Stimulatory Effects of CO_2 Laser, Er:YAG Laser and Ga-Al-As Laser on Exposed Dentinal Tubule Orifices

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Summary **We investigated the effects of lasers irradiation on the exposed dentinal tubule. Human tooth specimens with exposed dentinal tubule orifices were used. Three types of lasers (CO_2 laser, Er:YAG laser and Ga-Al-As laser) were employed. The parameters were 1.0 W in continuous-wave mode with an irradiation time of 30 s for the CO_2 laser, 30 mJ in continuous-wave mode with an irradiation time of 60 s for the Er:YAG laser, and 1.0 W in continuous-wave mode with an irradiation time of 60 s for the Ga-Al-As laser. A non-irradiated group was used as a control. After laser irradiation, the dentinal surface of each sample was observed using SEM. Afterwards, all samples were immersed in methylene blue dye solution in order to evaluate the penetration of the dye solution and observe the change in dentinal permeability after laser irradiation. SEM observation showed that the control group had numerous exposed dentinal tubule orifices, whereas these orifices were closed in the laser-irradiated groups. There was consistent dye penetration into the pulp chamber in the control group, whereas no dye penetration was evident in the laser-irradiated groups. Therefore, laser appears to be a promising treatment for reducing permeation through exposed dentinal tubules.**

***Key Words*: laser irradiation, SEM observation, free radical, dentinal tubules, dentinal hypersensitivity**

Introduction

Dentinal hypersensitivity may occur due to loss of the covering enamel and/or cementum after gingival recession, resulting in exposure of the cervical dentine with patency of the dentinal tubules. Dentin surface was dissolved by acids or active oxygen (H_2O_2) [*1*], and then dentinal tubule was exposed. It is thought that this condition will be occurred the dentinal hypersensitivity. However, the exact mechanism responsible for dentinal hypersensitivity is still unclear. The hydrodynamic theory [*2*], which the most widely accepted hypothesis, considers that the stimulus causing convective fluid flow in the dental tubules is related to the permeability

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of dentin. Therefore, one method of treatment is to block the exposed dentinal tubules, and the other is to reduce the excitability of sensory nerves. Pashley *et al.* [*3*, *4*] reported that potassium oxalate was effective for treatment of dentinal hypersensitivity. However, as potassium oxalate dissolves in a relatively short time, any beneficial effect is thought to be only short-term [*5*]. Recently, the clinical use of lasers has increased, and lasers are now employed frequently for endodontic treatment [*6–8*]. Energy from the long-wavelength, non-penetration type CO_2 and Er:YAG lasers is absorbed by water, and is used for surgical applications, treatment of dentinal hypersensitivity, and root canal therapy [*9–13*]. On the other hand, energy from the short-wavelength penetration-type Nd:YAG and Ga-Al-As lasers is not absorbed by water, and therefore suited for coagulation of deep tissue in the body, as well as for surgical treatment, formation of hard tissue, treatment of dentinal hypersensitivity and root canal therapy [*6*, *8*, *13–20*]. Zhang *et al.* [*11*] reported that CO_2 laser irradiation is useful for treatment of cervical dentinal hypersensitivity without thermal damage to the pulp. Lan *et al.* [*16*] reported that Nd:YAG laser treatment can be used to seal exposed dentinal tubules. However, issues such as the effects of laser irradiation on dentinal hypersensitivity have received little attention. Therefore, our research has focused on the transmission of photochemical energy to dentin via three types of lasers. Accordingly, we have performed in vitro investigations of dentinal hypersensitivity using three types of laser irradiation.

The purpose of this study was to evaluate morphologic differences in exposed dentinal tubule orifices in human tooth tissue before and after laser irradiation.

Materials and Methods

Samples

This study was approved by the ethics committee at the Nihon University School of Dentistry at Matsudo, No. EC 03-025. The samples were obtained from the root of an extracted human tooth according to the method of Tsujimoto *et al.* [*21*] Four blocks were obtained from the root of an extracted single cone tooth. The pulp was removed with a K-file, then 15% EDTA was applied to the dentinal wall for a period of 2 min to remove the smear layer. The samples were washed with pure water and blot dried with Kim-wipe paper, were kept in pure water at 37°C. Four blocks were used for each examination (1 cm × 1 cm block).

Laser irradiation

For the first set, a CO_2 laser apparatus (Panalas C10, Panasonic, Tokyo, Japan) with a wavelength of 10.6 µm and a maximum power output of 10 W was used. The samples were irradiated continuously at 1.0 W output power for 30 s (CO_2 laser group) with wet condition. For the second set, a Er:YAG laser apparatus (Erwin, Morita, Tokyo, Japan) with a wavelength of 2.94 µm was used. The samples were irradiated continuously at 30 mJ output power for 60 s with tapping water (Er:YAG laser group). For the third set, a high-energy Ga-Al-As laser apparatus (OSADA-LIGHTSURGE 3000, Osada, Tokyo, Japan) with a wavelength of 0.81 µm and a maximum power output of 3.0 W was used. The samples were irradiated continuously at 1.0 W output power for 60 s, was 60 J/cm^2 (Ga-Al-As laser group) with wet condition. The distance from the tip of the laser apparatus fiber to the sample was 1 cm. As a control, samples without any irradiation were used (control group). Laser irradiation was maintained in an room temperature at 25°C. In this laser irradiated condition, we were decided on pilot study data and the manufacturer's recommended.

Effect of temperature by laser irradiation

We measured change of a temperature in laser irradiation time using thermography (Nippon avionics, Tokyo, Japan).

SEM observation

The samples were dehydrated in a graded ethanol series from 40% to 100%, and in 3-methylbutyl acetate using the critical-point drying method. The dried samples were sputter-coated with a layer of Au-Pt and observed using an S-2150 SEM (Hitachi, Tokyo, Japan) at 15 kV [*1*].

Dye penetration test

Three coats of manicure were applied to all surfaces of the sample except the site of irradiation, and the sample was dried. The samples were then immersed in 2% methylene blue solution (pH 7.4) and left to stand at 37°C for 5 min. Then, the surface of each longitudinally cut sample was observed for dye penetration using a stereoscopic microscope.

Measurement of percentage dye penetration

We measured the distance from the dentin surface of the sample to the pulp chamber and also the distance of max dye penetration part into the dentinal tubule orifices, and calculated the percentage dye infiltration.

Statistical analysis

All values are presented as the mean ± SD, and the significance of differences was determined using Student's *t* test.

Results

Effect of temperature by laser irradiation

In the case of CO_2 laser irradiation, temperature was rised for 5.9°C. On the other hand, the temperature was

Table 1. Effect of temperature by laser irradiation. Dentin surfaces were irradiated by lasers, and the CO_2 laser irradiation caused a maximum temperature rise of 5.9°C. On the other hand, the Er: laser and Ga-Al-As laser irradiation did not cause temperature.

Laser	Irradiation time	Temperature rised (°C)
CO_2 laser	30 s	±5.9
Er:YAG laser	60 s	±0.3
Ga-Al-As laser	60 s	±0.6

almost not changed in both Er:YAG laser and Ga-Al-As laser irradiation (Table 1).

SEM observation

SEM observation showed that the control group had exposed dentinal tubule orifices, almost without a smear layer (Fig. 1a). Samples of dentinal tubules irradiated by the CO_2 laser showed closed dentinal tubule orifices (Fig. 1b). The surfaces of the samples irradiated with the Er:YAG and Ga-Al-As lasers showed sealing of the dentinal tubules (Fig. 1c, d).

Dye penetration test

The control group consistently displayed dye penetration into the pulp chamber (Fig. 2a). The Er:YAG laser group and the Ga-Al-As laser group exhibited slight dye penetration into the pulp chamber (Fig. 2c, d). In the CO_2 laser group, dye penetration into the dentin side tubules was observed (Fig. 2b).

Measurement of percentage dye penetration

The dye penetration after laser irradiation expressed as a percentage was 41.2% in the CO_2 laser group, 14.1% in the Er:YAG laser group, and 21% in the Ga-Al-As laser group. These degrees of dye penetration were significantly less than in the control group ($P<0.01$) (Fig. 3).

Discussion

The cause of dentinal hypersensitivity is not well understood, and as its symptoms are unspecified and subjective, there is no effective treatment. In addition, Kozuka *et al.* [*22*] reported that smear layer into dentinal tubules were omitted by active oxygen and free radical, and that a high concentration of H_2O_2 and longer application time increased the damage to intertubular dentin and peritubular dentin. It is commonly thought that smear layer is role of protect from external irritation. Consequently, the importance of obturating the dentinal tubules in certain clinical conditions is well established. Reducing the number of open tubules or decreasing their diameter is one goal of therapy

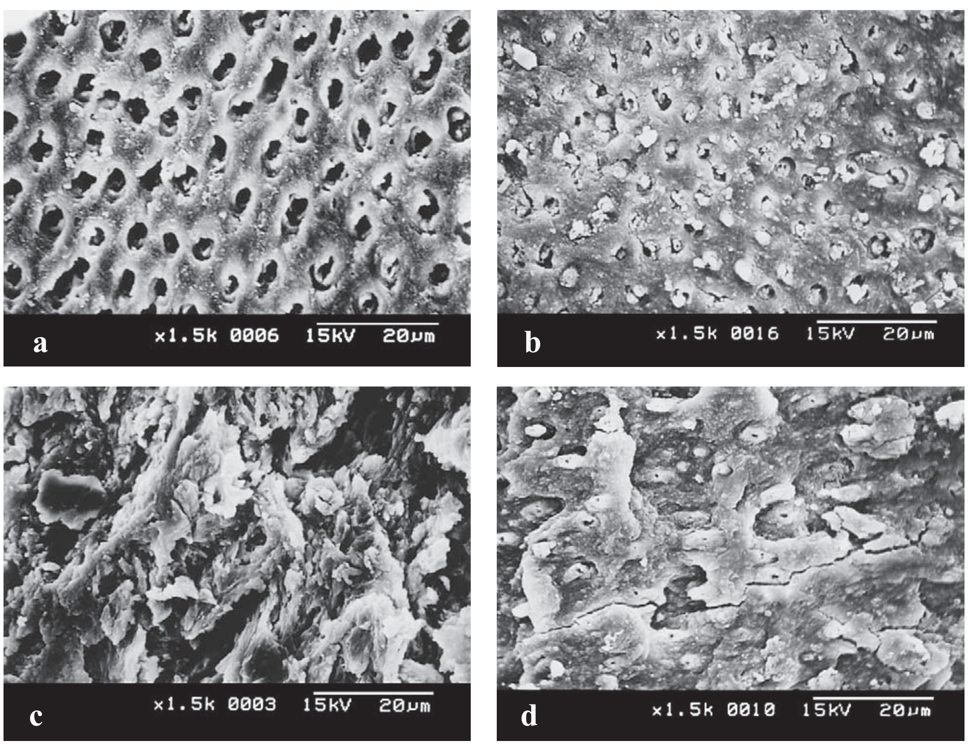

Fig. 1. SEM observation. a: control group, b: CO_2 laser group, c: Er:YAG laser group, d: Ga-Al-As laser group. Control shows exposed dentinal tubule orifices, almost without a smear layer (a). CO_2 laser irradiation has closed the dentinal tubule orifices (b). Er:YAG laser and Ga-Al-As laser treatment has sealed the dentinal tubules (c, d).

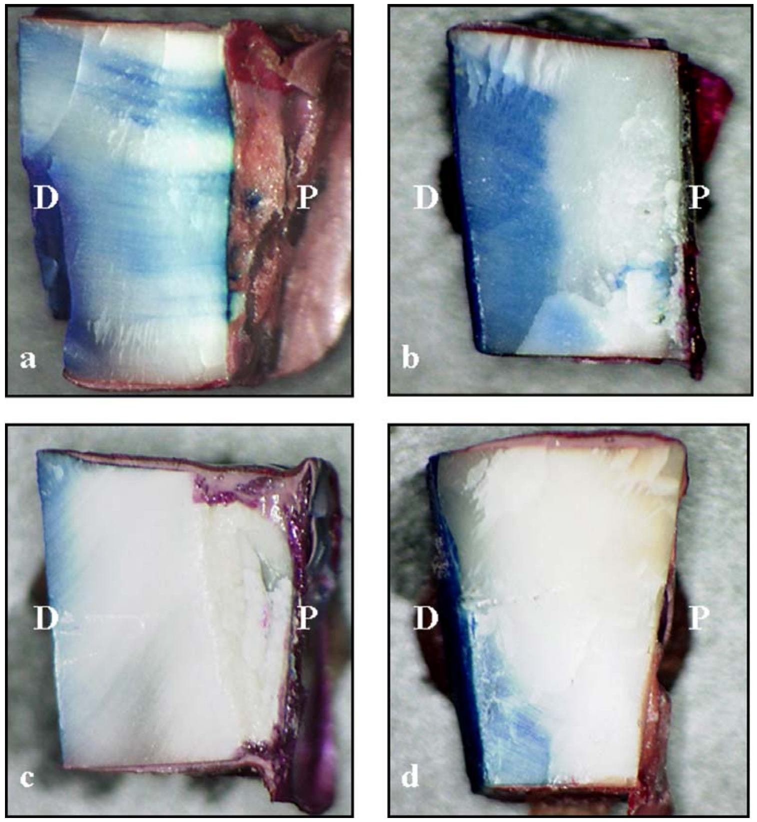

Fig. 2. Dye penetration test. D: dentin side, P: pulp side. a: control group, b: CO_2 laser group, c: Er:YAG laser group, d: Ga-Al-As laser group. The control group consistently displayed dye penetration test into the pulp chamber (a). The Er:YAG laser group and the Ga-Al-As laser group display slight dye penetration into the pulp chamber (c, d), while dye penetration into the dentin side tubules is observed in the CO_2 laser group (b).

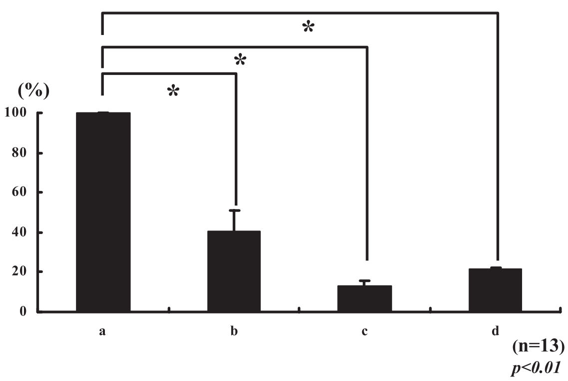

Fig. 3. Measurement of percentage dye penetration. a: control group, b: CO_2 laser group, c: Er:YAG laser group, d: Ga-Al-As laser group. The laser groups all show significantly lower percentage dye penetration than the control group (p<0.01).

for sensitive teeth. Potassium oxalate, resin-bonding and sodium fluoride are some therapeutic tubule-occlusive agents [*3*, *4*]. However, Kerns *et al.* [*5*] have reported that the effects of tubule occlusion are relatively short-term. Therefore, many investigators have been searching for other therapeutic agents or methods that are effective for treatment of dentinal hypersensitivity and are relatively long-lasting. The effects of laser irradiation on dentin in vitro have been studied because laser irradiation can close and seal dentinal tubules, and may possibly reduce dentinal hypersensitivity.

In this study, we examined the utility of three kinds of laser irradiation for closed on exposed dentinal tubule orifice by SEM observation of dye penetration into the dentin surface.

First of all, we checked change of temperature on the dentin surface when using laser apparatus. In the results, only CO_2 laser irradiation was raised temperature within 5.9°C, however others were not changed.

CO_2 laser irradiation of dentinal tubules resulted in smaller tubule orifices. Energy from the non-penetration-type CO_2 laser is absorbed by water, suggesting that water on the dentin surface is evaporated by the treatment. The effects of a CO_2 laser on dentinal hypersensitivity are caused by occlusion or narrowing of the dentinal tubules. Bonin *et al.* [*23*] reported that using the CO_2 laser at 1.0 W moderate energy density resulted mainly in the sealing of dentinal tubules, as well as a reduction of their permeability.

Energy from the non-penetration-type Er:YAG laser is

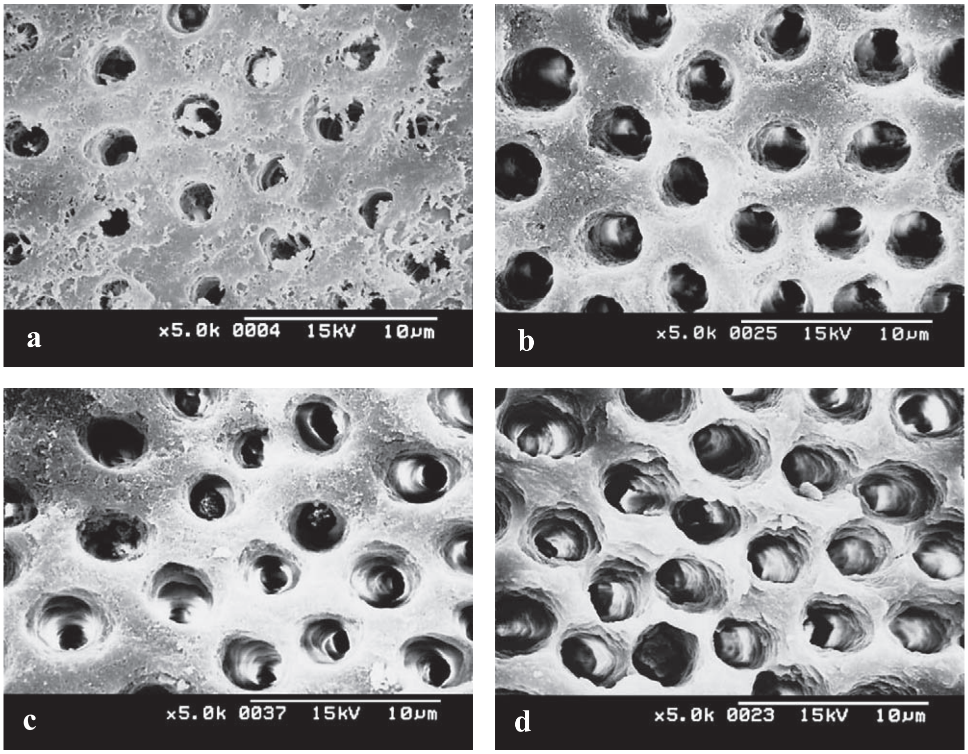

Fig. 4. Treatment with 10 M H_2O_2 after dentin surface. Typical scanning electron micrographs after treatment with 10 M H_2O_2. a: control group, b: H_2O_2 after 1 day, c: H_2O_2 after 5 days, d: H_2O_2 after 10 days.

also absorbed by water. In this study, Er:YAG laser irradiation of dentinal tubules resulted in sealing of their orifices, most likely due to destruction of the hydroxyapatite crystals on the dentin surface. The effects of the Er:YAG laser on dentinal hypersensitivity are due to sealing of the dentinal tubules. Aranha *et al.* [*12*] reported that Er:YAG laser irradiation at 60 mJ is useful for decreasing dentin permeability.

The exact mechanism responsible of dentinal hypersensitivity is still unclear. In the previous reports, free radicals were generated by He-Ne laser and Ga-Al-As laser irradiation with H_2O_2, NaClO [*24*] or cultured cells [*6*]. We supposed when using the non-penetration-type lasers (CO_2 and Er:YAG), free radicals were generated on the surface of dentin, then dentin structure was destroyed and crumbled. Finally, dentinal tubules were obturated these dentin rubbish. Kawamoto *et al.* [*1*] reported that the intertubular dentin and peritubular dentin were dissolved by higher concentrations H_2O_2 and if the H_2O_2 was applied for longer times. And they detected hydroxyl radical from H_2O_2. In this study, we also observed that the intertubular dentin and peritubular dentin were dissolved by H_2O_2 (Fig. 4).

Energy from the penetration-type Ga-Al-As laser is absorbed by water. Hamachi *et al.* [*19*] reported that Ga-Al-As laser treatment reduced thermal dentinal sensitivity by 88.5%, and was useful for treatment of dentinal hypersensitivity. In the present study, Ga-Al-As laser irradiation caused stenosis of the dentinal tubule orifices, most likely due to denaturation of the collagen in dentin. Therefore, the effects of the Ga-Al-As laser on dentinal hypersensitivity are due to stenosis of the dentinal tubules.

In the dye penetration test, the control group consistently displayed dye penetration into the pulp chamber, whereas the laser-irradiated groups did not. Dye penetration into the dentinal tubules was particularly restricted by the Er:YAG and Ga-Al-As lasers, and produced very similar dye penetration images. However, in the CO_2 laser-irradiated samples, slight dye penetration was observed into the dentin side tubules. Because the energy of the non-penetration-type CO_2 laser is absorbed by water, the dentin is dissolved in water on the dentin surface and evaporates on heating, possibly leading to blockade of the dentinal tubule orifices.

On the other hand, energy from the non-penetration-type Er:YAG laser is also absorbed by water. In this case too, the dentin is dissolved, but the water on the dentin surface is evaporated explosively by the laser energy. Energy from the penetration-type Ga-Al-As laser is absorbed by water. It is hypothesized that internal organic matter at the dentin surface is denatured by Ga-Al-As laser irradiation, and that this occludes the dentinal tubule orifices. The present results indicate that laser irradiation can be used to close exposed dentinal tubules. Many previous reports [*6*, *8*, *13*–*20*] have indicated that laser irradiation has curative effects in patients with dentinal hypersensitivity. Laser treatment for dentinal hypersensitivity may have long-term effectiveness, and is simple and quick, with no adverse side-effects. Characteristics make it an effective, robust and attractive therapy for dentinal hypersensitivity. We are studying that the relation of laser irradiation and free radicals generation.

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Abbreviations

SEM, scanning electron microscope; Er:YAG, erbium yttrium aluminum garnet.

References

[1] Kawamoto, K. and Tsujimoto, Y.: Effects of the hydroxyl radical and hydrogen peroxide on tooth bleaching. *J. Endod.*, **30**, 45–50, 2004.

[2] Dowell, P. and Addy, M.: Dentine hypersensitivity—a review: aetiology, symptoms and theories of pain production. *J. Clin. Periodontal.*, **10**, 341–350, 1983.

[3] Pashley, D.H. and Galloway, S.E.: The effects of oxalate treatment on the smear layer of ground surfaces of human dentine. *Arch. Oral. Biol.*, **30**, 731–737, 1985.

[4] Pashley, D.H., Livingston, M.J., Reeder, O.W., and Horner, J.: Effects of the degree of tubule occlusion on the permeability of human dentine in vitro. *Arch. Oral. Biol.*, **23**, 1127–1133, 1978.

[5] Kerns, D.G., Scheidt, M.J., Pashley, D.H., Horner, J.A., Strong, S.L., and Van, Dyke, T.E.: Dentinal tubule occlusion and root hypersensitivity. *J. Periodontol.*, **62**, 421–428, 1991.

[6] Matsui, S., Tsujimoto, Y., and Matsushima, K.: Stimulatory Effects of Hydroxyl Radical Generation by Ga-Al-As Laser Irradiation on Mineralization Ability of Human Dental Pulp Cells. *Biol. Pharm. Bull.*, **30**, 27–31, 2007.

[7] Matsui, S., Sakamoto, M., Kimura, M., Tsujimoto, Y., Ikemi, T., and Matsushima, K.: Effects of temperature elevation to mineralization ability on human dental pulp cells after irradiated by Ga-Al-As laser. *Jpn. J. Endod.*, **27**, 17–22, 2006.

[8] Ohbayashi, E., Matsushima, K., Hosoya, S., Abiko, Y., and Yamazaki, M.: Stimulatory Effect of Laser Irradiation on Calcified Nodule Formation in Human Dental Pulp Fibroblasts. *J. Endod.*, **25**, 30–33, 1999.

[9] Biamontil, A., Moscarini, M., and Stentella, P.: Regression rate of clinical HPV infection of the lower genital tract during pregnancy after laser CO_2 surgery. *Clin. Exp. Obstet. Gynecol.*, **33**, 93–95, 2006.

[10] Schwarz, F., Bieling, K., Nuesry, E., Sculean, A., and Becker, J.: Related Clinical and histological healing pattern of peri-implantitis lesions following non-surgical treatment with an Er:YAG laser. *Lasers. Surg. Med.*, **38**, 663–671, 2006.

[11] Zhang, C., Matsumoto, K., Kimura, Y., Harashima, T., Takeda, F.H., and Zhou, H.: Effects of CO_2 laser in treatment of cervical dentinal hypersensitivity. *J. Endod.*, **24**, 595–597, 1998.

[12] Aranha, A.C., Domingues, F.B., Franco, V.O., Gutknecht, N., and Eduardo, Cde, P.: Effects of Er:YAG and Nd:YAG lasers on dentin permeability in root surfaces: a preliminary in vitro study. *Photomed. Laser. Surg.*, **23**, 504–508, 2005.

[13] Malmstrom, H.S., McCormack, S.M., Fried, D., and Featherstone, J.D.: Effect of CO_2 laser on pulpal temperature and surface morphology: an in vitro study. *J. Dent.*, **29**, 521–529, 2001.

[14] Khabbaz, M.G., Makropoulou, M.I., Serafetinides, A.A., Papadopoulos, D., and Papagiakoumou, E.: Q-switched versus free-running Er:YAG laser efficacy on the root canal walls of human teeth: a SEM study. *J. Endod.*, **30**, 585–588, 2004.

[15] Aksenov, I.V.: Laser energy in abdominal surgery. *Khirurgiia.*, **7**, 41–44, 2006.

[16] Liu, H.C., Lin, C.P., and Lan, W.H.: Sealing depth of Nd:YAG laser on human dentinal tubules. *J. Endod.*, **23**, 691–693, 1997.

[17] Lan, W.H., Liu, H.C., and Lin, C.P.: The combined occluding effect of sodium fluoride varnish and Nd:YAG laser irradiation on human dentinal tubules. *J. Endod.*, **25**, 424–426, 1999.

[18] Lan, W.H., Lee, B.S., Liu, H.C., and Lin, C.P.: Morphologic study of Nd:YAG laser usage in treatment of dentinal hypersensitivity. *J. Endod.*, **30**, 131–134, 2004.

[19] Hamachi, T., Iwamoto, Y., Hirofuji, T., Kabashima, H., and Maeda, K.: Clinical Evaluation of GaAlAs-semicondoctor Laser in the Treatment of Cervical Hypersensitivity Dentin. *Jpn. J. Conserv. Dent.*, **35**, 12–17, 1992.

[20] Anjo, T., Ebihara, A., Takeda, A., Takashina, M., Sunakawa, M., and Suda, H.: Removal of two types of root canal filling material using pulsed Nd:YAG laser irradiation. *Photomed. Laser. Surg.*, **22**, 470–476, 2004.

[21] Tsujimoto, Y., Kozuka, M., Hitotsune, N., and Yamazaki, M.: Removal capability of smear layer of root canal by NaClO and H_2O_2 mixed solution. *Jpn. J. Endo.*, **18**, 19–24, 1997.

[22] Kozuka, M., Kawamoto, K., Miura, H., Tsujimoto, Y., and Yamazaki, M.: Effect of H_2O_2 for use of tooth bleaching to root canal dentin surface: Examination of temperature and application time by SEM. *Jpn. J. Endo.*, **22**, 16–19, 2001.

[23] Bonin, P., Boivin, R., and Poulard, J.: Dentinal permeability of the dog canine after exposure of a cervical cavity to the beam of a CO_2 laser. *J. Endod.*, **17**, 116–118, 1991.

[24] Kashima-Tanaka, M., Tsujimoto, Y., Kawamoto, K., Senda, N., Ito, K., and Yamazaki, M.: Generation of free radicals and/or active oxygen by light or laser irradiation of hydrogen peroxide or sodium hypochlorite. *J. Endod.*, **29**, 141–143, 2004.