP suspended in water on dry enamel and incorporation of NP within the bonding agent (SB), however, the addition of laser was a must for not getting devastating results far from controls. On the other hand, a still good match to control group was observed in the group where NP were suspended in ethanol and rubbed to etched enamel surface without laser exposure (Fig. 6). By looking at the above remarks, it could be drawn that at the meantime, restricting to addition of NP suspended in ethanol to etched enamel would be the best so far until further information and studies is carried to come up with a better manipulation method of incorporating a bonding agent with nanoparticles.

CONCLUSION

Within the limitation of this study, the addition of experimental nanoparticles with or without CO2 laser incorporation into enamel enhances its color preservation where some demineralization is expected during the process of bonding and debonding of orthodontic brackets, thus, improving the enamel esthetics.

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