

Laser Induced Explosive Vapor and Cavitation Resulting in Effective Irrigation of the Root Canal. Part 1: A Visualization Study

Jan Blanken, DDS,^{1†} Roeland Jozef Gentil De Moor, DDS, MSc, PhD,^{2**‡} Maarten Meire, DDS, MSc,^{2‡} and Rudolf Verdaasdonk, PhD^{3†}

¹Department of Dental Materials Sciences, Academic Centre for Dentistry Amsterdam (ACTA),

University of Amsterdam and VU University Amsterdam, Louwesweg 1, 1066 EA Amsterdam, The Netherlands

²Department of Operative Dentistry and Endodontology, Ghent Dental Laser Center, Dental School, Ghent University, De Pintelaan 185/P8, B-9000 Gent, Belgium

³Department of Medical Technology & Clinical Physics, University Medical Centre, PO Box 85500, 3508 GA Utrecht, The Netherlands

Background and Objectives: Limited information exists regarding the induction of explosive vapor and cavitation bubbles in an endodontic rinsing solution. It is also not clear whether a fiber has to be moved in the irrigation solution or can be kept stationary. No information is available on safe power settings for the use of cavitation in the root canal. This study investigates the fluid movements and the mechanism of action caused by an Er,Cr:YSGG laser in a transparent root model.

Material and Methods: Glass models with an artificial root canal (15 mm long, with a 0.06 taper and apical diameter of 400 µm) were used for visualization and registration with a high-speed imaging technique (resolution in the microsecond range) of the creation of explosive vapor bubbles with an Er,Cr:YSGG laser at pulse energies of 75, 125, and 250 mJ at 20 Hz using a 200 µm fiber (Z2 Endolase). Fluid movement was investigated by means of dyes and visualization of the explosive vapor bubbles, and as a function of pulse energy and distance of the fiber tip to the apex.

Results: The recordings in the glass model show the creation of expanding and imploding vapor bubbles with secondary cavitation effects. Dye is flushed out of the canal and replaced by surrounding fluid. It seems not necessary to move the fiber close to the apex.

Conclusion: Imaging suggests that the working mechanism of an Er,Cr:YSGG laser in root canal treatment in an irrigation solution can be attributed to cavitation effects inducing high-speed fluid motion into and out the canal. *Lasers Surg. Med.* 41:514–519, 2009.

© 2009 Wiley-Liss, Inc.

Key words: absorption; endodontics; Er,Cr:YSGG; fiber optics; laser dentistry; root canal; smear layer

INTRODUCTION

A wide spectrum of possible strategies exists for attaining the goal of removing the canal contents and eliminating infection. They all have in common that there is a chemo-

mechanical preparation for each strategy: at the basis root canal instruments are used for shaping and cleaning, in addition irrigants are needed for cleaning and disinfecting and especially in these areas that cannot be reached by instruments or are insufficiently cleaned. So irrigation is a very important part of root canal treatment procedures. Hand irrigation, however, is not so effective in the apical part of the root canal, nor in oval extensions, isthmuses, and anastomoses [1–7]. In order to enhance the spreading of the irrigant and to activate irrigants sonic and ultrasonic activation have been investigated and promoted [8–11].

Lasers have been proposed as or an alternative for the conventional approach in cleaning, disinfecting and even shaping of the root canal or as an adjuvant to conventional chemo-mechanical preparation in order to enhance debridement and disinfection [12–15].

Several wavelengths are associated with bactericidal effects [16–19]. Some are used to remove or to modify the smear layer after root canal preparation [20–24]. All these studies have in common that the desired effects are the result of photo-thermal effects since the laser devices were used without air and/or water cooling and depending on the laser-tissue/target interaction also more or less on absorption. Because of the rather high intensities required for disinfection and smear layer removal, potential concerns exist regarding heating of dentin. Various coolants and irrigants can be used during intra-canal laser treatment to reduce thermal stress to the radicular dentin and to the periodontium [25].

Another manner to remove smear layer and to disrupt the biofilm is the use of ultrasound: with a small file or

[†]Assistant Professor.

[‡]Professor.

*Correspondence to: Roeland De Moor, PhD, Department of Operative Dentistry and Endodontology, Dental School, Ghent University, Ghent University Hospital, De Pintelaan 185/P8, B-9000 Gent, Belgium. E-mail: roeland.demoor@ugent.be

Accepted 19 June 2009

Published online 28 July 2009 in Wiley InterScience

(www.interscience.wiley.com).

DOI 10.1002/lsm.20798

smooth wire (size 10–20) oscillating freely in the root canal to induce powerful acoustic microstreaming smear layer can be removed from the root canal walls as the result of the production of shear stresses along the root canal wall [11]. It was also demonstrated that ultrasound may result in acoustic cavitation, that is, the creation of new bubbles or the expansion, contraction and/or distortion of pre-existing bubbles in a liquid. So explosions and implosions generating pressure waves which create shear stress along the root canal walls, which may be sufficient to remove smear layer and biofilm are of interest. A previous study of Blanken and Verdaasdonk [26] estimated that when an Er,Cr:YSGG laser is used within the canal with plain endodontic tip (Biolase Z4 Endotip), fluid movement within the root canal occurs immediately following each pulse, with fluid speeds up to 20 m/second (72 km/hour). The working mechanism of the Er,Cr:YSGG laser in the root canal was attributed to vapor bubble expansion and implosion with secondary cavitation effects inducing these high-speed fluid motions into and out the canal. It was also demonstrated that the thermal components were moderate. As more research is needed to clarify the underlying physical mechanisms the present study was undertaken using high-speed imaging at a timescale relevant to the cleaning process.

MATERIALS AND METHODS

High-Speed Imaging Set-Up

Pulsed laser induced vapor bubble formation and cavitation are very fast processes that evolve within microseconds. To visualize this process very fast cameras are needed or a high-speed imaging technique. In this study, an alternative high-speed imaging technique was used which was developed and described by Verdaasdonk et al. [27] With this method, laser–tissue interaction is visualized in real time by capturing images at preset delays during the course of action.

The optical set-up is shown in Figure 1. At the onset of a laser pulse from an Er,Cr:YSGG laser, (Waterlase Millennium, Biolase Technology, San Clemente, CA) (2,780 nm), a signal was sent through an amplifier and a programmed time-delay box to trigger a flash lamp (Portable stroboscope Type 4912, maximum 200 Hz) (Brüe & Kjaer, Naerum,

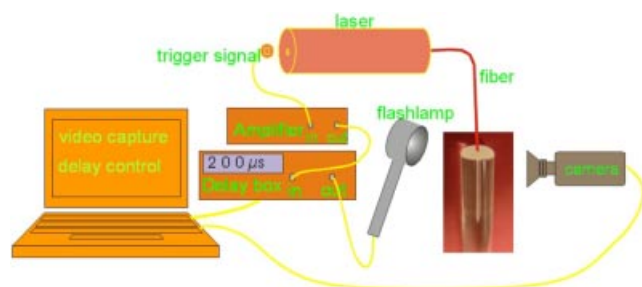


Fig. 1. Optical set-up: the Er,Cr:YSGG laser is attached to through an amplifier and a time delay box to a flash lamp. The light source provides 1 microsecond light flashes with a repetition rate up to several hundreds Hz. [Figure can be viewed in color online via www.interscience.wiley.com.]

Denmark). This light source provides 2 microseconds light flashes with a repetition rate up to several hundreds Hz. This delay box was programmed to extend the delay at every next laser pulse with preset steps of 1 microsecond up to millisecond. The captured images are combined to a movie sequence to show the dynamics of the vapor bubbles in a time range from 1 to 300–3,000 microseconds. The aiming beam from the laser was shut off which would interfere with the imaging. A 25 frames per second CCD camera (C-cam technologies BC 1 15-u-B-40, Vector International, Leuven, Belgium) with a collimating and an imaging lens was used to capture an image of the target with the time resolution of the flash (2 microseconds).

By video editing these recordings (Mpeg Video Wizard Womble Multimedia, Cupertino, CA, USA), films and snap shots were made of cavitation bubbles during their life cycle. In this manner every stage of the life cycle of a vapor bubble down to 1 microseconds could be visualized.

Root Canal Models

To simulate and visualize the conditions within a root canal, glass cylinder and glass blocks with a lumen shaped like a root canal were used. The inner diameter of the apex was 400 μm , the taper was 0.06 mm and the length of the canal was 15 mm. To create controlled conditions, the glass models were submerged in water while making the recordings to prevent interference from air. The canal was filled with water, sodium hypochlorite 2.5% or a red colored dye and remaining small air bubbles were removed as thoroughly as possible. The root canal irrigant was degasified to prevent the formation of gas bubbles during the experiments.

Fiber Tip

Fiber tips made of silica with a diameter of 200 μm and lengths of 25.28 and 33 mm as are used in endodontic treatment were used. The fiber tips were fixed in the hand piece of the Er,Cr:YSGG laser and positioned above the water container. The tip was either submerged in water or put into the root canal model.

Experiment 1—Video Recordings in the Root Canal Model for Visualization of the Cavitation

High-speed imaging was performed of two times (water vs. NaOCl) 15 sets of five glass blocks per investigated power setting, submerged under water in a transparent container. Only 200 μm , Z2 fibers were used. Video sequences were recorded of bubble expansion and implosion in a time range of 10–1,000 microseconds, typically with steps of 10 microseconds. The pulse energy ranged from 12.5 to 125 mJ at 20 Hz (0.25–2.5 W) (a series of five blocks was investigated per increase of 12.5 mJ). The pulse energies have to be corrected for energy loss with a calibration factor of 0.3 for the 200 μm fibers according to the manufacturer's information.

Experiment 2—Test With Dyes

Thirty canals (6 \times 5 canals) were filled with a red dye and the glass blocks were submerged under water. The 200 μm

fiber was inserted. Two modalities were studied. In the first experiment (T1) the fiber was moved out of the canal by hand in 5 seconds. The fiber was reinserted in the canal and the procedure was repeated. The laser was only fired when the fiber was moved out of the canal, away from the apex. In the second experiment (T2) the fiber was kept stationary at 5 mm distance from the apex of the canal. The laser was activated for 5 seconds. After a 5 seconds pause this procedure was repeated until the canal was clean. In a previous study it was demonstrated that at least 75 mJ was needed to remove dye from a root canal within seconds. Pulse energies investigated in this study were 75 mJ (T1.1, T2.1), 125 mJ (T1.2, T2.2), and 250 mJ (T1.3, T2.3) at 20 Hz.

RESULTS

Experiment 1—Video Recordings in the Root Canal Model for Visualization of the Cavitation

A number of representative images are shown. Figures 2 and 3 show the captured images of expanding and imploding vapor bubbles inside the root canal model from 0 to 750 μ J for a sequence of 12.5 mJ at 20 Hz and 75 mJ at 20 Hz. In both figures the onset of the explosive vapor bubble is shown in the third frame from the left. Figures 4 and 5 show an air occlusion being compressed when the bubble grows and expands (25 and 50 mJ at 20 Hz). In Figure 6 sequence at 125 mJ at 20 Hz is shown over a 0–2,000 microseconds time period. The water during the exposure time of around 130 microseconds is turned instantly into water vapor. The small canal prevents the vapor from expanding freely laterally, pushing the water both forward and backward in the canal. The forward pressure can be easily observed in the first three frames of each sequence showing an air bubble, present in the canal, being compressed to a flat disk. Since the water obstructs the expansion of vapor in the forward direction, the bubble also grows backwards along the fiber. The pressure inside the bubble remains high for a long time, since it has to fight against the resistance of the irrigant which has to be displaced in the small canal. This process delays the dynamics of expansion and implosion. The expanding bubble is clearly visible. Figures 2–6 show the presence of an air bubble included at the apex of the canal. During implosion of the vapor bubble, the formation of new bubbles can be observed near the apex (Figs. 5–6) attributed to cavitation effects due to low pressure as a secondary effect

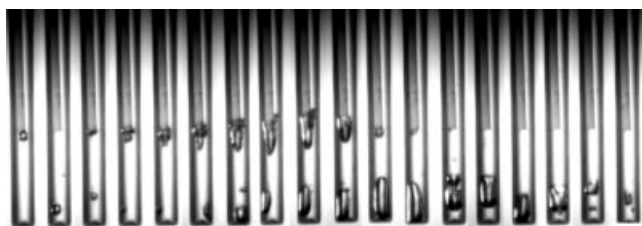


Fig. 2. High-speed sequence in an artificial root canal in a glass block—fiber of 200 μ m, 12.5 mJ, 20 Hz, 0–750 microseconds from the start of the pulse.

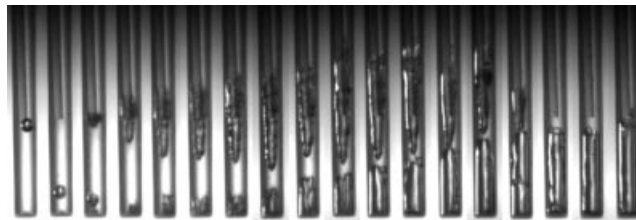


Fig. 3. High-speed sequence in an artificial root canal in a glass block—fiber of 200 μ m, 75 mJ, 20 Hz, 0–750 microseconds from the start of the pulse.

of the imploding vapor bubble. Fluid turbulence remains for a longer time after the actual laser pulses up to several milliseconds.

The findings were identical for the cavitation effects in both water and NaOCl in the root canals.

Figure 7 shows the trend line of the lifetime of a vapor bubble in the root canal model until implosion for the 200 μ m fiber used at 20 Hz. In Figure 8 the fluid velocity was calculated based on the measurement of bubble growth and collapse versus time. At 75 mJ fluid velocities of 21 m/second were derived.

Experiment 2—Test With Dyes

At 75 mJ all dye was removed from the canal during five times 5 seconds of laser activation moving the fiber out of the canal. At 125 and 250 mJ, three and one movements were needed to free the canal from dye.

When the fiber was kept stationary half way in the canal with pulse energies of 125 and 250 mJ the same radiation dose was needed. Only the low energy setting of 75 mJ was too low to totally free the canal from dye. More than six times 5 seconds activation time were applied and even then the canal was only partially free from dye.

In Figure 9 the effect of a sequence of 250 mJ at 20 Hz and moving the fiber out of the canal is demonstrated. A clean canal is seen within one sequence of 5 seconds.

The findings described for the removal of the dye are summarized in Table 1.



Fig. 4. An air inclusion being compressed when the bubble grows and expands—settings: 25 mJ, 20 Hz.

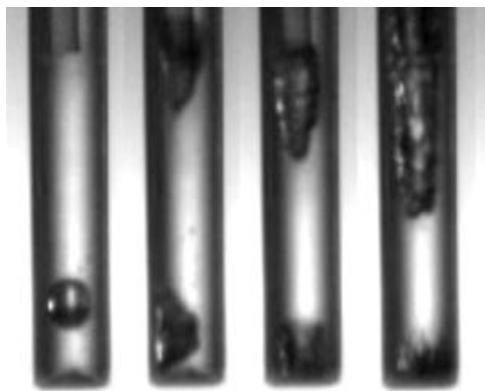


Fig. 5. An air inclusion being compressed when the bubble grows and expands—settings: 50 mJ, 20 Hz.

DISCUSSION

The high-speed imaging method as applied in this study enables capturing of images with microsecond resolution. Although each image is captured from a different pulse of the laser, the dynamics of the bubble formation has proven to be reproducible in the in vitro setting used for this study.

The present high-speed recordings have demonstrated that vaporization of the irrigant will result in the formation of vapor bubbles, which expand and implode with secondary cavitation effects. An interesting finding from the endodontical point of view is that the creation of bubbles is identical in both water and the sodium hypochlorite solution. The presence of water or the sodium hypochlorite solution is necessary. When the radiation is not absorbed by water there was no bubble, no cavitation, no pressure being build-up, and no fluid motion.

The Er,Cr:YSGG laser emits its energy in pulses of about 130 microseconds long. At the beginning of the laser pulse, the energy is absorbed in a 2 μm -thick layer that is instantly super heated to boiling temperature (100°C) at high pressure and turned into vapor. This vapor at high pressure starts to expand at high speed and provides an opening in front of the fiber for the laser light. As the laser continues to emit energy, the light passes through the bubble and evaporates the water surface at the front of the bubble. In this way it “drills” a channel through the liquid until the pulse ends after about 140 microseconds. This mechanism

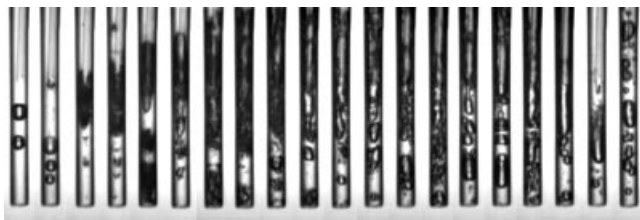


Fig. 6. High-speed sequence in a artificial root canal in a glass block-fiber of 200 μm , 125 mJ, 20 Hz, 0–2,000 microseconds from the start of the pulse.

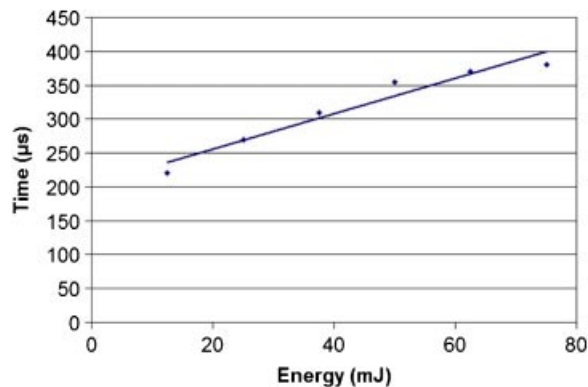


Fig. 7. Trend line of the lifetime of a vapor bubble in the root canal model until implosion (200 μm fiber, 20 Hz, different powers).

is well known and has been referred to as “the Moses effect in the microsecond region” by van Leeuwen et al. [28].

As the energy source stops, the vapor cools and starts condensing, while the momentum of expansion creates a lower pressure inside the bubble. Liquid surrounding the bubble is accelerated to fill in the gap. Near the fiber tip, where the expansion started, the bubble implosion begins, first resulting in separation of the bubble from the fiber. Consequently, the water seems to rush into the bubble from the back, making the imploding bubble shaped like a sickle. After 260 microseconds, the process of implosion is finished and the bubble has vanished. This bubble mechanism has shown to be reproducible at each pulse in a free water environment. A free expansion of the bubble laterally is not possible in the root canal model, and hence the water is pushed forward and backward in the canal. The forward pressure can be easily observed in the first three frames of each sequence showing an air bubble, present in the canal, being compressed to a flat disk. Since the water obstructs the expansion of the vapor in the forward direction, the bubble grows backwards along the fiber. The pressure

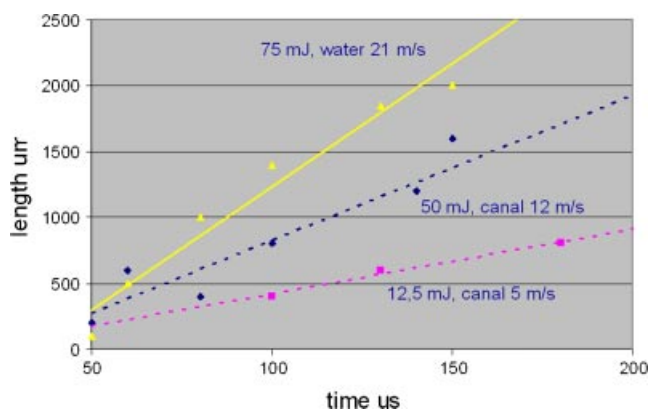


Fig. 8. Fluid velocities calculated from the high-speed imaging sequences. [Figure can be viewed in color online via www.interscience.wiley.com.]

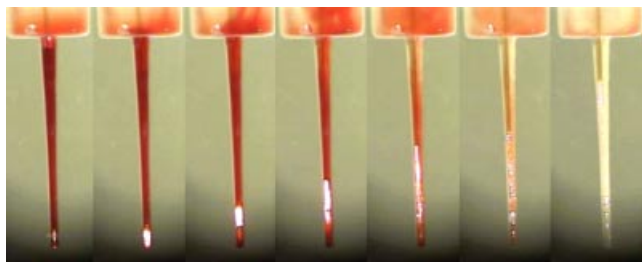


Fig. 9. Removal of the red dye out of the canal—fiber is moved out of the canal, power settings: 250 mJ/20 Hz—1 sequence of 5 seconds. [Figure can be viewed in color online via www.interscience.wiley.com.]

inside the bubble remains high for a long time, since it has to fight against the resistance of the water which has to be displaced in the small canal. This process delays the dynamics of expansion and implosion compared to a free water situation. In a previous study it was demonstrated that this process takes three times longer [26]. So the lateral and forward expansion in the root canal is limited by the root canal wall, while the backward expansion is blocked by the fiber making the lumen of the canal even smaller. These differences with a free water situation result in the creation of shear stress along the wall of the canal, which should be sufficient to remove smear layer. With the present high-speed fluid motion secondary cavitation bubbles will also be induced at irregularities along the root canal wall. The implosion of the primary and secondary bubbles creates microjets in the fluid aimed at the wall with very high forces locally. This process is in fact similar to what happens in ultrasound baths for cleaning of instruments. This mechanism might also contribute to the disruption of cells and the smear layer at the wall.

Data obtained from the models filled with a dye showed that the difference between keeping the fiber stationary in the canal or moving it out of the canal was absent for higher pulse energies. Previous research [26] showed vapor bubbles up to 4.5 mm length caused by this laser and for the 200 μ m fiber used in this study 3.0–3.5 mm bubble lengths. This means that the laser fiber can be kept a number of millimeters away from the apex of a tooth to have the bubble effect. The present findings are confirmed by George and Walsh [25] and Matsuoka et al. [29] who emphasized that the use of pulsed lasers to create pressure waves in irrigant fluids within the root canal may result in apical extrusion of liquid if the fiber tip is held too close to

TABLE 1. Overview of the Efficacy of the Removal of the Red Dye Out of the Root Canal With Different Protocols

| Pulse energy (mJ) | Fiber moved out of the canal (second) | Fiber kept stationary (second) |
|-------------------|---------------------------------------|--------------------------------|
| 75 | 5 × 5 | > 6 × 5 |
| 125 | 3 × 5 | 3 × 5 |
| 250 | 1 × 5 | 1 × 5 |

the apical foramen which is confirmed in other studies by the authors.

Studies using Er,Cr:YSGG lasers with water spray coolant have demonstrated that some collateral damage appeared to be evident when high energy levels were used [30,31]. An explanation might be that due to the dimensions of the vapor bubbles at these higher energy levels there is no water between fiber and canal wall to absorb the emitted energy that can be absorbed therefore by hydroxyapatite in the canal wall. Another explanation might be wall contact of the apex of the fiber.

CONCLUSION

This study clearly indicates that an Er,Cr:YSGG laser when used with a water spray or an NaOCl solution creates explosive vapor bubbles and cavitation effects. This is due to a phase change that is caused by the efficient absorption of the mid Infrared wavelength by water. These bubbles have a volume that is about 1,600 times the original volume. In a narrow canal therefore a pressure gradient will arise that will act as a fluid pump. When the vapor bubble implodes the process works opposite.

Fluid velocities are very high and are likely to cause secondary cavitation along canal walls where irregularities are present that can cause shear stresses in the fluid passing it.

ACKNOWLEDGMENTS

The authors want to thank Rick Mansvelt Beck from the department of biomedical engineering of the University Medical Centre in Utrecht for his help and cooperation.

REFERENCES

- Ram Z. Effectiveness of root canal irrigation. *Oral Surg Oral Med Oral Pathol* 1977;44:306–311.
- Salzgeber RM, Brilliant JD. An in vitro evaluation of the penetration of an irrigation solution in root canals. *J Endod* 1977;3:394–398.
- Abou-Rass M, Patonai FJ. The effects of decreasing surface tension on the flow of irrigating solutions in narrow root canals. *Oral Surg Oral Med Oral Pathol* 1982;3:524–526.
- Druttman AC, Stock CJ. An in vitro comparison of ultrasonic and conventional methods of irrigant replacement. *Int Endod J* 1989;22:174–178.
- Cameron JA. Factors affecting the clinical efficiency of ultrasonic endodontics: A scanning electron microscopy study. *Int Endod J* 1995;28:47–53.
- Lee S-J, Wu M-K, Wesselink PR. The efficacy of ultrasonic irrigation to remove artificially placed dentin debris from different sized simulated plastic root canals. *Int Endod J* 2004;37:607–612.
- Burleson A, Nusstein J, Reader A, Beck M. The in vivo evaluation of hand/rostry/ultrasound instrumentation in necrotic, human mandibular molars. *J Endod* 2007;33:782–787.
- Lumley PJ, Walmsley AD, Laird WRE. Streaming patterns around endosonic files. *Int Endod J* 1991;24:290–297.
- Roy RA, Ahmad M, Crum LA. Physical mechanisms governing the hydrodynamic response of an oscillating ultrasonic file. *Int Endod J* 1994;27:197–207.
- Sabins RA, Johnson JD, Hellstein JW. A comparison of the cleaning efficacy of short-term sonic and ultrasonic passive irrigation after hand instrumentation in molar root canals. *J Endod* 2003;29:674–678.

11. van der Sluis LMW, Wu M-K, Versluis M, Wesselink PR. Passive ultrasonic irrigation of the root canal: A review of the literature. *Int Endod J* 2007;40:415–426.
12. Kimura Y, Wilder-Smith P, Matsumoto K. Lasers in endodontics: A review. *Int Endod J* 2000;33:173–185.
13. Stabholz A, Sahar-Helft S, Moshonov J. Laser in endodontics. *Dent Clin North Am* 2004;48:809–832.
14. Meire M, De Moor RJG. Lasers in endodontics: Laser disinfection, an added value ? *ENDO* 2007;1:159–172.
15. De Moor RJG, Torbeyns D, Meire M. Lasers in endodontics. Part 2: Root canal wall cleanliness and modification. *ENDO* 2009;3:19–33.
16. Moritz A, Gutknecht N, Goharkhay K, Schoop U, Wernisch J, Sperr W. In vitro irradiation of infected root canals with a diode laser: Results of microbiologic, infrared spectro-metric, and stain penetration examinations. *Quint Int* 1997;28:205–209.
17. Moritz A, Gutknecht N, Schoop U, Goharkhay K, Dörtbudak O, Sperr W. Irradiation of infected root canals with a diode laser in vivo: Results of microbiological examinations. *Lasers Surg Med* 1997;21:221–226.
18. Schoop U, Moritz A, Kluger W, Nedjelic N, Georgopoulos A, Sperr W. The Er:YAG laser in endodontics: Results of an in vitro study. *Lasers Surg Med* 2002;30:360–364.
19. Schoop U, Kluger W, Moritz A, Patruta S, Goharkhay K, Sperr W, Wernisch J, Gattringer R, Mrass P, Georgopoulos A. Bactericidal effect of different laser systems in the deep layers of dentin. *Lasers Surg Med* 2004;35:111–116.
20. Takeda FH, Harashima T, Kimura Y, Matsumoto K. Comparative study about the removal of smear layer by three types of laser devices. *J Clin Laser Med Surg* 1998;16:117–122.
21. Depraet FJHW, De Bruyne MAA, De Moor RJG. The sealing ability of an epoxy resin root canal sealer after Nd:YAG laser irradiation of the root canal. *Int Endod J* 2005;38:302–309.
22. Camargo SE, Valera MC, Camargo CH, Fonseca MB, Menezes MM. Effects of Nd:YAG laser irradiation on root canal wall dentin: A scanning electron microscopic study. *Photomed Laser Surg* 2002;23:399–404.
23. Gurbuz T, Ozdemir Y, Kara N, Zehir C, Kurudirek M. Evaluation of root canal dentin after Nd:YAG laser irradiation and treatment with five different irrigation solutions: A preliminary study. *J Endod* 2008;34:318–321.
24. Moura-Netto C, Antonio MPS, Moura AAM, Davidowivcz H, Carvalho CF, Lage-Marques JL. Morphologic analysis of dentin surfaces on apical third after Nd:YAG laser and diode laser irradiation. *Photomed Laser Surg* 2008;26:263–266.
25. George R, Walsh LJ. Apical extrusion of root canal irrigants when using Er:YAG and Er,Cr:YSGG lasers with optical fibers: An in vitro dye study. *J Endod* 2008;34:706–708.
26. Blanken JW, Verdaasdonk RM. Cavitation as a working mechanism of the Er,Cr:YSGG laser in endodontics: A visualisation study. *J Oral Laser Appl* 2007;7:97–106.
27. Verdaasdonk RM, van Swol CFP, Grimbergen MCM, Rem AI. Imaging techniques for research and education of thermal and mechanical interactions of lasers with biological and model tissues. *J Biomed Optics* 2006;11: 041110-1/13.
28. van Leeuwen TG, van de Veen MJ, Verdaasdonk RM, Borst C. Non contact tissue ablation by Holmium:YSGG laser pulses in blood. *Lasers Surg Med* 1991;11:26–34.
29. Matsuoka E, Jayawardena JA, Matsumoto K. Morphological study of the Er,Cr:YSGG laser for root canal preparation in mandibular incisors with curved root canals. *Photomed Laser Surg* 2005;23:480–484.
30. Ali MN, Hossain M, Nakamura Y, Matsuoka E, Kinoshita J-I, Matsumoto K. Efficacy of root canal preparation with Er,Cr:YSGG laser irradiation with crown-down technique *in vitro*. *Photomed Laser Surg* 2005;23:196–201.
31. Althundasar E, Ozelik B, Cehreli ZC, Matsumoto K. Ultramorphological and histochemical changes after Er,Cr:YSGG laser irradiation and two different irrigation regimes. *J Endod* 2006;32:465–468.