The sealing ability of an epoxy resin root canal sealer after Nd:YAG laser irradiation of the root canal

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Abstract

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Aim To evaluate *ex vivo* the effect of Nd:YAG laser irradiation with and without black ink on instrumented root canal walls, and the degree of both coronal and apical microleakage of filled root canals.

Methodology Seventy-two single-rooted teeth were instrumented up to a size 40 K-file, and then divided into six groups of 10 teeth: groups 1 and 4 remained unlased and acted as control groups, groups 2 and 5 were treated with a Nd:YAG laser (Fidelis Plus, Herzele, Belgium), groups 3 and 6 were treated with a laser and black ink; the remaining 12 teeth served as positive and negative controls. The laser was operated at 1.5 W, 15 Hz, four times for 5 s with a 20-s interval. Groups 4–6 were filled using cold lateral condensation of gutta-percha and AH26. After storage in water for 48 h at 37 °C, through-and-through leakage (L in $\mu L\ day^{-1}$) was measured for 48 h under a pressure of 1.2 atm using a fluid transport model and recorded as $L = 0$ (L1), $0 < L \leq 10$ (L2), $L > 10$ (L3). After the assessment of leakage with the fluid transport model, the teeth were immersed in rhodamine B solution for 48 h at 37 °C. Apical and coronal dye leakage was scored after longitudinal splitting of these teeth. All teeth of groups 1–3 were split longitudinally and observed under SEM for evaluation of remaining smear layer.

Results Through-and-through leakage was only observed in the group lased with black ink (two samples – L2). Apical and coronal dye leakage was observed in all groups; there were no statistically significant differences amongst the three experimental groups. The through-and-through leakage, measured with the fluid transport model in two teeth of group 6, was confirmed in the dye leakage test (rhodamine B dye was observed along the total length of the root filling). There was evidence of melted and ablated root canal dentine in the laser-treated groups. These findings were more obvious in root canals lased in association with black ink. All apical foramina in the lased group remained patent.

Conclusions Nd:YAG laser irradiation with black ink increased the amount of melted and ablated dentine areas compared with that without black ink. Nd:YAG lasing in association with black ink did not result in a reduction of either coronal or apical microleakage in root filled teeth.

Keywords: apical leakage, coronal leakage, dye leakage, fluid transport model, Nd:YAG laser.

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Introduction

The three-dimensional sealing of a properly chemo-mechanically prepared root canal system is one of the major objectives of root canal treatment. On the basis of current biological understanding, the root filling should fulfil its role in three ways: (i) by blocking

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communication between the oral cavity and the periradicular tissues (the inhibition of coronal leakage), (ii) the entombment of surviving bacterial cells in the root canal system, and (iii) the inhibition of an influx of fluid from the periapical tissues (Sundqvist & Figdor 1998).

The Nd:YAG laser has been evaluated for its application in Endodontology over a number of years. A variety of studies have shown that direct Nd:YAG laser irradiation on dentine surfaces produced melting and recrystallization which, in turn, caused the dentine to be less permeable (Dederich *et al.* 1984, Levy 1992, Stabholz *et al.* 1992, Miserendino *et al.* 1995, Goodis *et al.* 1997). In association with these findings an improvement in the cleanliness of canal walls has been reported (Levy 1992) with the potential to remove the smear layer entirely (Goodis *et al.* 1993, Harashima *et al.* 1997). Indeed, Takeda *et al.* (1998) reported that in some cases the smear layer may melt and fuse onto the dentinal walls.

In view of the concepts of 'dentine melting' (Takeda *et al.* 1998) and 'reduction of the permeability of dentine' (Stabholz *et al.* 1992) a number of investigators have attempted to seal the apical foramen using Nd:YAG laser irradiation (Weichman *et al.* 1972, Saunders *et al.* 1995, Park *et al.* 2001, Gekelman *et al.* 2002). The evaluation of the effect of prior Nd:YAG laser irradiation on apical sealing of root fillings has been reported (Goya *et al.* 2000, Park *et al.* 2001, Carvalho *et al.* 2002, Gekelman *et al.* 2002). The Nd:YAG laser irradiation appeared to improve significantly the quality of the apical seal and thus to reduce apical leakage following root filling. Data regarding the evaluation of coronal leakage of root fillings in association with prior laser irradiation of the root canal walls is limited.

As it has been shown that Nd:YAG laser irradiation with black ink may lead to an enhanced smear layer removal and to the development of glazed root canal wall dentine (Goya *et al.* 2000), the primary aim of this study was to compare the effect of Nd:YAG laser irradiation at moderate intensity with and without black ink to that of conventional root canal cleaning and preparation in terms of degree of smear layer removal and root canal wall debridement.

The second aim was to evaluate through-and-through leakage using a fluid transport model, and both coronal and apical leakage by means of dye leakage testing in laser irradiated teeth with and without black ink compared with nonlaser treated root canals.

Materials and methods

Seventy-two extracted human straight single-rooted teeth with mature apices were used. Both carious (limited occlusal and/or interproximal lesions without pulp exposure) and noncarious teeth were included. All teeth were stored in 10% formalin until the sample was completed. In order to exclude teeth with multiple root canals, all teeth were radiographed from two angles before root canal treatment. Surface organic debris was removed by submerging the teeth in 2.5% sodium hypochlorite for 8 h. Subsequently, they were washed with tap water for 1 h and stored in saline until used.

Sample preparation

The crowns were removed 2 mm above the cemento-enamel junction with a high-speed fissure bur and water spray. After gross removal of pulp tissue, a size 15 Flexofile (Dentsply Maillefer, Baillaigues, Switzerland) was introduced into the canal until it could be seen in the major apical foramen. The working length was determined by subtracting 1 mm from this length.

The root canals were prepared by means of a crown-down/step-back technique by one operator (FD) (De Moor & De Boever 2000, De Moor & Hommez 2002, De Moor & De Bruyne 2004). The coronal half of the root canals was preflared with Gates Glidden drills (Dentsply Maillefer) in a larger to smaller sequence (numbers 4-3-2) and the canals were irrigated copiously with 2.5% sodium hypochlorite with a 27-gauge endodontic needle (Monoject; Sherwood Medical, St Louis, MO, USA). Smear layer was removed using File Eze (Ultradent Products Inc., South Jordan, UT, USA) during root canal preparation. The apical half of the canal was then prepared with the step-back technique up to a size 40 master file. The canals were dried with paper points and the patency of the apical foramen was confirmed with a size 10 Flexofile. The roots were than randomly divided into six experimental groups of 10 roots each, six positive controls and six negative controls. Three groups of 10 teeth (groups 1, 2 and 3) were used for the study of surface modifications on root canal walls, the other three groups of 10 teeth were root filled (groups 4, 5 and 6).

Laser treatment

Groups 1 and 4, which served as the controls were not lased. In groups 2 and 5, the root canals were

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irradiated with a pulsed Nd:YAG laser (Fidelis Plus, Herzele, Belgium). The laser beam was emitted at a wavelength of 1064 nm and a flexible fibre (diameter 0.30 mm) delivery system was used. The parameters used were those suggested by the manufacturer: an output of 1.5 W, 100 mJ per pulse and a pulse frequency of 15 Hz. The tip of the fibre was introduced 1 mm short of the working length without activating the laser. At this time the laser was activated and the fibre moved coronally in circular movements over the root canal wall at the standardized output of 1.5 W and 15 Hz. The total time of exposure per canal was 5 s. This procedure was performed four times with a time interval of 20 s. In groups 3 and 6, the teeth were irradiated by the same method as groups 2 and 4 after an absorbent paper point, soaked with India ink (Pelikan, Hannover, Germany), was introduced to working length and the walls of the root canal were coated.

Scanning electron microscopic observation

The teeth of groups 1, 2 and 3 were grooved longitudinally on the buccal and lingual surface with a rotating diamond disk of small diameter under continuous water cooling and then carefully fractured and sectioned with a sharp chisel. Care was taken to include the apical foramen in the fracture line. The sections were dehydrated by a series of graded ethanol solutions, and then sputter-coated with a gold layer of approximately 15 μm thickness. The sections were viewed to evaluate the cleansing effect of the laser. Scanning electron microscopic (SEM) photomicrographs (JEOL JSM840, Tokyo, Japan) were taken at 15 kV in each experimental group in the apical, the middle and coronal third of the roots of areas with and without smear layer. The remaining smear layer on the root canal walls was scored blind on a 4-grade scale (according to Goya *et al.* 2000) (Table 1) using a method modified from Gutmann *et al.* (1994).

Root canal filling: cold lateral gutta-percha condensation

The root canals of groups 4, 5 and 6 were filled by means of the lateral gutta-percha condensation technique by one operator (De Moor & Hommez 2002, De Moor & De Bruyne 2004). A standard size gutta-percha cone (Dentsply Maillefer) that matched the master apical file (ISO 40) was fitted to working length with tug back. AH 26 sealer (Dentsply De Trey, Konstanz, Germany) was mixed according to the manufacturer's instructions and placed in the canal with a paper point to working length. The master cone was coated with AH 26 and gently seated at working length. Lateral condensation was then carried out using size 20 and 25 accessory gutta-percha cones with endodontic finger spreaders (Dentsply Maillefer) placed in the first instance to within 1 mm of the working length. The gutta-percha cones coated with sealer were laterally condensed until they could not be introduced more than 3 mm into the root canal.

Following obturation, the gutta-percha was removed from the coronal cavity up to the level of the cemento-enamel junction with a warm instrument (PK Thomas Waxing Instrument, N° PKT-2; Hu Friedy, Leimen, Germany) and vertically condensed with Machtou pluggers (Dentsply Maillefer). A small cotton pellet was sealed in the access cavities of the experimental roots with Ketac-Fil (Espe, Seefeld, Germany). The samples were then stored in water for 48 h at 37 °C.

Leakage study

Fluid transport model

After 48 h the Ketac-Fil restorations (of groups 4, 5 and 6) and the cotton pellets were removed. The root surfaces were covered with two layers of clear acrylic nail varnish except the apical 2–3 mm and the coronal opening. The roots were then attached to a fluid transport model as described by Wu *et al.* (1993a) and

Table 1 Criteria for degree of remaining smear layer

Score	Criterion
1	Little or no smear layer; covering <25% of the specimen; tubules were visible and patent, or almost complete laser melting
2	Little to moderate or patchy mounts of smear layer; covering 25–50% of the specimen; many tubules visible and patent, or laser melting, or scattered laser melting
3	Moderate amounts of scattered or aggregated smear layer; covering 50–75% of the specimen; minimal to no tubule visibility or patency, or scattered laser melting
4	Heavy smear layer covering >75% of the specimen; no tubule orifices visible or patent, or no visible laser melting

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De Bruyne *et al.* (2005). The roots were connected to a plastic tube with Al-Fix Gel (Novatio Belgium n.v., Olen, Belgium) at both ends and additionally sealed with Quick-Bond (Novatio). The plastic tubes on either side of the specimen were filled with distilled water. A standard glass capillary tube was connected to the plastic tube at the outlet side of the specimen. Using a syringe, water was sucked back into the open end of the glass capillary and an air bubble was created. A head space pressure of 1.2 bar from the inlet side, which was the coronal side of the tooth, was applied to force the water through the voids along the filling, thus displacing the air bubble in the capillary tube. Microleakage (L in μL day^{-1}) was measured for 48 h under a pressure of 1.2 atm and recorded as $L = 0$ (L1), $0 < L \leq 10$ (L2), $L > 10$ (L3).

Rhodamine dye leakage

After obtaining the results from the fluid transport model, the teeth were disconnected and dried. The remnants of the Al-Fix Gel and the Quick-Bond were removed with a sharp scalpel. The cleaned external root surfaces of the teeth of groups 4, 5 and 6 were covered with clear acrylic nail varnish. All surfaces were coated with the nail varnish except the apical 2–3 mm and the coronal opening. The teeth were then immersed in Rhodamine B dye (0.6% solution) (Sigma-Aldrich Chemie GmbH, Steinheim, Germany) at 37 °C for 48 h, after which they were thoroughly washed with running water. The roots were then bisected longitudinally as described previously and observed by stereoscopy (Stemi SR; Zeiss, Oberkochen, Germany) at ×6 magnification with calibrated ocular scale.

Controls

In addition to the specimens of the experimental groups, the root canals of another six teeth were prepared and obturated. The coronal opening as well as the apex were covered with cyanoacrylate glue before all the surfaces of the root were coated with two layers of varnish. These were the negative controls (three for the assessment of coronal leakage by means of the fluid transport model and three for the rhodamine dye leakage testing). In the remaining six teeth, the root canals were not obturated and sealed neither apically nor coronally prior to leakage testing; these were the positive controls.

Statistical analysis

Statistical analysis was performed using the Kruskal–Wallis test for nonparametric data to determine whether there were statistically significant differences between the groups. If statistically significant differences were present, pairs of groups were compared using Dunn tests.

Results

Scanning electron microscopic observation

Table 2 shows the amount of remaining smear layer in all groups. The highest amount of remaining smear layer was found in the unlased group, which served as the control. Group 3 which was the group coated with black ink prior to laser treatment showed the least amounts of remaining smear layer. The scores in the two laser-treated groups were significantly reduced compared with the control ($P < 0.001$).

A typical finding in the lased groups was that the canal walls had open dentinal tubules and melted surfaces; in general the canal walls were free of smear layer and debris (Fig. 1). Nevertheless, a limited number of areas with remnants of smear layer were found (Fig. 2). Figure 3 shows a cross-section at the level of the apical stop. The typical appearance as shown in Fig. 1 can be recognized. Moreover, there are spherical particles of melted and fused smear layer with a glass-like structure.

There was a significant difference between the groups. Groups 2 and 3 had significantly fewer remnants of smear layer than group 1. There was no statistically significant difference between groups 2 and 3.

Leakage study

The negative control teeth showed no transportation of the air bubble in the fluid transport model nor dye penetration. The positive control teeth showed immediate transportation of the air bubble in the fluid transport model and dye penetration along the entire length of the root canal.

Agreement amongst examiners (FDP and MDB) for interpretation of the leakage results for both fluid

Table 2 Smear layer scores

	Score			
Group	1	2	3	4
1 (control)	0	0	2	8
2 (laser without Indian ink)	6	3	1	0
3 (laser with Indian ink)	9	1	0	0

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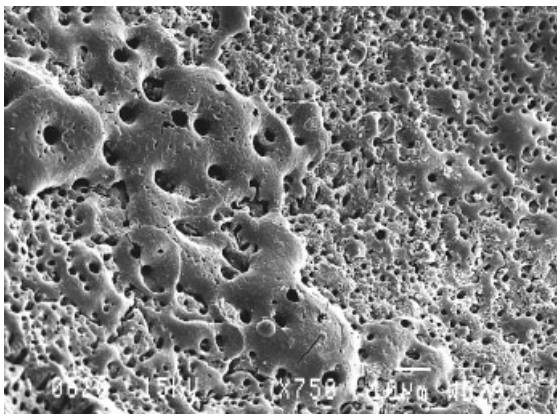

Figure 1 SEM photograph from group 3; area at the apical third of the root canal; patent dentinal tubules are found next to a melted surface area (original magnification ×750, bar represents 10 μm).

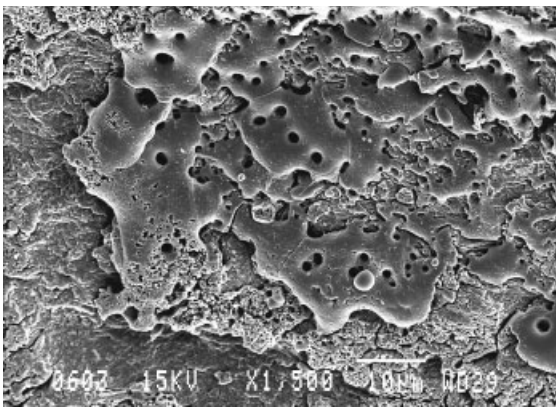

Figure 2 SEM photograph from group 2; areas (at the middle third of the root canal) with remnants of smear layer are observed next to melted surfaces (original magnification ×1500, bar represents 10 μm).

transport model and dye leakage was evaluated using Cohen's kappa ($\kappa = 0.92$). No significant differences amongst the observers were scored, so that the calculation of the average leakage values of the two observers for each root was justified.

Fluid transport model

The leakage results are shown in Table 3. Groups 4 and 5 had no leakage; in group 6, which was the group treated with black ink prior to laser irradiation, two teeth had moderate leakage (L2). No statistically significant differences were found between the different groups.

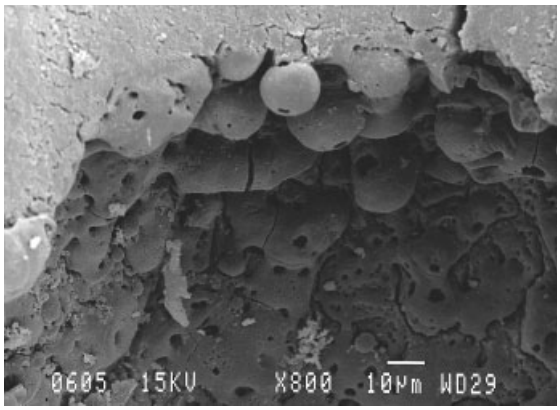

Figure 3 SEM photograph from group 2; area (at the level of the apical stop) with melted and ablated smear layer. A number of spherical particles with a glass-like structure are present (original magnification ×800, bar represents 10 μm).

Table 3 Scores for through-and-through leakage (fluid transport model)

	Score		
Group	L1	L2	L3
4 (control)	10	0	0
5 (laser without Indian ink)	10	0	0
6 (laser with Indian ink)	8	2	0

Table 4 Scores for apical leakage (dye leakage)

	Score					
Group	0	1	2	3	4	5
4 (control)	1	1	4	2	2	0
5 (laser without Indian ink)	1	1	2	2	3	1
6 (laser with Indian ink)	1	1	3	1	2	2

Leakage scores: 0, no leakage detected; 1, up to 0.5 mm; 2, 0.5–1 mm; 3, 1–2 mm; 4, >2 mm leakage; 5, through-and-through leakage pattern.

Dye leakage

As the data indicated a non-normal distribution, leakage was assigned using the following categories: apical leakage: 0 = no leakage detected; 1 = up to 0.5 mm; 2 = 0.5–1 mm; 3 = 1–2 mm; 4 = >2 mm; 5 = through-and-through leakage (Table 4); coronal leakage: 0 = no leakage detected; 1 = up to 1 mm; 2 = 1–2 mm; 3 = 2–4 mm; 4 = >4 mm; 5 = through-and-through leakage (Table 5). No statistically significant differences were found between the different groups. The two teeth showing leakage in the fluid transport model (L2 – group 6) did not show

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Table 5 Scores for coronal leakage (dye leakage)

	Score					
Group	0	1	2	3	4	5
4 (control)	0	0	4	3	3	0
5 (laser without Indian ink)	0	0	2	7	0	1
6 (laser with Indian ink)	0	0	3	2	3	2

Leakage scores: 0, no leakage detected; 1, up to 1 mm; 2, 1–2 mm; 3, 2–4 mm; 4, >4 mm leakage; 5, through-and-through leakage pattern.

infiltration of the dye in eventual cracks, so that the possibility of through-and-through leakage due to a crack could be excluded.

Discussion

The smear layer is a combination of organic and inorganic debris present on the root canal wall after instrumentation (McComb & Smith 1975). This layer has been described as being superficial on the dentinal surface and packed into the dentinal tubules (Gutmann & Witherspoon 2002). Biologically, the presence of the smear layer has been postulated to be an avenue for leakage and a source of substrate for bacterial growth and ingress (Mader *et al.* 1984, Aktener *et al.* 1989). When canals were obturated with thermoplasticized gutta-percha and sealer, the frequency of bacterial penetration in the presence of the smear layer has been shown to be significantly higher than with smear layer removal (Behrend *et al.* 1996). When the smear layer is not removed, it may slowly disintegrate and dissolve around leaking canal filling materials, or it may be removed by bacterial by-products such as acids and enzymes (Sen *et al.* 1995).

Technically the smear layer may interfere with the penetration of gutta-percha into the tubules and the adhesion and penetration of root canal sealers into dentinal tubules. Significant tubular penetration of gutta-percha and sealers has been shown with thermoplasticized obturations (Gutmann 1993). Studies have also shown a decreased incidence of microleakage with gutta-percha and sealer obturations when the smear layer was removed and the gutta-percha was chemically or thermally softened before obturation (Gençoglu *et al.* 1993, Karagöz-Kücükay & Bayirli 1994). A reduction of apical leakage after removal of the smear layer was also reported when using nonchemically or nonthermally softened gutta-percha (Kennedy *et al.* 1986).

Removal of the smear layer in root canals after Nd:YAG laser irradiation and an associated change of the structure of the root canal surfaces has been demonstrated by a number of investigators (Dederich *et al.* 1984, Miserendino *et al.* 1995, Harashima *et al.* 1997, Khan *et al.* 1997, Goya *et al.* 2000), as well as an increase in dentine microhardness, making it more resistant to acid demineralization (White *et al.* 1991). Melted and recrystallized root dentine (Dederich *et al.* 1984, Miserendino *et al.* 1995, Goya *et al.* 2000), and deposition of resolidified silica glass (Miserendino *et al.* 1995) were described. Care has to be taken with the interpretation of some of these findings, as in the study by Dederich *et al.* (1984) the teeth were split longitudinally prior to lasing and therefore the impact of the laser beam was perpendicular to the root-canal wall. This does not represent the clinical application of the beam within an intact root canal. The fibre-optic cable is kept parallel to the root canal wall, with the sides of the fibre in contact with the wall. Such clinical application reduces the energy density the dentine receives, and so may produce a different effect from the findings observed by Dederich *et al.* (1984).

Laser irradiation produces different effects on the same tissue at different parameters and the same laser can produce varying effects in different tissues (Goya *et al.* 2000). These effects are dependent on the power and the mode of the energy delivery system, type and condition of target tissue, size and form of the optical fibre through which the laser beam is transmitted. The contradictory findings as presented by Bahcall *et al.* (1992, 1993) and Saunders *et al.* (1995), who did not demonstrate the removal of smear layer and debris are in this respect to be explained as a result of the different laser parameters used.

As absorption of Nd:YAG laser is enhanced by black ink, it potentiates laser effects on root canal walls (Zhang *et al.* 1998). Morioka *et al.* (1984) tested different black materials to absorb the laser beam at enamel surfaces and found that a waterproof Indian ink was most suitable for a pulsed Nd:YAG laser. Based on this finding, a number of authors advocated the use of black ink to paint the root canal walls prior to lasing with the Nd:YAG laser (Zhang *et al.* 1998, Koba *et al.* 1999). In the present study, it was seen that the use of the Nd:YAG laser at moderate intensity in association with black ink resulted in the cleanest root canal surfaces. The smear layer was ablated or melted, and dentinal tubules were occluded with laser-melted and recrystallized tooth substance. Whereas Goya *et al.* (2000) stated that these surfaces resulted in a better contact between filling material and root canal wall dentine, resulting in a significantly reduced

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leakage in the apical third of the root canals, this finding could not be confirmed in the present study. Moreover, although no statistically significant differences could be demonstrated for coronal leakage assessed with both rhodamine dye and the fluid transport model, a trend towards more (fluid transport model) and higher (dye) leakage than the control (unlased group) and the group of roots lased without Indian ink was observed.

In this study two different methods were used to assess leakage. Previous studies (Wu & Wesselink 1993, Pommel *et al.* 2001, De Bruyne *et al.* 2005) had emphasized the lack of or a limited correlation amongst the results obtained with different leakage evaluation methods. Therefore, dye leakage testing was chosen especially for the assessment of apical leakage and was on the other hand also a means of evaluation of the presence of eventual cracks and fissures in the root. The fluid transport model was chosen as a means of evaluation for through-and-through leakage. A limitation of the fluid transport model is that it does not give information on the extent of coronal voids. Additional information in this respect can than be provided by coronal dye leakage testing.

For reasons of completeness, it has to be mentioned that lasing in combination with black ink under the conditions of the present *in vitro* study resulted in each case in the development of fine fire flashes. The clinical impact of the present observation of fire flashes beyond the apical foramen into the periapical tissues when using the Nd:YAG at moderate intensity and exceeding the apical foramen into the periapical tissues has until now not yet been investigated. Therefore, it cannot be recommended to use the Nd:YAG laser in combination with black dye.

Conclusions

Under the conditions of this *in vitro* study: (i) the smear layer was removed by pulsed Nd:YAG laser irradiation significantly more than in the nonlased specimens ($P < 0.001$); (ii) the use of black ink resulted in a more pronounced removal of smear layer; (iii) Nd:YAG laser irradiation with black ink did not result in a statistically significant reduction of both apical and coronal leakage and cannot be recommended.

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