



## Effects of Diode Laser 940 nm with and without 5 % Sodium Fluoride White Varnish with Tri-calcium Phosphate on Dentin Permeability (In vitro study)

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**Abstract : Introduction:** It is found that hypersensitive teeth have a larger number and wider patent tubules than those of non-sensitive teeth. **Objective:** The aim of this study is to compare between the effects of diode laser at different power densities, with and without sodium fluoride on the sealing of exposed dentinal tubules and dentin permeability. **Materials and methods:** 118 teeth were used. Samples were divided into three major groups. The first consisted of 100 teeth used for permeability test. The second consisted of 16 teeth for measuring external surface temperature elevation while irradiation. The third, in turn, consisted of one pair of teeth observed under SEM for dentine surface morphology analysis. **Results:** For dentin permeability measurement, the results revealed a highly significant difference between the control group and 2, 3 W with varnish groups. For the external surface temperature elevation, the results revealed that temperature rise, for both laser alone and with varnish groups, was between (67-97.9 °C) at 1.6, 2 W. SEM analysis revealed that approximately optimum sealing of tubules occurred in 2W with the varnish group. **Conclusions:** The combined application of 940 nm diode laser at 2W with 809.7 W/cm<sup>2</sup> power densities, with sodium fluoride white varnish show a significant improvement in their effects on dentin permeability reduction as compared to each treatment alone.

**Keywords :** Dentin Permeability, Diode Laser, NaF White Varnish

### Introduction

Hypersensitive teeth have a larger number with wider diameter dentinal tubules (DTs) than those of nonsensitive teeth (Kerns et al., 1991). Regarding Brannstrom's hydrodynamic theory (Brännström, 1963), the movement of fluid along the DTs stimulates the mechano-receptors in or near the pulp, and the occlusion or narrowing the patent DTs diminishes dentin permeability and respectively reduces the grade of DH (Rimondini et al., 1995). Thus, the permeability of dentin becomes an important part in dentin hypersensitivity treatment. Different desensitizing agents have been recommended to treat DH, but studies showed that they are ineffective or short time lasting (Kishore et al., 2002). Recently, the clinical application of lasers has increased. In the treatment of DH, high energy

densities (high power level) lasers (diode, Nd: YAG, Er: YAG, Er Cr: YSGG, and CO<sub>2</sub> lasers), were reported to provoke melting effect with the recrystallization of dentine inorganic component resulting in the occlusion of dentinal tubules. But actually this does not happen. Later, it has been found that laser treatment reduces sensitivity by the coagulation of protein of dentin organic components that lead to DTs sealing (Trushkowsky and Oquendo, 2011). Therefore, the aim of this study is to evaluate, concerning dye penetration ratio, the effect of using diode laser (940nm) with or without 5 % NaF varnish with TCP on dentin permeability.

### Methods

#### Sample Collection

In our study, 118 sound adult human maxillary premolar teeth with two roots extracted for orthodontic purpose have been used. The age of

patients was between (18-25 years) for standardization. They were kept in 4°C distilled water containing 0.1% thymol to hinder microbial growth until use.

**Samples Treatment**

Samples were divided into ten subgroups, the control group which did not receive any

treatment, NaF white varnish (Clinpro White Varnish, 3M ESPE, USA) group, four 940 nm diode laser EPIC™ (BIOLASE, San Clement, CA, USA, power output: 10 Watt) without varnish groups and four laser with varnish groups at 0.8W, 1.6W, 2W and 3W (Table 1) .

**Table 1:** Samples Treatment

Power density(W/cm <sup>2</sup> )	Exposure time(sec)	Spot size(mm)	varnish	power	laser	Groups
–	–	–	–	–	–	control
–	–	–	+	–	–	varnish
323.8	10	0.561	–	0.8	+	0.8W
647	10	0.561	–	1.6	+	1.6W
809.7	10	0.561	–	2	+	2W
1214.57	10	0.561	–	3	+	3W
323.8	10	0.561	+	0.8	+	0.8W+V
647	10	0.561	+	1.6	+	1.6W+V
809.7	10	0.561	+	2	+	2W+V
1214.57	10	0.561	+	3	+	3W+V

The used laser settings: continuous, noncontact mode 1 mm distance (which was fixed by the aid of orthodontic wire), exposure time 10 seconds , power densities 323.8 , 647, 809.7 ,1214.57 W/cm<sup>2</sup> , for the following output power: 0.8, 1.6, 2 , 3 W respectively, laser fiber tip diameter: 300 µm. Just one session for each sample was held.

**Sodium Fluoride White Varnish with TCP Preparation and Application**

For the varnish groups, 5% NaF varnish with TCP was used, after thoroughly mixing, two thin coats were applied and allowed to set in the presence of saliva. The artificial saliva was prepared following the protocol utilized by

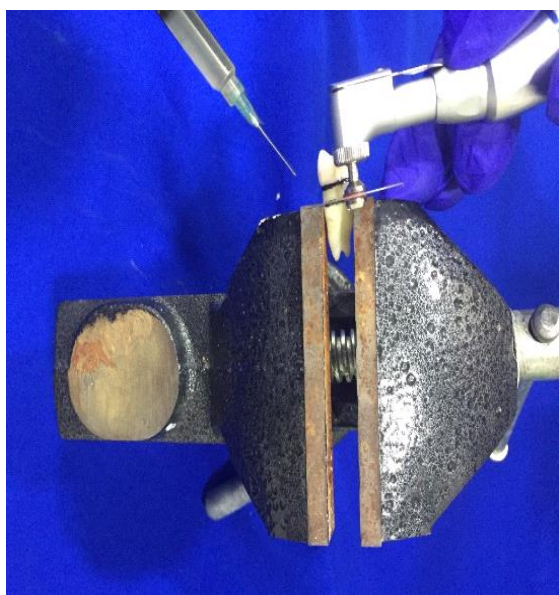
Karlinsey et al(Karlinsey et al., 2012). After irradiating the samples with laser (with the same parameters above), they were immersed in artificial saliva for 24 h to the certify complete treatment period as instructed by the manufacture. Then the surface area treated with varnish was brushed manually using soft brush (Sensodyne, Glaxo Smirth Kline co., UK) for 10 sec using the modified bass technique (Poyato-Ferrera et al., 2003), with 10 strokes for at 45°, in apico-coronal direction with gentle hand pressure to simulate patient teeth brushing.

**Samples Preparation for the Permeability Test**

A hundred teeth were used for permeability measurement. After removing cementum with a

periodontal curette by 70 times strokes as stated by Coldiron et al.(Coldiron et al., 1990) with a periodontal curette, two horizontal sectioning were made using a diamond disk mounted on low speed hand piece (250 rpm) under running distilled water. First section was done at the cement-enamel junction and the second one was done 3 mm apical to the first one (Figure 1). The specimens were immersed in 17% EDTA solution for 1 minute for smear layer removal according to manufacture instruction, then washed with distilled water in the ultrasonic cleaner for 15 minutes and dried with a 5 sec air blast.

After marking an area of 12 mm<sup>2</sup> (3X4 mm) on specimen buccal surface to determine the area for laser irradiation, the specimens were coated with three coats of nail varnish except the marked area.



**Fig.(1):** Two horizontal sectioning were made using a diamond disk mounted on low speed hand piece

## Tests

### Permeability Test

To estimate dye penetration, the samples were immersed in 2 % methylene blue dye for one hour at room temperature. Then, the samples were washed under tap water for 1 minute and left to dry, according to Al-Maliky et al protocol (Al-maliky et al., 2014)

The samples were stabilized by a holder. Then, they were sectioned using a diamond disk in the occlusal-apical direction in the midpoint of mesio-distal width using a diamond disk mounted on low speed hand piece (250 rpm)

under running distilled water , the disk was replaced every ten samples. The resultant specimens were placed on a sheet wax and evaluated using a stereomicroscope (Hamilton, Altay Scientific, Rome, Italy) under a magnification of X40. The examined specimen photo introduced into computer software KAD-KAS measure pictures (CAD-KAS Kessler Computer software GbR, Germany) V 1.0 on Microsoft Windows 10 operation system For the standardization of the samples measurements, the ratio of dye penetration, from the outer surface of the dentine toward the pulp chamber, was estimated as follow:

$$\text{Dye penetration ratio} = \frac{\text{Length of dye penetration inside the dentin}}{\text{Whole dentin thickness of the sample}} \times 100$$

Whole dentin thickness of the sample

### SEM Test

To analyze dentin surface morphology changes, SEM test was done. Samples were mounted on a holder for stabilization during laser irradiation. After lasing, samples fixation and dehydration, in an ascending ethanol series, were done following the protocol used by Marchesan et al (Marchesan et al., 2008).

After dehydration, the specimens were left to dry for 24h, then fixed on aluminum stub and metallized with a layer of gold, using vacuum evaporation . The samples were analyzed by SEM (Inspect S50) with 2000x (50µm), 5000x (20µm) magnification, at 20 KV.

### Absorption Spectrum of Varnish Assessment

The absorption spectrum of varnish (Clinpro White Varnish) was determined with a Spectrophotometer (UV-9200, Biotech co. Ltd, UK) calibrated in the spectral range from 900 to1000 nm. Two thin coating of varnish on a glass slide was analyzed, and then the absorbance was calculated as follow:

$$\text{Varnish absorbance} = \text{the resulted absorbance (varnish and glass)} - \text{glass slide absorbance}$$

### External Surface Temperature Measurement

In order to verify the laser effect on the dentin surface, external surface temperature assessment was made, samples were mounted on a holder for stabilization during laser irradiation. A thermal camera (Flir i5) was positioned at 10 cm away from the sample as recommended by the manufacturer, and a thermal image was taken during laser irradiation to measure temperature elevation of the external tooth surface Figure 2.



**Fig. (2):** Setup of External Surface Temperature Assessment

**Results**

**Permeability Assessments**

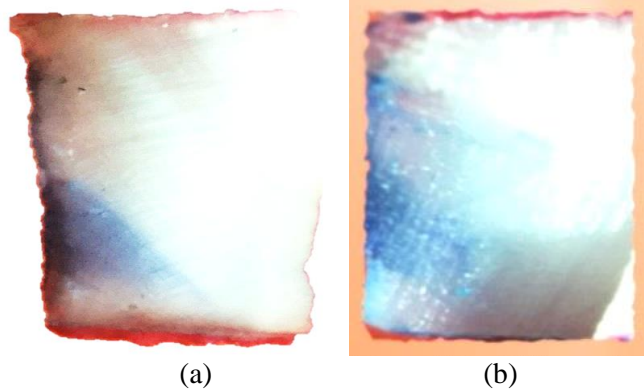
The data obtained from permeability ratio measurement from all the tested groups were statistically analyzed by SPSS Statistics 23 (IBM, NY, USA).

First, descriptive statistics was done to find the means, standard deviations, maximum and minimum value (see Table 2).The minimum recorded values were 0.42, 0.39 with group 2W+V and 3W+V group.

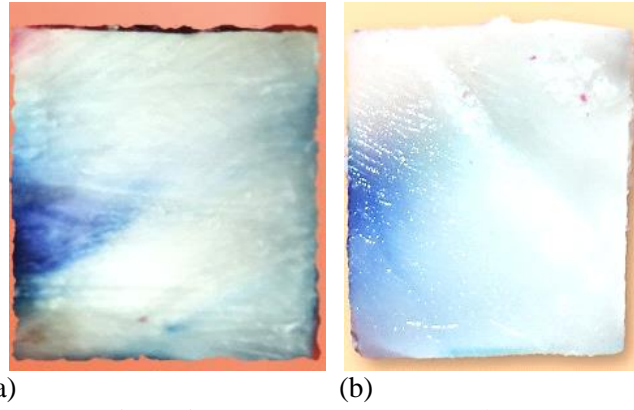
The combined laser with varnish group showed less penetration compared to laser alone groups using same parameters. Maximum dye penetration among tested groups was shown in (Figures 3-7).

**Table (2):** Descriptive Statistics for Dye Penetration Ratio among All Tested Groups

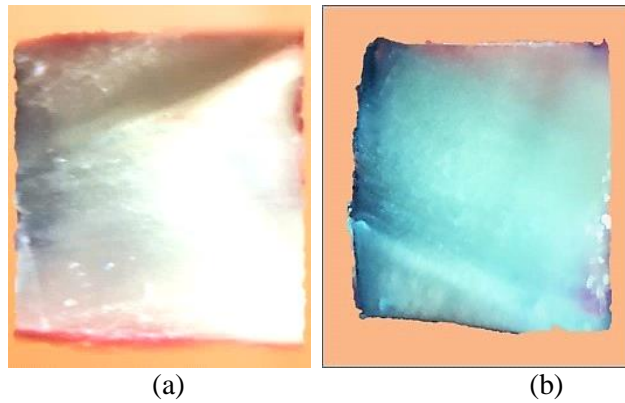
Maximum	Minimum	Std. Error	Std. Deviation	Mean	N	Groups
50.8000	9.5300	3.8438989	12.1554757	29.224200	10	Control
42.2000	2.8300	4.6194412	14.6079558	20.548000	10	Varnish
42.8000	2.0200	4.3760297	13.8382209	22.587000	10	0.8W
33.1000	1.8500	3.4182660	10.8095064	13.555000	10	0.8W+V
26.0000	1.2200	2.5248353	7.9842304	12.773000	10	1.6W
25.4000	2.2100	2.5192610	7.9666027	9.841100	10	1.6W+V
23.1000	.6000	2.3402743	7.4005973	9.722000	10	2W
19.3000	.4200	1.8257140	5.7734145	7.694000	10	2W+V
20.6000	.4600	2.2036920	6.9686858	9.534000	10	3W
17.2000	.3900	1.6425609	5.1942336	7.632000	10	3W+V



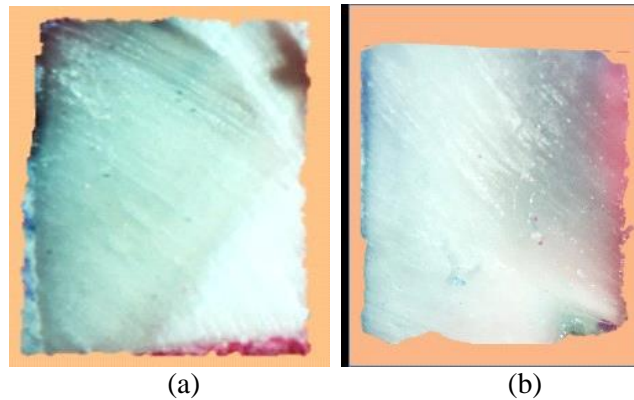
(Figure 3). Maximum dye penetration ratios among (a) control group, (b) varnish alone group under microscopic examination. Magnification X40.



(Figure 4). Maximum dye penetration ratios among (a) 0.8W group (b) 0.8W+V group under microscopic examination. Magnification X40.



(Figure5). Maximum dye penetration ratios among (a) 1.6W group (b) 1.6W+V group under microscopic examination. Magnification X40.



(Figure 6). Maximum dye penetration ratios among (a) 2W group (b) 2W+V group under microscopic examination. Magnification X40.



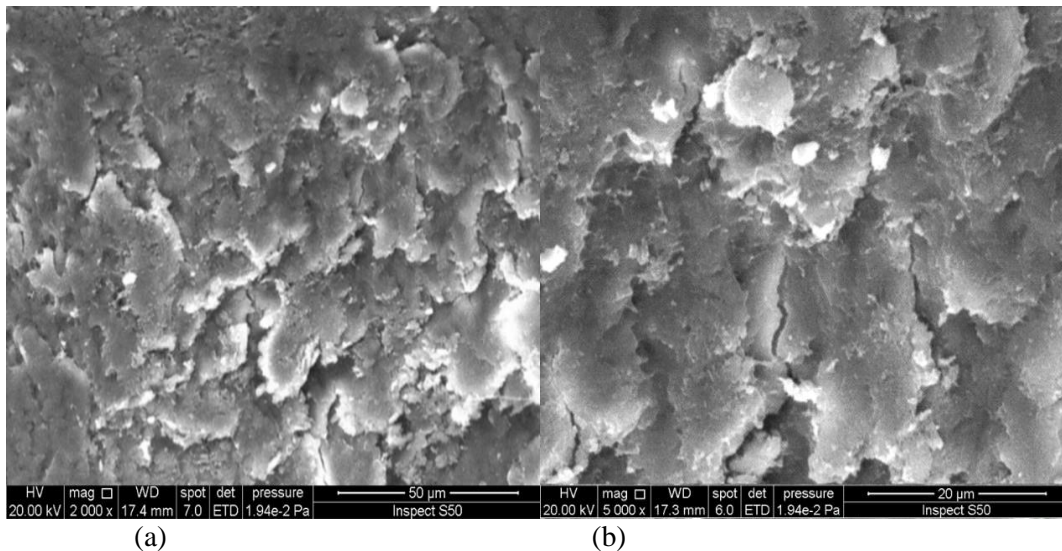
(Figure 7). Maximum dye penetration ratios among (a) 3W group, (b) 3W+V group under microscopic examination. Magnification X40.

To check if the acquired data had normal distribution, Shapiro-Wilk test was implemented, and the test statistics disclosed that data were normally distributed ( $P > 0.05$ ). To examine if the groups were statistically different, One-way ANOVA test was used and the obtained descriptive level was (0.000), which revealed that the groups were highly significantly different. To investigate the difference between each group, multiple comparisons were done using Dunette T test. The results revealed that there

was significant difference between control group and 1.6W+V, 2W and 3 W groups, while the high significant difference occurred between control group and 2W+V, 3W+V ( $P=0.008$ ).

## 2- SEM Evaluation.

SEM analysis of the examined specimens showed surface structural changes. At 2W+V almost optimum sealing of tubules was observed without signs of carbonization or cracking. Figure (9 a, b).



**Fig.9:** Scanning electron microscopic (SEM) views of treated dentin by the diode laser 2W with varnish. Almost an optimum sealing of DTs were observed without signs of carbonization or cracking. Magnification: 2000x, 5000x.

## NaF white Varnish Spectrum Assessment

Data of absorption spectrum analysis for Clinpro white varnish in the range of (900-1000nm) was illustrated in (Figure 10). Data revealed that varnish absorbance for 940nm was 0.808, which was higher than the absorbance for 980nm.

(Figure 10). Clinpro white varnish absorption spectrum for (900-1000nm)

## Surface Temperature Measurements

Surface temperature assessment for laser without and with varnish groups as measured by thermal camera are shown in (Table 4). Data revealed that surface temperature was ranged between 67-97.9°C for 1.6, 2 W and exceed 200 °C at 3W for both laser alone and with varnish groups.

**Table (4):** Surface temperature measurements (°C) for laser without and with varnish groups

Mean	#2	#1	Groups
50.7	51.5	49.9	<b>0.8W</b>
71.05	75.1	67	<b>1.6W</b>
87	83.6	90.4	<b>2W</b>
203.85	185.7	222	<b>3W</b>
59.75	61	58.5	<b>0.8W+V</b>
79.65	78.1	81.2	<b>1.6W+V</b>
96.75	95.6	97.9	<b>2W+V</b>
225.85	208.7	243	<b>3W+V</b>

## Discussion

The patency of dentinal tubules is interrelated with dentin hypersensitivity. The main goal of a successful treatment is the partial or complete sealing of DTs (Tunar et al., 2014). In this study

we tried to evaluate the ability of diode laser 940nm laser alone or combined with NaF white varnish with TCP to decrease DTs patency concerning dentin permeability.

A laser wavelength of 800 and 980nm has a poor absorption in water and hydroxyapatite (Gutknecht, 2007) with a little absorption in dental tissues (by its mineral structures such as phosphate and carbonate) (Alfredo et al., 2009). This low absorption prevails scattering, or diffused transmission of the laser radiation through the dentin, and important thermal effects (Schoop et al., 2006).

This research used continuous wave mode to ensure scanning the whole dentin surface, noncontact mode to protect the optical fiber from contamination with varnish and damage by the surface heat, in oblique manner to reduce a direct pulp exposure by the part of laser radiation that was not absorbed by dentin (Umana et al., 2013), or varnish.

#### **Permeability Test**

This investigation was performed to evaluate quantitatively the permeability of human dentin discs before and after application of varnish, laser or laser with varnish.

The result revealed that there was a significant difference between control group and 1.6W+V, 2W, 3W groups ( $P < 0.05$ ), while the high significant difference occurred between control group and 2W+V, 3W+V groups ( $p < 0.01$ ), and no significant difference for the varnish alone group with control group. This may indicate that the permeability decreases as power density increases which could be due to increasing the absorbed energy and hence more laser effect on dentin surface and consequently more sealed DTs, and the application of NaF white varnish had a synergistic effect in reducing dentinal permeability when used with laser. These results reflect that in the combination groups the sealing effect on the DTs is higher than each treatment alone, that is attributed to the NaF varnish mechanism of action, which relies on interactions occurring between NaF in varnish and calcium ions from saliva or varnish resulting in calcium fluoride crystals ( $\text{CaF}_2$ ) precipitation on the dentinal tubules that could mechanically occlude the tubules opening. Besides, diode laser absorption was intensifying with varnish addition, due to the increased mineral content, and hence more thermal effect on dentin surface. In addition, a high increase in fluoride uptake depth in dentin after absorption

of laser irradiation by varnish (figure 10), as reported by Mei et al (Mei et al., 2015), leading to more calcium fluoride formation, more adhesion and consequently better sealing effect and according to Hagen-Poiseuille's law, the dominating factor in permeability is the tubular radius, which influences the dentinal fluid flow in the fourth power. A reduction in dentinal fluid flow (permeability) diminishes the nerve endings excitability upon stimulus that evoked fluid movements and pain perception.

These findings agree with Rizzante et al.'s study (Rizzante et al., 2016), which demonstrated a better result in permeability reduction with increased 980 nm diode laser irradiation power, which is the close wavelength to 940 nm, and the combination of diode laser irradiation with a fluoride varnish ensuring a greater reduction in permeability.

#### **SEM analysis**

In order to examine the dentin surface morphology changes, SEM analysis was made. SEM revealed that approximately optimum sealing of DTs occurred in 2 W+V group. The low absorption of 940 nm diode laser by dentin (Gutknecht, 2007) led to surface temperature elevation to an accepting level (67-97.9 °C) for 1.6, 2 W laser with and without varnish groups, as examined by thermal camera (Table 4), that promote protein denaturation of dentin organic compound. The coagulated protein tend to expand (Rees and Robertson, 2001), as a consequence, lead to DTs diameter reduction or occlusion. This high increase in fluoride uptake depth of varnish by laser reflects its resistance to be brushed away by manual tooth brush after 10s brushing that was done after lasing, while it could be brushed away when used alone. The combination treatment appeared to be a step forward to reduce the shortcoming of either treatment alone.

These results accord with Liu et al study (Liu et al., 2013), who found that 2 W/CW 980-nm diode laser, having a close wavelength to 940nm diode laser, was a suitable power parameter due to its rapid sealing of the exposed dentinal tubules and its safety to the dental tissue.

These findings come in accordance with Lan et al, Tosun et al studies (Lan et al., 1999, Tosun et al., 2016), which revealed that the combination treatment of sodium fluoride varnish and Nd: YAG laser, which occurred in NIR region as

940nm diode laser, resulted in more occluded dentinal tubule orifices on dentin surface structure than when used alone, and the varnish could be brushed away when used alone especially from the center of orifices .

#### Surface temperature measurement

In order to assess which photothermal interaction took place on the dentin surface related to different used laser parameters settings, surface temperature was measured using thermal camera.

The results revealed that the temperature rise was between (67-97.9 °C) for 1.6, 2 W laser with and without varnish groups which means that protein denaturation of dentin organic components occurred, and it exceeded 200 °C at 3W for both laser alone and with varnish groups which indicated surface carbonization occurred (Knappe et al., 2004). Results match Trushkowsky et al. study(Trushkowsky and Oquendo, 2011).

#### Conclusion

The combined application of NaF varnish with TCP with 940nm diode laser at 2W+V, continuous mode, irradiation speed: about 1.2 mm/sec for 10 sec, laser fiber tip diameter: 300 µm, improves dentinal tubules sealing effects of both varnish, and diode laser.

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## تأثيرات ليزر الدايبود 940 نانومتر مع وبدون طلاء 5٪ فلوريد الصوديوم الأبيض مع ثلاثي فوسفات الكالسيوم على نفاذية العاج (دراسة مختبرية)

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**الخلاصة:** المقدمه: لقد وجد أن الأسنان شديدة الحساسية لديها عدد أكبر و قطر أوسع من أنابيب العاج من تلك المتواجدة في الأسنان غير الحساسة. **الهدف:** هدفت هذه الدراسة إلى المقارنة بين تأثيرات ليزر الدايبود بكثافات طاقة مختلفة، مع أو بدون طلاء فلوريد الصوديوم على غلق الانابيب العاجية وعلى نفاذية العاج. **المواد وطرق العمل:** استخدمت للدراسة 118 عينة من الأسنان. تم تقسيم العينات إلى ثلاث مجموعات رئيسية. تتكون الأولى من 100 سن لقياس نفاذية العاج. بينما تتكون الثانية من 16 سن لقياس الارتفاع الحراري للسطح عند التشعيع. وتتكون الثالثة من زوج من الاسنان لوحظت تحت المجهر الإلكتروني الماسح لتحليل التغيرات السطحية للعاج. **النتائج:** أظهرت نتائج تقييم نفاذية العاج وجود فرق معنوي كبير بين مجموعة السيطرة ومجموعتي 2 و 3 واط مع الطلاء. وقد أظهرت قياسات الارتفاع الحراري ، لكل من مجموعات الليزر وحده ومع الطلاء، كان ما بين (67-97.9 درجة مئوية) عند 1.6 و 2 واط. وكشف تحليل المجهر الإلكتروني الماسح أن ما يقرب من الاغلاق المحكم للانابيب حدث في مجموعة 2 واط مع الطلاء. **الاستنتاجات:** استنتجا للدراسة المعنية، تم التوصل الى ان التطبيق المشترك لليزر الدايبود 940 نانومتر باستخدام 2 واط كثافة الطاقة 809.7 واط / سم<sup>2</sup> مع طلاء فلورايد الصوديوم أظهرت تحسنا كبيرا في تأثيرها على الحد من نفاذية العاج مقارنة بكل معامل وحده .