A Study on Root Canal Cleansing by Nd:YAG Laser with Black Dye Solution

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Purpose: To investigate in vitro the removal of smear layer, plug, debris, and pulp tissue by Nd:YAG irradiation with different potencies and times, with or without black ink on the apical root canal wall.

Materials and Methods: Twenty-five extracted human teeth were divided into 5 groups after endodontic treatment. G1: no laser irradiation, control; G2: Nd:YAG laser 1 W, 1 s, 20 pps, 50 mJ; G3: Nd:YAG laser 2 W, 1 s, 20 pps, 50 mJ; G4: Nd:YAG laser 1 W, 2 s, 20 pps, 50 mJ, black ink; G5: Nd:YAG laser 2 W, 1 s, 20 pps, 50 mJ, black ink. All teeth were sectioned and analyzed under light microscope and SEM.

Results: Statistically significant differences were observed among the 5 groups, p = 0. 0027(p < 0.05). No significant difference was found between G1 (control) and G2 (1 W, 1 s), p = 0.8345 (p > 0.05), nor between G3 (2 W, 1 s) and G5 (2 W, 1 s, black ink), p = 0.0758 (p > 0.05). However, a marked difference was seen between G4 (1 W, 2 s, black ink) and G5 (2 W, 1 s, black ink), p = 0.0367 (p < 0.05). A statistically significant difference (p < 0.05) was found between G3 (no black ink) and G5 (black ink) regarding carbonization.

Conclusion: Nd:YAG irradiation was effective in the removal of smear layer, plug, debris, and pulp tissue, even without an initiator (black ink) in the potencies of 1 W, 2 s and 2 W, 1 s, but the use of black ink caused less carbonization in the potency of 2 W and 1 s, which may suggests it is safer. However, the treatment with 1 W, 2 s was considered more efficient than the treatment with 2 W, 1 s. Nevertheless, Nd:YAG laser treatment was not sufficiently effective with 1 W, 1 s.

Keywords: Nd:YAG laser, black ink, smear layer, plug, debris, carbonization.

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McComb and Smith[1] were the first investigators to show the presence of a smear layer in instrumented root canals. Since then, many studies have been performed in an attempt to find ways of removing the smear layer, because it was believed that its presence prevents the complete cleansing and disinfection of the root dentin, as well as the perfect contact between the filling material and the root dentin wall.[2-4]

It has been demonstrated that different kinds of chelating agents[5] (EDTA, citric acid) and several lasers[6-11] can be used for the removal of the smear layer. Saito et al[12] suggested that chelating agents promote the removal of the smear layer more efficiently in the coronal and middle thirds than in the apical third of the canals, even when different irrigation times and solution volumes were employed.[5,13-14] Therefore, it has been accepted that the incomplete removal of the smear layer and insufficient cleaning of the canal walls led to failed root canal treatments.

The use of Nd:YAG laser irradiation in the root canal has revealed a number of advantages of this method, including the removal of the smear layer and debris, as well as a bactericidal effect. Additionally, it has been demonstrated to be useful during the steps of cleaning and shaping the canal walls, and to promote a good apical sealing after apicoectomy.[6-9] Nevertheless, attention must be paid to the thermal effects when using the laser in vivo,[15-16] since the consequences of laser irradiation depend on different factors such as power level, frequency, air, water, angulations, movement, distance, fiber shape, duration of exposure and color of dentin.[17] The Nd:YAG laser has a wavelength of 1064 nm. Although this laser light is poorly absorbed by water, this wavelength is easily absorbed by protein, pigmented tissue and dark surfaces. As a consequence, a black dye such as India ink was used in several studies to enhance the effect of lasing, and painting the root canal wall with black ink was recommended when this laser is used in order to increase the absorption of the laser energy at the dentin surface.[10-17]

The purpose of this in vitro study was to evaluate the removal of debris, plugs, pulp tissue and smear layer using the Nd:YAG laser with different potencies by using the light microscope and the scanning electron microscope (SEM) and to assess the effects of the black ink in the procedure.

MATERIALS AND METHODS

Twenty-five extracted human permanent teeth with a single root and a closed apex were used for this study. The crowns were separated from the root at the cementoenamel junction using a diamond disk (Isomet, Buehler; Lake Bluff, IL, USA). The working length of each root canal was established at 1 mm short of the apical foramen with a #15 K-file. Each root canal was irrigated with 1 ml of 5.25% sodium hypochlorite after each file, and shaped with Gates-Glidden drills I, II and III at the third middle of the canals. Using ISO 2/100 tapered manual K-files, the apical third of the canals were prepared and apical collar resistance was established with a finishing #40 file and the #15 K-file as a patency file up to the foramen, by one operator. The present study did not incorporate the EDTA rinse so as not to influence debris and smear layer removal, but to just test the efficacy of Nd:YAG laser as the smear layer remover.

The teeth were randomly divided into 5 groups:

Group 1: Control – no laser irradiation.
Group 2: Nd:YAG laser 1 W, 1 s, 20 pps, 50 mJ.
Group 3: Nd:YAG laser 2 W, 1 s, 20 pps, 50 mJ.
Group 4: Nd:YAG laser 1 W, 2 s, 20 pps, 50 mJ + black ink
Group 5: Nd:YAG laser 2 W, 1 s, 20 pps, 50 mJ + black ink

The black ink (Kaimei; Urawa, Japan) used in this study is the traditional ink for Japanese calligraphy, composed of carbon black and polyvinyl alcohol, and was applied inside the canal with a sterilized cotton point before the laser irradiation in groups 4 and 5, and there was no excess of the dye through the apex. For the Nd:YAG laser irradiation, a dLase 300 (American Dental Laser; Birmingham, MI, USA) with a flexible fiber of 0.32 mm diameter and a wavelength of 1064 nm was used by introducing the fiber inside the canal up to 1 mm short of the foramen and irradiated without movement.

The roots were divided longitudinally into halves using diamond burs, and subsequently split with scissors to expose the root canal walls. All the specimens were analyzed and photographed under a light microscope, then dehydrated in a graded series of alcohol and subsequently stocked with silica. For the SEM (JEOL, JSM-5500; Tokyo, Japan) analysis, the root canal walls were coated with a 15-µm platinum-palladium layer (E-1030 Ion Sputter, Hitachi; Tokyo, Japan). All teeth were examined by the SEM, and classified in accordance with the criteria presented in Table 1. These criteria are based on those used by Arisu et al[17] with a few adjustments for this research; the representative area for the score was the apical stop just above the constriction, where the laser directly irradiated.

The optical microscope analysis was performed to examine carbonization, which is like burning, producing great structural changes that may interfere with the success of treatment, because the irradiation was beyond the amount necessary. On the other hand, the melted dentin may be seen just with SEM analysis; it corresponds to melted and ablated smear layer, and may show spherical particles with a glass-like structure (glazing). Such morphological changes are expected with laser irradiation and may be beneficial for sealing of the root canal complex and preventing leakage.

Table 1 Criteria

Score	Criteria
0	No plug. No debris. No pulp tissue. No smear layer. All dentin tubules were open.
1	No plug. Almost no debris. Few debris particles with diameter less than 20 µm. No pulp tissue. No smear layer. All dentin tubules were open.
2	No plug. Moderate debris. Large quantities of debris particles with diameters less than 20 µm; and/or No pulp tissue. Moderate smear layer. Dentin tubules covered with a thin smear layer.
3	Plug. Plug in the foramen was present; and/or Debris. Large amounts of debris particles whose diameters were greater than 20 µm; and/or Pulp tissue. Remnant of pulp tissue still present; or/and Smear layer. A thick smear layer closed all dentin tubules.

RESULTS

In Group 1 (control), carbonization was absent, the apical seat (meaning the surface of the apical stop just above constriction) formation showed three cases of "moderate" and two cases of "less", and the criteria indicated three cases of "3" and two cases of "2". In Group 2 (1 W, 1 s, no black ink), carbonization was found in three cases, the apical seat formation showed two cases of "moderate", two cases of "over" and one case of "less", and the criteria indicated three cases of "3", one case of "2" and one case of "1". In Group 3 (2 W, 1 s, no black ink), carbonization was found in three cases, the apical seat formation showed two cases of moderate and three cases of less, and the criteria indicated four cases of "0" and one case of "1". In Group 4 (1 W, 2 s and black ink), carbonization was found in one case, the apical seat formation showed five cases of "moderate", and the criteria indicated five cases of "0". In group 5 (2 W, 1s and black ink), carbonization was found in one case, the apical seat formation showed one case of "moderate" and four cases of "less", and the criteria indicated one case of "0", two cases of "1", one case of "2" and one case of "3".

All the results are shown in Table 2, and images from light microscopy (Figs 1 to 6) and SEM (Figs 7 to 14) illustrate the different apical seat formation, the surface of the apical stop just above the constriction, and cases where carbonization was evident.

Table 2 Results of all samples

	Teeth	Criteria	Apical seat	Carbonization
Group 1	1	2	moderate	–
Control	2	3	less	–
	3	2	less	–
	4	3	moderate	–
	5	3	moderate	–
Total		13		
Average		2.6		
Group 2	6	3	moderate	–
Nd:YAG	7	2	over	+
1 W,1 s	8	3	over	+
20 pps	9	3	less	–
	10	1	moderate	+
Total		12		
Average		2.4		
Group 3	11	0	moderate	+
Nd:YAG	12	0	less	+
2 W, 1 s	13	0	moderate	+
20 pps	14	0	less	–
	15	1	less	–
Total		1		
Average		0.2		
Group 4	16	0	moderate	–
Nd:YAG	17	0	moderate	–
1 W, 2 s	18	0	moderate	+
20 pps	19	0	moderate	–
Black ink	20	0	moderate	–
Total		0		
Average		0		
Group 5	21	1	less	–
Nd:YAG	22	0	moderate	–
2 W, 1 s	23	1	less	–
20 pps	24	3	less	+
Black ink	25	2	less	–
Total		7		
Average		1.4		

Figs 1, 2 and 3 show respectively: (A) Sample from group 2 with apical seat less, (B) sample from group 4 with apical seat moderate and (C) sample from group 2 with apical seat over.

Figs 4 (group 2, A), **5** (group 3, B) **and 6**(group 4, C) show carbonization.

The Kruskal-Wallis test revealed statistically significant differences among the 5 groups, with $p = 0.0027$ ($p < 0.05$) (Table 3). On the other hand, comparisons between Group 1 (Fig 7) (control) and Group 2 (1 W, 1 s) by the Mann-Whitney U-test showed no significant differences with $p = 0.8345$ ($p > 0.05$).

No significant difference ($p = 0.0758$) was found between Groups 3 and 5 (Fig 8) when the Mann-Whitney U-test was applied ($p > 0.05$), suggesting that there is no statistical difference between the use of black ink in the potency of 2 W and 1 s of exposure time.

Moreover, comparisons between Groups 4 (Fig 9) and 5 (Figs 10 to 12) by the Mann-Whitney U-test are shown in Table 4. A marked difference could be observed ($p = 0.0367$; $p < 0.05$), showing that the use of the same total energy is more effective when the parameters of 1 W and 2 s were utilized rather than 2 W and 1 s.

Regarding carbonization, comparisons between Groups 3 (no black ink) and 5 (black ink) by the Mann-Whitney U-test showed a statistically significant diference with $p < 0.05$, suggesting that the use of black ink caused less carbonization (Table 5) in the potency of 2 W and 1 s.

DISCUSSION

In this study, extracted human permanent teeth with a single root, closed apex and straight canal were used in order to simulate clinical conditions of root canal treatment. This investigation assessed the efficacy of laser irradiation to remove smear layer, plug, pulp tissue and debris during endodontic treatment, as well as its effects on dentinal tissue.

Some restrictions may apply to this in vitro comparison with a small sample size of 5 teeth per group. Biological outcomes such as unpredictable overall consequences of the laser treatment according to pain or inflammation outside the apical foramen could not be examined. Moreover, further studies in this area are needed in order to demonstrate possible differences of

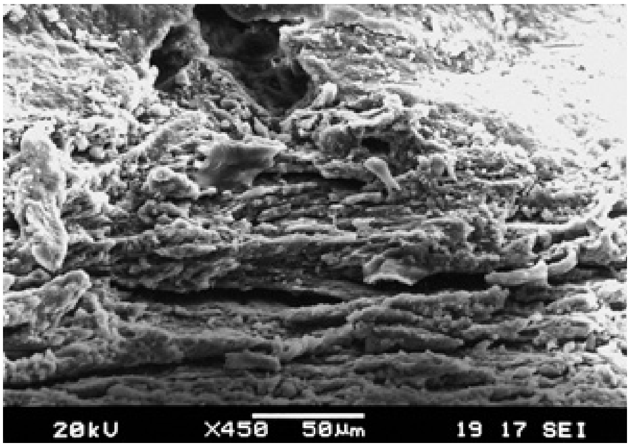


Fig 7 Sample of group 1 (control) showing great amounts of smear layer in the root canal wall.

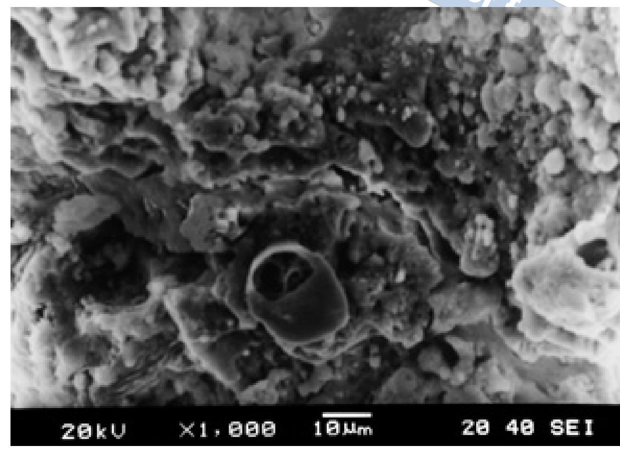


Fig 8 Sample of group 5 showing mixture of black ink, melting dentin and smear layer.

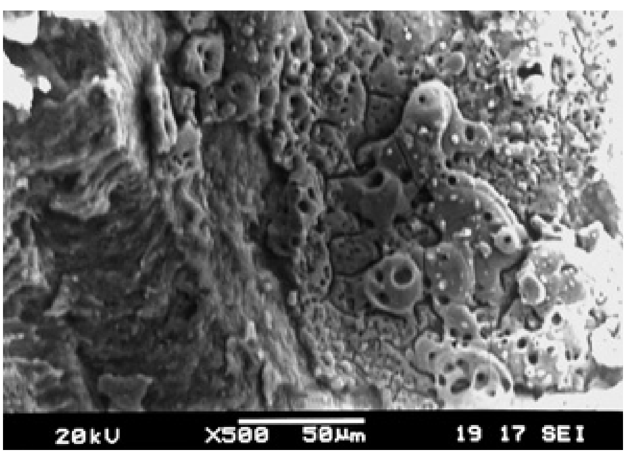


Fig 9 Sample of group 4 with melting dentin resulted from 1 W and 2 s and black Ink of Nd:YAG laser treatment.

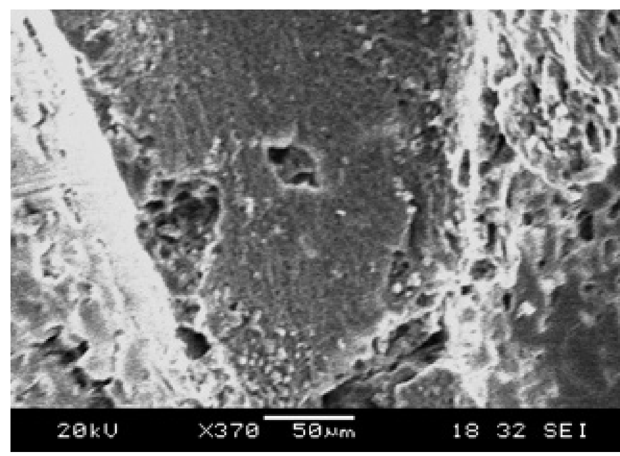
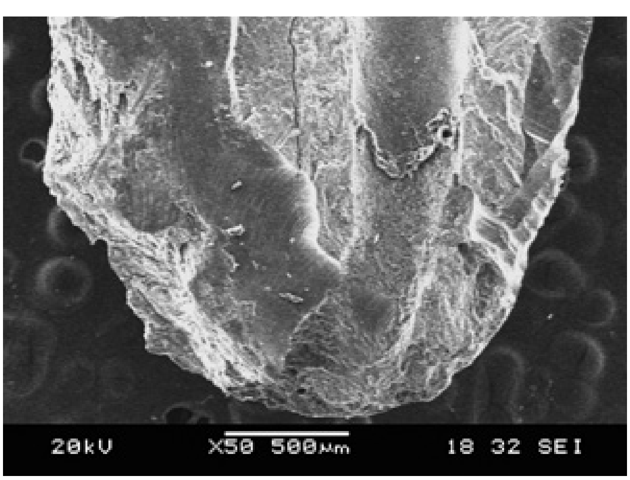
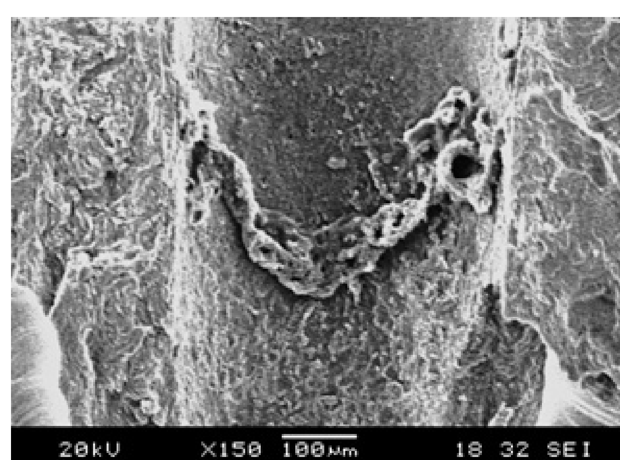


Fig 10 Sample of group 5 shows an entrance of accessory canal clear.





Figs 11 and 12 Sample of group 5 shows the limit between the laser irradiated portion and the nonirradiated portion of the root canal.

Table 3 Cleanliness of root canal wall

	Treatment	Black ink	Average score	Standard deviation
Group 1	Control	–	2.6	0.548
Group 2	1 W, 1 s, 20 pps	–	2.4	0.894
Group 3	2 W, 1 s, 20 pps	–	1.0	0.447
Group 4	1 W, 2 s, 20 pps	+	0	0
Group 5	2 W, 1 s, 20 pps	+	1.4	1.140

Table 4 Comparison of cleanliness of root canal wall, groups 4 and 5

	Treatment	Black ink	Average score	Standard deviation
Group 4	1 W, 2 s, 20 pps	+	0	0
Group 5	2 W, 1 s, 20 pps	+	1.4	1.140

Table 5 Comparison of groups 3 and 5, carbonization using black ink or not

	Treatment	Black ink	Carbonization
Group 3	2 W, 1 s, 20 pps	–	60%
Group 5	2 W, 1 s, 20 pps	+	20%

the laser treatment on various types of root canals (curved or straight, wide and thin), or diverse clinical situations (closed apex or incomplete root formation).

Although many applications of the Nd:YAG laser have already been established, there is no consensus on the adequate potency to be used without risks of injuries to periodontal tissues. It is well known that the interactions of laser irradiation with dental tissues do not depend solely on the potency, but also on frequency, fiber shape, duration of exposure, color of dentin,[17] air, water, irrigation solutions, angulations, movement and distance.[18,19] Nevertheless, the Nd:YAG laser permits easy control of the energy applied, and the fine optic fiber (0.32 mm) is very convenient for endodontic uses. In 1999, Takeda et al[20] described that the teeth used in their experiments were prepared up to a size #60 K-file, which was performed to allow access for the laser tips. However, the authors correctly predicted that in the future laser tips would be made smaller, and excessively enlarging the root canal would be unnecessary.

Marques et al[21] showed that pulsed Nd:YAG laser has the ability of melting the dentin wall using an output of 1 to 3 W. Conversely, Saunders et al[22] reported that pulsed Nd:YAG laser irradiation with a power of 0.75 to 1.7 W did not melt the wall of the root canal and failed to remove the debris. Moura-Netto et al[23] found dentin fusion and resolidification, with partial smear layer and debris removal with Nd:YAG laser irradiation at 1.5 W, 100 mJ and 15 Hz on continuous mode. Koba et al[16] demonstrated that the parameter of 2 W and 20 pps for 2 seconds would be suitable for infected root canals without any thermal injury to periapical tissue, although this high energy level is not appropriate for teeth just after pulpectomy. This in vitro research showed that laser treatments of 2 W and 20 pps for 1 s and 1 W and 20 pps for 2 seconds are effective for removing smear layer, plugs, debris and pulp tissue, but the laser treatment of 1 W for 1 s is not sufficient. The latter treatment consisted of a short supply of energy and absence of black ink dressing, suggesting that the energy could not be effectively absorbed.

Furthermore, another relevant finding of this study concerns the variation of efficacy under different time exposures and varying potencies. The same amount of energy was utilized for Groups 4 and 5 (1 W and 2 s and 2 W and 1 s, respectively), but the rate of efficiency was shown to be superior for the former group. It may be inferred that a longer exposure under a lower potency reduces the risk of tissue damage during clinical use since the elevation of the temperature is predictably lower.

On the other hand, in order to buffer the heat generated during laser irradiation and to improve the irradiation effect, root canals can be filled with either sodium hypochlorite, which readily dissolves organic substances,[19,24,25] or EDTA, which effectively dissolves the inorganic content.[16,19,24] This fact was corroborated by Ikarugi et al,[19] since the authors did not find any carbonization or marked loss of the root canal wall or apical seat damage when the canal was filled with sodium hypochlorite or EDTA before irradiation with Nd:YAG laser using the potencies of 1 W and 3W for 5 seconds, and the comparison between the use of black ink and irrigation solutions when using the Nd:YAG irradiation is the topic of another study already underway in our department.

Moreover, the Nd:YAG laser has a wavelength of 1064 nm. Although the laser light is poorly absorbed by water, this wavelength is easily absorbed by protein, pigment tissue and dark surfaces,[10-17] mainly hemoglobin and melanin.[27] Morioka et al[28] compared the absorption rate of a laser beam at the enamel surface with different black absorbent materials and found that a waterproof India ink was the most suitable initiator for pulsed Nd:YAG lasers. Painting the root canal with waterproof black ink was recommended when this laser is to be used for root canal treatment, so that more laser energy would be absorbed at the dentin surface.[10] This is an important point to be considered, because if the root canal wall or apical periodontal tissue is excessively heated, severe tissue injury, such as carbonization and burn, can occur, negatively affecting the prognosis of the root canal therapy.[19] However, the present study revealed no statistical difference between the use or not of black ink in the potency of 2 W and 1 s of exposure time, maybe because this energy is too low, and no significant results were seen. On the other hand, the use of black ink showed significantly less carbonization than the group where the black dye was not utilized. One possible explanation for this fact is that the black ink is supposed to penetrate only in the smear layer, and the absorption of laser energy is limited on this part. Since it does not penetrate deeply into healthy dentin, it causes less carbonization.

Goya et al[29] described that Nd:YAG laser irradiation at the parameters of 2 W and 20 pps for 4 s could significantly remove the smear layer, and the use of black ink enhanced the removal; furthermore, laser irradiation with black ink reduced apical leakage significantly. On the other hand, Depraet et al[30] observed that Nd:YAG laser irradiation of 5 s at 1.5 W, 100 mJ, 15 Hz in association with black ink resulted in the cleanest root canal surfaces, but not in a statistically significant reduction of either apical or coronal leakage. Furthermore, the lasing in combination with black ink resulted in development of fine fire flashes, and so cannot be recommended.

A few studies reported adverse effects of the use of Nd:YAG. Bahcall and colleagues[31] performed root canal enlargement using the Nd:YAG laser, with parameters of 3 W and 25 pps for 30 s on canine teeth and reported heat-induced inflammation of apical periodontal tissue, bone resorption, and root resorption. However, it has already been established that the Nd:YAG laser can promote disinfection and sterilization as a result of the heating effect,[9] preventing pain after treatment due to the efficient removal of the bacteria at the apex.[16] More advantages include the sealing of the dentinal tubules by melting dentin,[32] as well as the removal of debris and smear layer from instrumented root canals by vaporizing tissues.[33] The nature of the interaction between laser irradiation and tissue depends on the absorption of light and its conversion into heat;[10] thus the laser irradiation parameters have to be very well defined to perform an effective treatment with only benefits and without damage.

It has been reported that laser irradiation that causes marked dentin loss and carbonization in root canals might hinder the tight adhesion between the root canal wall, and the filling material can be hindered. Thus, it is necessary to adjust the laser energy to avoid these effects. In order to prevent loss and carbonization inside the root canal and promote cleaning effects avoiding to dentin damage, it will be necessary to develop a method to selectively stain the smear layer, lower the pulse count by reducing peak power, and increase the irradiation effect by either lowering the laser tip output or shortening the single irradiation time.[19]

It is possible to say that the use of laser in clinical dentistry is increasing. Some kinds of lasers are being used for many applications, such as pit and fissure sealing, removal of dentin caries, improving bond strength to dentin on adhesive restorative procedures, osteotomies, gingivectomies, treatment of periodontal pockets, treatment of dentin hypersensitivity, reducing tumor size as a primary treatment, frenectomy, debonding orthodontic brackets, excision of oral pyogenic granuloma, treatment of oral ulcerative lesions, peri-implantitis, maxillofacial surgery for patients taking anticoagulants, etc.[11,34-47] Undoubtedly, new beneficial laser therapies will be discovered in the near future.

CONCLUSION

This in vitro investigation demonstrated that Nd:YAG laser irradiation is effective on the removal of smear layer, plug, debris and pulp tissue, even without an initiator at the potencies of 1 W and 2 s and 2 W and 1 s. However, the treatment under the former parameters proved to be more effective and safer. Nevertheless, Nd:YAG laser treatment was not sufficiently effective when the parameters of 1 W and 1 s were utilized. Also, the use of black ink caused less carbonization in the group where the potency of 2 W and the exposure of 1 s were utilized, suggesting a lower risk of tissue damage.

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