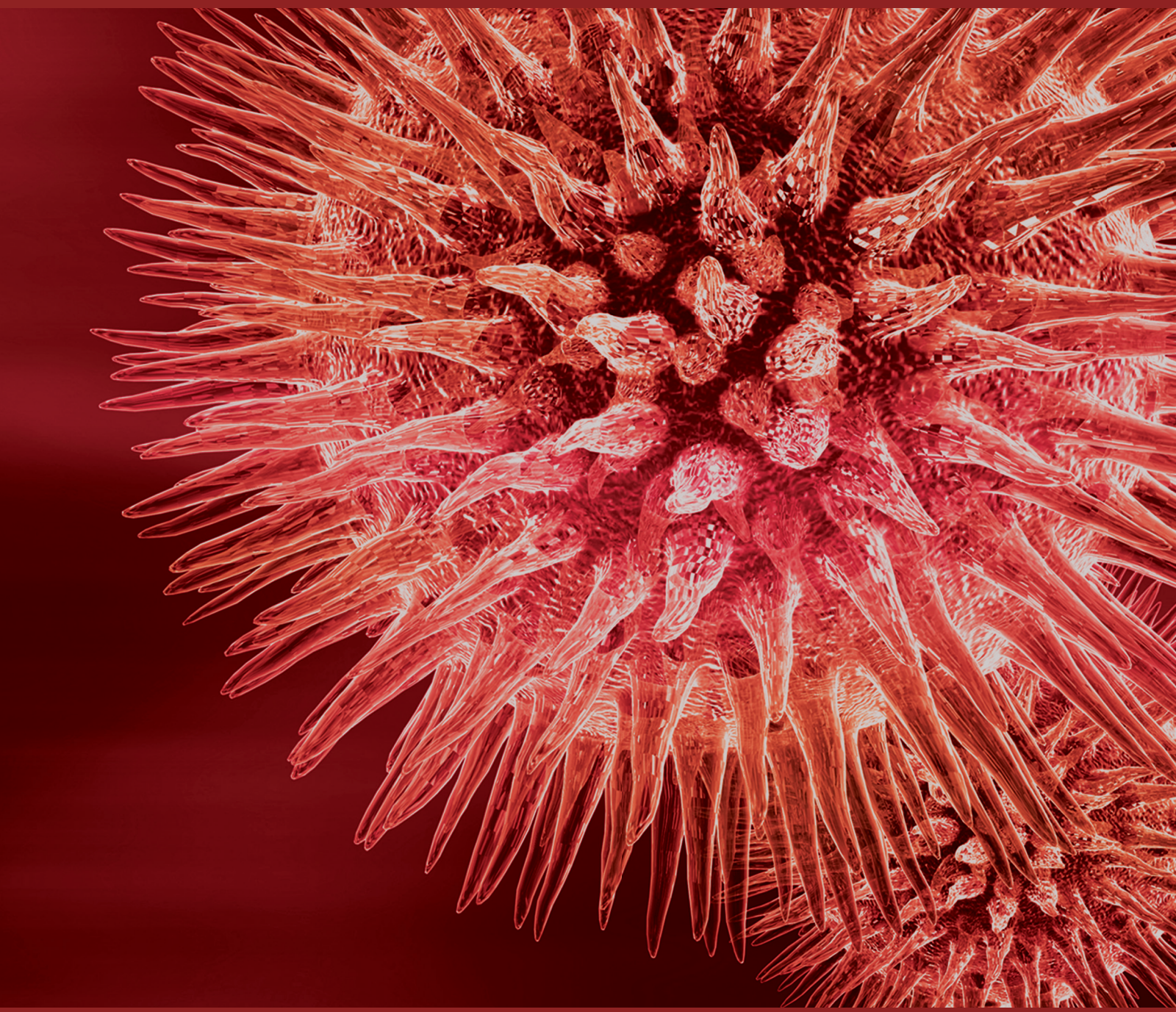
BioMed Research International

# Oral Biology, Oral Pathology, and Oral Treatments

Guest Editors: Samir Nammour, Toni Zeinoun, Kenji Yoshida, and Aldo Brugnera Junior



# Oral Biology, Oral Pathology, and Oral Treatments

BioMed Research International

# Oral Biology, Oral Pathology, and Oral Treatments

Guest Editors: Samir Nammour, Toni Zeinoun, Kenji Yoshida, and Aldo Brugnera Junior

# Contents

Hindawi Publishing Corporation
BioMed Research International
Volume 2016, Article ID 2849795, 1 page
http://dx.doi.org/10.1155/2016/2849795

*Editorial*

# Oral Biology, Oral Pathology, and Oral Treatments

**Samir Nammour,[1] Toni Zeinoun,[2] Kenji Yoshida,[3] and Aldo Brugnera Junior[4]**

[1]*Department of Dental Science, Faculty of Medicine, University of Liège, Quai Godfroid Kurth 45, 4020 Liege, Belgium*
[2]*Department of Oral and Maxillofacial Surgery, Faculty of Dentistry, Lebanese University, Beirut, Lebanon*
[3]*Department of Oral and Maxillofacial Surgery, School of Dentistry, Aichi-Gakuin University, 2-11 Suemori-dori, Chikusa-ku, Nagoya, Aichi-ken 464-8651, Japan*
[4]*CITS-Center of Health Technological Innovation and Biomedical Engineering Institute, Unicastelo, São José dos Campos, SP, Brazil*

Correspondence should be addressed to Samir Nammour; s.namour@ulg.ac.be

Received 6 June 2016; Accepted 6 June 2016



Oral biology, oral pathology, and oral treatments are interesting fields in dentistry. The rapid evolution of technologies and the continuous apparition of new materials and products available for practitioners oblige searchers to evaluate their impact on oral tissues and teeth. The evaluation of the biocompatibility of new products is essential to avoid any tissues damage caused by an eventual toxicity or side effects of therapeutic products or materials.

This special issue is a compendium of different studies and fundamental and clinical researches. Some papers are focused on the microbiological evaluation of the effect of low level laser therapy (LLLT) in peri-implantitis treatment, a new diagnostic approach using microRNAs as salivary markers for periodontal diseases, evaluation of safety irradiation parameters of Nd:YAP laser beam during an in vitro endodontic treatments, a literature overview about the effects of Nd:YAG 1064 nm and diode 810 nm and 980 nm in infected root canals, efficacy of ultrasonic and Er:YAG laser in removing bacteria from the root canal system, a comparative study of microleakage on dental surfaces bonded with three self-etch adhesive systems treated with the Er:YAG laser and bur, and the study of laser Doppler measurement of flow variability in the microcirculation of the palatal mucosa.

We hope that the content of this special issue allows readers to understand the interaction of materials with oral tissues and provides to practitioners new therapeutic methods for their daily practices.

*Samir Nammour*
*Toni Zeinoun*
*Kenji Yoshida*
*Aldo Brugnera Junior*

Hindawi Publishing Corporation
BioMed Research International
Volume 2016, Article ID 1386158, 6 pages
http://dx.doi.org/10.1155/2016/1386158

*Research Article*

# A Preliminary In Vitro Study on the Efficacy of High-Power Photodynamic Therapy (HLLT): Comparison between Pulsed Diode Lasers and Superpulsed Diode Lasers and Impact of Hydrogen Peroxide with Controlled Stabilization

**Gianluigi Caccianiga,[1,2] Marco Baldoni,[1] Carlo Angelo Ghisalberti,[3] and Alessio Paiusco[1,2]**

[1]*School of Medicine and Surgery, University of Milano-Bicocca, Milan, Italy*
[2]*Faculty of Medical Sciences, LUDES HEI Foundation, Malta*
[3]*Department of Biomedical Sciences for Health, University of Milan, Milan, Italy*

Correspondence should be addressed to Gianluigi Caccianiga; gianluigi.caccianiga@unimib.it

Received 5 January 2016; Revised 10 June 2016; Accepted 4 July 2016

Academic Editor: Toni Zeinoun



*Aim*. In periodontology lasers have been suggested for the photodynamic therapy (PDT): such therapy can be defined as the inactivation of cells, microorganisms, or molecules induced by light and not by heat. The aim of this study was to evaluate results of PDT using a 980 nm diode laser (Wiser Doctor Smile, Lambda SPA, Italy) combined with hydrogen peroxide, comparing a pulsed diode laser (LI) activity to a high-frequency superpulsed diode laser (LII). *Materials and Methods*. Primary fibroblasts and keratinocytes cell lines, isolated from human dermis, were irradiated every 48 h for 10 days using LI and LII combined with SiOxyL$^{+}$™ Solution (hydrogen peroxide (HP) stabilized with a glycerol phosphate complex). Two days after the last irradiation, the treated cultures were analyzed by flow cytofluorometry (FACS) and western blotting to quantify keratin 5 and keratin 8 with monoclonal antibodies reactive to cytokeratin 5 and cytokeratin 8. Antimicrobial activity was also evaluated. *Results*. Both experimental models show the superiority of LII against LI. In parallel, stabilized HP provided better results in the regeneration test in respect to common HP, while the biocidal activity remains comparable. *Conclusion*. The use of high-frequency lasers combined with stabilized hydrogen peroxide can provide optimal results for a substantial decrease of bacterial count combined with a maximal biostimulation induction of soft tissues and osteogenesis.

## 1. Introduction

Laser versatility in dentistry, alternatively to or combined with scalpels, rotary instruments, and other surgical protocols, ensures less painful and invasive treatments, being also more precise and efficient and showing a high hemostatic control.

The benefit of this approach has been underlined for more than a decade [1]. Combined with traditional instruments, lasers can be used in all dentistry areas: oral surgery, implantology, periodontology, conservative dentistry, dental aesthetics, and endodontics, provided proper integrations of the application protocols are foreseen [2].

In periodontology lasers have been suggested for the photodynamic therapy (PDT): such therapy can be defined as the inactivation of cells, microorganisms, or molecules induced by light and not by heat.

PDT requires a light source (laser), a photosensitizer (a substance containing oxygen), and oxygenated tissues. Oxygen in fact is the crucial molecule for performing PDT. "Photodynamic" implies the application of luminous photonic dynamics on biological molecules [2–5].

The mechanism of action of PDT foresees the interaction of light with the dye the target tissues have been imbibed with. The dyed molecules adapt to the bacterial membrane of microorganisms [6, 7]. The laser light activates the dye

molecule or photosensitizer, while the resulting reaction with oxygen releases triplet oxygen with 2 unpaired, parallel-spin electrons [8, 9]. Given the coupling of 2 unpaired, opposite-spin electrons, the interaction between triplet oxygen and laser energy results in the formation of singlet oxygen, which determines the oxidation of the lipid membrane of bacteria and their cell death [2, 10–13].

To date only lasers with high penetration depth (600 to 1100 nm) have been taken into consideration for PDT, since they are scarcely absorbed by water and hydroxyapatite and in particular diode lasers. Thanks to such low absorption level, wavelengths comprised within this range can penetrate in tissues up to 2 cm. This can be especially suitable for the treatment of pathologies characterized by high bacterial dissemination, like periodontal diseases, whereas mechanical treatment protocols can only act on the directly treated surfaces, such as the hard tissues of the tooth (cement and dentine) and the hard and soft tissues of the periodontium comprised within the treatment site. The possibility of a deeper penetration could be useful to eradicate those bacteria that are involved in the pathology but that are not necessarily contiguous with the sick tooth.

Under normal setting conditions, diode lasers with power beyond 2 Watts (HLLT: High Level Laser Therapy) show a high thermal effect [14]; that is the reason why research has basically tested low-power diode lasers (LLLT: Low Level Laser Therapy) with energy pulses comprised within milliseconds (pulsed lasers) or continuously emitted energy pulses that cannot produce a significant temperature rise (above 45 centigrade degrees) and that are managed together with dyed photosensitizers, with typical absorption ranges in long wavelength bands.

However, it has been noticed that classic PDT is only partially effective in diseases showing deep bacterial infiltration [15]. This can be ascribed to the scarce peak power applied, below 2 Watts, as well as to the scarce penetration capacity of the laser light in tissues imbibed with photosensitizer, with a biocidal effect that can only be limited to the external and/or superficial areas in nonsurgical or open surgeries, for example, in the surgical treatment of peri-implantitis.

Although it does not show significant advantages in respect to surgery, classic PDT with pulsed or continuous LLLT and blue photoactivators seems to have a positive effect on inflammatory indexes [16].

The various photosensitizing chromophoric agents have been compared on *S. mutans* strains as an oral biofilm model. Toluidine blue ortho (TBO) was the only one able to substantially reduce a bacterial load of 3 Log, while others, such as methylene blue (MB), malachite green (MG), eosin (EOS), erythrosine (ERI), and rose Bengal (RB), proved to be less efficient [17].

The nonsurgical periodontal therapy combining a 980 nm laser with hydrogen peroxide is gaining more and more consensus in clinical dental practice as shown in Rey protocol [18, 19].

The benefits of hydrogen peroxide as opposed to classic photodynamic therapy (PDT) performed with photoactivating agents with absorption within the visible band consist in a higher bioavailability and deeper penetration in the biofilms as well as in the scarce interference in respect to the irradiation performed (Figure 1) [18].

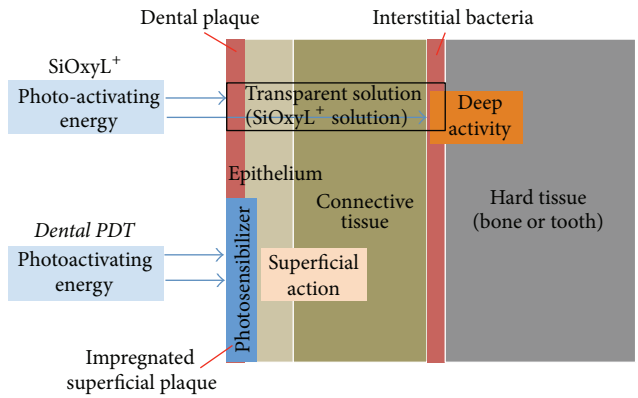



Figure 1: Benefits of the transparent photosensitizer.

The aim of this study was to evaluate the efficacy of low-frequency diode lasers (LI) compared to high-frequency lasers (LII), related also to effects of stabilizers contribution on hydrogen peroxide properties. The advantage to use LII could be to have more efficacy than LI in order to deliver singlet oxygen when laser meets hydrogen peroxide (more than 7000 times per second compared to 50/500 impacts). High frequency could improve the activity of the impacts inside the soft tissues and the efficacy of decontaminating effects of HLLT.

## 2. Materials and Methods

All tests were made by the same investigator.

*2.1. Reagents.* The 3% hydrogen peroxide stabilized with 200 ppm acetanilide (catalogue number 323381, HP-C), the 30% nonstabilized hydrogen peroxide, the sodium phosphate monobasic hexahydrate, and the glycerol phosphate disodium salt hydrated were purchased from Sigma-Aldrich (Milan, Italy).

The hydrogen peroxide solution with physiologic stabilization (HP-GC) is prepared by diluting with bidistilled water the nonstabilized $H_2O_2$ solution in 1 : 10 v/v and by dissolving the triad glycerin/monosodium phosphate/glycerol phosphate disodium with a 50/7/1 molar ratio in a quantity equivalent to 3.7% p/p of the solution.

*2.2. Irradiation Sources.* The irradiation sources were as follows:

(i) (LI) 980 nm diode laser (Wiser Doctor Smile, Lambda SpA, Italy) with 400 micron fiber, set to 2.5 Watts, (mean energy 0.625 W), and $T_{ON}$ 5 milliseconds and $T_{OFF}$ 15 milliseconds, with 50 Hertz frequency and application time 50 seconds. Operator used the same way to irradiate all samples.

(ii) (LII) High-frequency laser (Wiser Doctor Smile, Lambda SpA, Italy), set to "decontamination," with 400-micron fiber, with characteristics as shown under

Table 1: High-frequency laser technical specifications.

| | |
|---|---|
| Laser source | Semiconductor |
| Wavelength | 980 nm |
| Max power | 7 W |
| Power resolution | Digital 0.1 W to 7.0 W, resolution 0.1 W |
| Available pulses | Peak power > 2 W, mean power < 0.8 W, frequency > 8 KHz |
| Settings allowed | Decontamination, regeneration, peri-implantitis, light biostimulation, medium biostimulation |

Table 1 and application time 50 seconds (mean energy 0.5 W, frequency > 7000 Hz).

*2.3. Evaluation of the Biostimulating Effect.* Primary fibroblasts and keratinocytes cell lines (Matched Set-Cryopreserved Dermal Fibroblasts and Keratinocytes, Tebu-Bio™) isolated from human dermis are placed, respectively, in the culture media Euroclone™ and Tebu-bio (Human Adult Keratinocyte Growth Medium KM-2).

The culture of the lines is confluent-type (70–80% min) in a 1 : 1 mix of the two culture media (final FBS + 5%, named "A"). In racks equipped with 12 1 cm wells, single-layer fibroblasts (0.5 × 105 cells/well) and keratinocytes ($1 \times 10^5$ cells/well) are seeded or grown in an "organotypic" coculture.

0.3 mL of HP-GP solution, HP-C solution, or distilled water is added, respectively, to the cultures (control). Culture media are changed every 48 hours matching the irradiation treatment that is performed with LI and LII at 48 h intervals for 10 days.

The interval of 48 h in vitro is the minimum but also sufficient to allow the fibroblasts and keratinocytes to double their population. In fact, the average time of cell-doubling for keratinocytes is 40.5 h, something more for fibroblasts. In this way, the new generation of the cells has the time to express and produce keratin 5 and keratin 8, proteins of reinforcement of the junctional epithelium, stimulated by moderate stress factors.

According to common protocols in an vitro study, two days after the last irradiation, the treated cultures are analyzed by flow cytofluorometry (FACS) and western blotting to quantify keratin 5 and keratin 8 with monoclonal antibodies reactive to (cyto)keratin 5 and (cyto)keratin 8 (KRT 5/8, Antibodies-Online™).

*2.4. Verification of the Biocidal Activity.* The test is performed with current methods [18] on cultures of typical strains causing infections in the oral cavity.

Selection of the pathogenic strains is as follows:

(i) *Haemophilus actinomycetemcomitans* CIP 52103T ("HA").

(ii) *Bacteroides forsythus* CIP 105219T ("BF").

(iii) *Porphyromonas gingivalis* CIP 103683T ("PG").

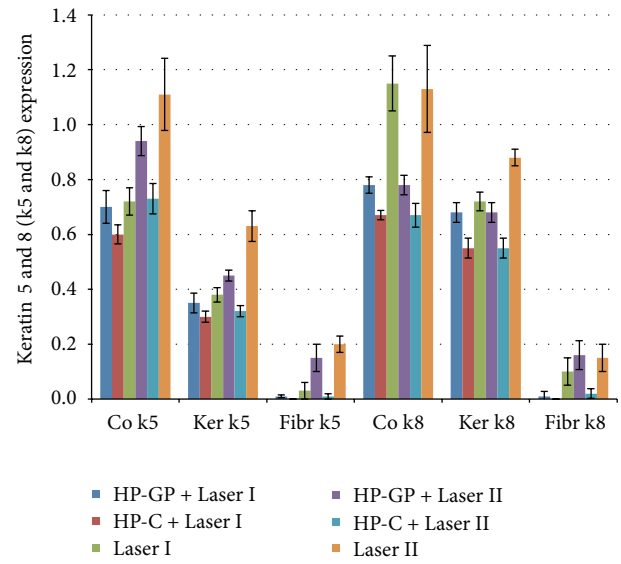



Figure 2: Cytostimulating activity. Western blot expression of keratin 5 (k5) and keratin 8 (k8) from organotypic coculture (Co), keratinocytes (Ker), and fibroblasts (Fibr) after 6x irradiation with (blue bars) Laser I alone; (red bar) Laser I with hydrogen peroxide-glycerol phosphate complex (HP-GP); (green bar) Laser I with common hydrogen peroxide (HP-C); (violet bar) Laser II alone; (light blue bar) Laser II with hydrogen peroxide-glycerol phosphate complex (HP-GP); and (orange bar) Laser I with common hydrogen peroxide (HP-C) Laser I. Experiments were performed 3 times. Data are given as mean ± standard deviation (s.d.).

(iv) *Micromonas micros* CIP 105294T ("MM").

(v) *Fusobacterium nucleatum* CIP 101130T ("FN").

A 30 $\mu$L suspension for each strain is placed in 1.5 mL Eppendorf tubes with 5% of culture medium and is treated with LI and LII with 10 s irradiations along the test tube, specifically 5 s of vertical motion and 5 s of rotary motion. Washing is performed with 1 part of hydrogen peroxide solution in 2 parts of culture solution with a 3 min contact time, checking for any temperature increase. At the end the population density is measured in CFUs (colony-forming units).

*2.5. Statistical Analysis.* All experiments were performed 3 times. Differences between groups were determined by ANOVA. $p$ values of less than $0.05^*$ are considered significant. Data are given as mean ± standard deviation (s.d.). All statistical analyses were performed employing the statistical algorithms in Microsoft™ Excel™ per Mac, release 14.6.5.

## 3. Results

The comparative evaluation of biostimulation data (Figure 2) and biocidal efficiency data (Figure 3) indicates a higher efficiency of high-frequency lasers (LII) in respect to diode lasers (LI). The same experimental kit shows the effects of the stabilizers contribution on hydrogen peroxide properties. The use of hydrogen peroxide in a glycerol phosphate complex (HP-GP) provides a substantial decrease of the bacterial load

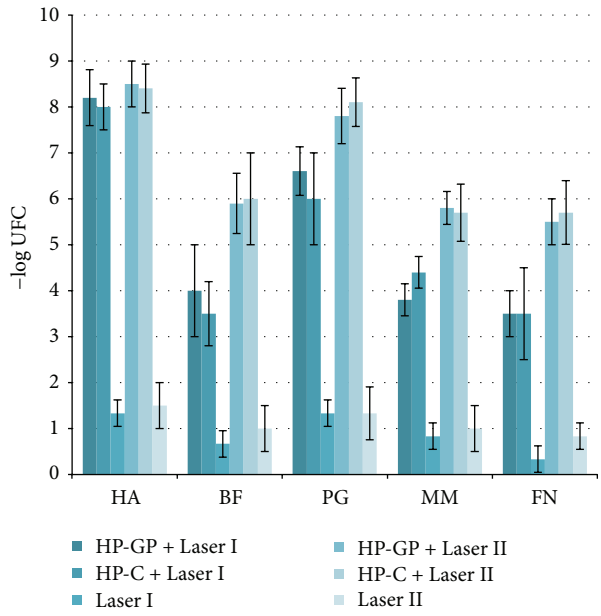



Figure 3: Sanitizing activity on the main bacteria involved in periodontal diseases. Decrease of pathogenic bacteria expressed as Unit Forming Colonies (UFC) of *Actinobacillus actinomycetemcomitans* (HA), *Bacteroides forsythus* (BF) or *Tannerella forsythensis*, *Porphyromonas gingivalis* (PG), *Micromonas micros* or *Peptostreptococcus micros* (MM), and *Fusobacterium nucleatum* (FN) upon treatment with Laser I + HP-GP; Laser I + HP-C; Laser I; Laser II + HP-GP; Laser II + HP-C; and Laser II. Experiments were performed 3 times. Data are given as mean ± standard deviation (s.d.).

that can be compared to that of the common hydrogen peroxide (HP-C) and to the minimization of the cytotoxic impact thanks to the particular physiologic-like composition (Figure 4).

In more detail, the expression of keratin 5–8 upon repeated LI exposure was slightly higher in the presence of hydrogen peroxide-glycerol phosphate complex (HP-GP) compared to common hydrogen peroxide (HP-C) in keratinocytes and fibroblast cultures and cocultures. The absence of HP as in control group (Laser I) slightly improved biostimulation, although the differences were not statistically significant ($p = 0.3646$). Overall higher biostimulation was attained from the data with LII, yet the same pattern is observed. HP-GP performed better than HP-C, while Laser II produces a higher expression of keratin 5 to keratin 8. Again, the difference between tests and control group was not statistically significant ($p = 0.0415$). Conversely, high sanitizing efficiency in the in vitro model was attained by either glycerol phosphate-stabilized hydrogen peroxide or common hydrogen peroxide in conjunction with Laser I/II, which afforded almost negligible decrease of pathogenic contamination (Figure 3).

## 4. Discussion

PDT performed with pulsed or continuous LLLT seems to show clear efficacy limits due to the following reasons:

(a) The very low power (below 1 Watt) cannot ensure a proper bactericidal efficacy on microorganisms that are responsible for periodontal diseases.

(b) The laser penetration capacity is limited, due to the energy absorbed in tissues imbibed with dyed photosensitizer [18, 20, 21].

Nonetheless, LLLT shows a good biostimulation effect: the purpose of laser-assisted biostimulation is to stimulate the activity of the cells designated to the regeneration of tissues [18, 20, 21] lost because of the aggression of oral pathogens. Moreover, laser biostimulation significantly activates the proliferation and differentiation of adult mesenchymal stem cells in the line required in the defect area caused by the periodontal disease [17, 22–24].

With HLLT pulsed (LI) the very long pulse time (within the milliseconds range) can emit frequencies that do not exceed 7000 Hz; this reduces the activation capacity of the hydrogen peroxide's derivate ($SiOxyL^+$ solution) and the correspondent release of singlet oxygen, which is crucial to ensure a decontaminating effect on microorganisms.

The use of HLLT with the "$SiOxyL^+$ HLL Technology," a superpulsed laser, goes beyond the limits of conventional PDT, since it allows combining the high peak power required to eliminate pathogens in the oral cavity (higher than 2 W) with a low mean power (below 0.8 W) that is suitable to promote laser-assisted biostimulation, whereas temperature does not exceed 45°C and remains inside the range of tissue vasodilation.

Moreover, a frequency higher than 7 KHz as determined by the pulse length in microseconds (superpulsed laser) triggers thousands of activation events per second of the $SiOxyL^+$ solution, resulting in continuous production of singlet oxygen that causes the cell death of the pathogenic bacteria that are responsible for infection diseases in the mouth [18, 20–22].

The use of diluted solutions of hydrogen peroxide combined with a 980 nm laser seems able to provide for a deep sanitization [18, 20]. Hydrogen peroxide is characterized by a moderate antibacterial capacity, and the laser increases its efficiency thanks to the photodynamic action due to the activation of peroxide. In fact, the transfer of energy from the laser to the $H_2O_2$ molecule results in its homolytic scission to $OH^-$ (hydroxyl-radical) or its decomposition to $H_2O$ and $^1O_2$ (singlet oxygen).

The limits of this method, if any, are to be ascribed to the hydrogen peroxide quality, specifically to the type of stabilizers that are required to avoid the decomposition of the aqueous solution of $H_2O_2$. When irradiated, stabilizers such as colloidal tin, silver nitrate, organophosphates, nitrates, and acetonitrile may generate free radicals and have therefore irritating effects.

It seemed appropriate to further increase the balance between antiseptic and regenerating properties. Laboratory methods were employed in order to evaluate a hydrogen peroxide composition with the best ratio between stability, antibacterial action, and low impact (nonnegative contribution) to laser biostimulation.

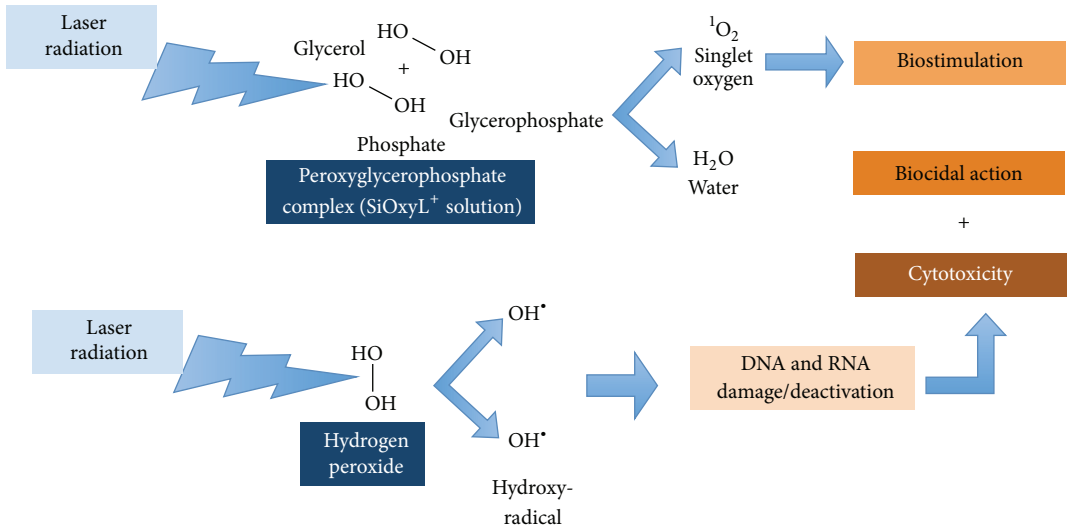



Figure 4: Mechanism of action of SiOxyL+ HLL Technology (High-Frequency Diode Laser Wiser and SiOxyL+ solution).

Hydrogen peroxide at 10 volumes 3% has no cytotoxic effect on human cells, as can instead occur with peroxide at 20 volumes; however, biostimulation implementation can be an important aim in therapies using HLLT Technology.

The addition of a complex containing peroxide-glycerol phosphate is based on the fact that this component promotes fibroblasts cellular vitality. So composition of common hydrogen peroxide was modified evaluating the adjunctive benefits of this complex, creating SiOxyL+ solution.

Tests of cell viability, made on fibroblasts and keratinocytes, effectively showed an activity implementation of these cells compared to the use of common hydrogen peroxide at 10 volumes 3%. Nowadays there are no similar studies published in literature, but some in vivo studies performed on periodontal disease and bone regeneration showed the excellent tissue response to HLLT performed with SiOxyL+ solution.

## 5. Conclusions

A solution to optimize such therapies seems to be the use of high-frequency lasers (LII) combined with hydrogen peroxide stabilized with glycerol phosphate complex (HP-GP) that provides optimal results for a substantial decrease of the bacterial load combined with a maximal biostimulation induction of soft tissues and osteogenesis.

## Competing Interests

The authors declare that they have no competing interests.

## References

[1] G. Rey, J. Bruneteaud, G. Bourg-Heckley, J. Levy, and G. Quentel, "Applications and compared Lasers in dental surgeries," *Implantology*, pp. 56–72, 2004.

[2] H. Gursoy, C. Ozcakir-Tomruk, J. Tanalp, and S. Yilmaz, "Photodynamic therapy in dentistry: a literature review," *Clinical Oral Investigations*, vol. 17, no. 4, pp. 1113–1125, 2013.

[3] P. Meisel and T. Kocher, "Photodynamic therapy for periodontal diseases: state of the art," *Journal of Photochemistry and Photobiology B: Biology*, vol. 79, no. 2, pp. 159–170, 2005.

[4] B. W. Sigusch, A. Pfitzner, V. Albrecht, and E. Glockmann, "Efficacy of photodynamic therapy on inflammatory signs and two selected periodontopathogenic species in a beagle dog model," *Journal of Periodontology*, vol. 76, no. 7, pp. 1100–1105, 2005.

[5] A. Pfitzner, B. W. Sigusch, V. Albrecht, and E. Glockmann, "Killing of periodontopathogenic bacteria by photodynamic therapy," *Journal of Periodontology*, vol. 75, no. 10, pp. 1343–1349, 2004.

[6] R. R. A. Hayek, N. S. Araújo, M. A. Gioso et al., "Comparative study between the effects of photodynamic therapy and conventional therapy on microbial reduction in ligature-induced periimplantitis in dogs," *Journal of Periodontology*, vol. 76, no. 8, pp. 1275–1281, 2005.

[7] A. A. Takasaki, A. Aoki, K. Mizutani et al., "Application of antimicrobial photodynamic therapy in periodontal and periimplant diseases," *Periodontology 2000*, vol. 51, no. 1, pp. 109–140, 2009.

[8] M. Wilson, "Lethal photosensitisation of oral bacteria and its potential application in the photodynamic therapy of oral infections," *Photochemical & Photobiological Sciences*, vol. 3, no. 5, pp. 412–418, 2004.

[9] J. M. De Almeida, L. H. Theodoro, A. F. Bosco, M. J. Hitomi Nagata, M. Oshiiwa, and V. G. Garcia, "In vivo effect of photodynamic therapy on periodontal bone loss in dental furcations," *Journal of Periodontology*, vol. 79, no. 6, pp. 1081–1088, 2008.

[10] K. Konopka and T. Goslinski, "Photodynamic therapy in dentistry," *Journal of Dental Research*, vol. 86, no. 8, pp. 694–707, 2007.

[11] A. Azarpazhooh, P. S. Shah, H. C. Tenenbaum, and M. B. Goldberg, "The effect of photodynamic therapy for periodontitis: a systematic review and meta-analysis," *Journal of Periodontology*, vol. 81, no. 1, pp. 4–14, 2010.

[12] T. Maisch, "Anti-microbial photodynamic therapy: useful in the future?" *Lasers in Medical Science*, vol. 22, no. 2, pp. 83–91, 2007.

[13] R. Polansky, M. Haas, A. Heschl, and G. Wimmer, "Clinical effectiveness of photodynamic therapy in the treatment of periodontitis," *Journal of Clinical Periodontology*, vol. 36, no. 7, pp. 575–580, 2009.

[14] M. Umana, D. Heysselaer, M. Tielemans, P. Compere, T. Zeinoun, and S. Nammour, "Dentinal tubules sealing by means of diode lasers (810 and 980 nm): a preliminary in vitro study," *Photomedicine and Laser Surgery*, vol. 31, no. 7, pp. 307–314, 2013.

[15] J. D. Carroll, M. R. Milward, P. R. Cooper, M. Hadis, and W. M. Palin, "Developments in low level light therapy (LLLT) for dentistry," *Dental Materials*, vol. 30, no. 5, pp. 465–475, 2014.

[16] G. P. Bombeccari, G. Guzzi, F. Gualini, S. Gualini, F. Santoro, and F. Spadari, "Photodynamic therapy to treat periimplantitis," *Implant Dentistry*, vol. 22, no. 6, pp. 631–638, 2013.

[17] G. Caccianiga, G. Cordasco, A. Leonida et al., "Periodontal effects with self ligating appliances and laser biostimulation," *Dental Research Journal*, vol. 9, supplement 2, pp. S186–S191, 2012.

[18] G. Caccianiga, A. Cambini, G. Rey, A. Paiusco, T. Fumagalli, and M. S. Giacomello, "The use of Laser diodes superpulses in Implantology," *European Journal of Inflammation*, vol. 10, no. 2, pp. 97–100, 2012.

[19] G. Rey, "L'apport du laser dans le traitement des poches paradontales," *Implantodontie*, vol. 38, pp. 27–34, 2000.

[20] G. Caccianiga, E. Urso, R. Monguzzi, K. Gallo, and G. Rey, "Efecto bactericida del láser de diodo en periodoncia," *Avances en Odontoestomatologia*, vol. 24, no. 2, pp. 157–166, 2008.

[21] G. Caccianiga, E. Urso, K. Gallo, and G. Rey, "Efecto bactericida del làser Nd:YAP. Estudio in vitro," *Avances en Odontoestomatologia*, vol. 23, no. 3, pp. 127–133, 2007.

[22] A. Leonida, A. Paiusco, G. Rossi, F. Carini, M. Baldoni, and G. Caccianiga, "Effects of low-level laser irradiation on proliferation and osteoblastic differentiation of human mesenchymal stem cells seeded on a three-dimensional biomatrix: in vitro pilot study," *Lasers in Medical Science*, vol. 28, no. 1, pp. 125–132, 2013.

[23] J. T. Hashmi, Y.-Y. Huang, S. K. Sharma et al., "Effect of pulsing in low-level light therapy," *Lasers in Surgery and Medicine*, vol. 42, no. 6, pp. 450–466, 2010.

[24] A. Pejcic, D. Kojovic, L. Kesic, and R. Obradovic, "The effects of low level laser irradiation on gingival inflammation," *Photomedicine and Laser Surgery*, vol. 28, no. 1, pp. 69–74, 2010.

Hindawi Publishing Corporation
BioMed Research International
Volume 2016, Article ID 8421656, 10 pages
http://dx.doi.org/10.1155/2016/8421656

*Review Article*

# Laser Application in Dentistry: Irradiation Effects of Nd:YAG 1064 nm and Diode 810 nm and 980 nm in Infected Root Canals—A Literature Overview

**Yves Saydjari,[1,2] Thorsten Kuypers,[2] and Norbert Gutknecht[1]**

[1]*Department of Conservative Dentistry, Periodontology and Preventive Dentistry, RWTH Aachen University, Pauwelsstraße 30, 52074 Aachen, Germany*
[2]*Praxis für Laserzahnheilkunde Dres. Jung & Kuypers, Neusser Strasse 600, 50737 Köln, Germany*

Correspondence should be addressed to Yves Saydjari; yves.saydjari@rwth-aachen.de

Received 4 January 2016; Revised 18 April 2016; Accepted 15 May 2016

Academic Editor: Toni Zeinoun



*Objective*. In endodontics, Nd:YAG laser (1064 nm) and diode laser (810 nm and 980 nm) devices are used to remove bacteria in infected teeth. A literature review was elaborated to compare and evaluate the advantages and disadvantages of using these lasers. *Methods*. Using combined search terms, eligible articles were retrieved from PubMed and printed journals. The initial search yielded 40 titles and 27 articles were assigned to full-text analysis. The studies were classified based upon laser source, laser energy level, duration/similarity of application, and initial and final bacterial count at a minimum of 20 prepared root canals. Part of the analysis was only reduced microorganisms and mechanically treated root canals upon preparation size of *ISO 30*. All studies were compared to evaluate the most favorable laser device for best results in endodontic therapy. *Results*. A total of 22 eligible studies were found regarding Nd:YAG laser 1064 nm. Four studies fulfilled all demanded criteria. Seven studies referring to the diode laser 980 nm were examined, although only one fulfilled all criteria. Eleven studies were found regarding the diode laser 810 nm, although only one study fulfilled all necessary criteria. *Conclusions*. Laser therapy is effective in endodontics, although a comparison of efficiency between the laser devices is not possible at present due to different study designs, materials, and equipment.

## 1. Introduction

The bacterial contamination of the root canal system in a tooth is the main factor of pulpal and periapical lesions [1]. The polymicrobial flora comprises an almost equal proportion of gram-negative and gram-positive bacteria [2, 3]. Those that are highly pathogenic like *Escherichia coli* produce toxic substances such as proteolytic enzymes or endotoxins, which affect and damage the surrounding periodontics [4, 5]. Endodontic therapy in dentistry involves decimating these bacteria.

In the past, the removal of an infected tooth was the only method of therapy success. In the 1940s, penicillin was used to treat infected teeth and periodontal tissues [6]. However, this intervention eliminated the symptoms rather than the main cause, while unnecessary antibiotic resistances were also created.

For decontamination, the infected teeth were treated by chemical-mechanical preparation to achieve a complete removal of the entire pulp tissue [7]. In addition to the mechanical treatment of the root canals, antibacterial rinsing solutions and drugs like calcium hydroxide applied into the root canal were used for the supportive decontamination. Sodium hypochlorite (NaOCl) proved to be an efficient rinsing solution [8]. A direct contact between chemical agents and microorganisms is required to gain its bactericidal effect.

None of the known chemical agents are currently able to satisfy all demanded requirements of root canal rinsing solutions [9].

A lege artis primary root canal treatment lies—according to the published success—between 70 and 85% [10]. The accessory side channels leaving the main canal in the area of apex occur approximately 70% in all teeth, primarily complicating success, as shown in Figure 1.

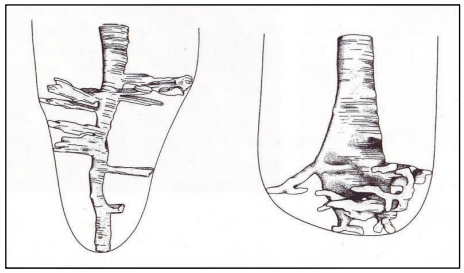


Figure 1: Schematic representation of the canal ramifications of teeth 13 and 24 by Blechschmidt and Meyer. A portion leads to the periodontal ligament, while another ends blindly in the dentin [20].

If certain sections of a tooth are insufficiently prepared, infected tissue remains, which can lead to an exacerbation of the inflammatory process. Moreover, an effective antiseptic rinsing is not possible if the preparation size is too low [9].

The limited penetration depth (approx. 100 $\mu$m) of chemical substances restricts the bacterial reduction in deeper dentin layers [11].

To remove the smear layer formed by the mechanical preparation to prevent a recolonization of the root canal system [12, 13], an extra rinsing fluid like chelate ethylenediaminetetraacetate (EDTA) or citric acid is necessary. In this case, a laser-supported root canal treatment could be an added value. Michiels et al. were able to demonstrate a significant higher reduction of reinfection of root canals after smear layer modification by the Nd-YAG laser versus an EDTA solution [14]. This result shows that the laser can also reduce the risk of leakage after root canal filling and its consequences.

In addition, adverse effects like toxicity, bad taste, and unpleasant odor of irrigation solutions have been shown in several clinical trials [15].

Spratt et al. proved in 2001 that the rinsing solution is only able to have an adequate bactericidal effect in reducing the biofilm through long exposure time [16].

Another important factor is that root canals are noncircularly sectioned yet have an oval cross section, which restricts a mechanical treatment with round instruments. A disinfecting rinsing solution combined with a laser could also provide valuable assistance to effectively remove any remaining tissue and bacteria.

Samiei et al. showed statistical differences in their in vitro study about mechanical stepback technique and laser cleaning of the root canals in teeth. The cleaning efficacy of combined laser and rotary was better than the single stepback technique [17].

Calcium hydroxide has also been proven particularly effective in root canals. This antibacterial product should remain in the root canal for at least seven days to achieve the best effect. In this context, Archilla et al. demonstrated that only a single Nd:YAG laser session is necessary to eliminate the same amount of endotoxin as calcium hydroxide is able to achieve in seven days [18]. The laser as adjunct in endodontic therapy could offer new possibilities regarding the problems described above, preventing a reinfection with its following consequences.

The laser development occurred in the 1950s, shortly after which it was used in medicine and primarily in the field of ophthalmology and dermatology. In 1971, the first $CO_2$ laser was used in endodontics to seal the apical foramen [19].

The term laser (English for light amplification by stimulated emission of radiation) is an acronym describing its operating principle indeed. It acts as a light amplifier and promotes the exponential reproduction of photons due to induced emission. Each laser has various purposes in dentistry, depending upon different wavelengths.

The effects of laser irradiation in biological tissue depend on various factors [20].

*(1) Laser*

(i) wavelength and absorption in tissue,

(ii) mode of operation CW (clocked, pulsed, and Q-switched),

(iii) energy or power output (single-pulse energy/power Watt per $cm^2$),

(iv) active time (e.g., pulse duration),

(v) repetition rate (Hz),

(vi) application method of the laser (contact/noncontact, focused/defocused, and rapid movements/at one point),

(vii) time of application.

*(2) Surrounding Media*

(i) air,

(ii) water,

(iii) blood.

*(3) Tissue*

(i) absorption coefficient corresponding to laser wavelength,

(ii) thermal conduction coefficient.

The laser light can be reflected on the surface *(reflexion)* or emerge after penetrating the tissue *(transmission)*. There also may be *remissions* and *diffusions* in the irradiated tissue.

The higher the absorption, the lesser the penetration depth and thermal side effects, since the energy is absorbed by the tissue absorption and its associated processes.

Laser energy can be delivered in various forms, whereby the operating mode depends on the kind of power output:

(i) continuous power output = continuous wave = CW,

(ii) chopped mode,

(iii) free running pulse,

(iv) Q-switch mode.

Table 1: Lasers in dentistry.

| Laser device | Use in dentistry | Wavelength |
|---|---|---|
| Neodymium:YAG laser (Nd:YAG laser) | Surgery, endodontics, and periodontics | 1064 nm |
| Erbium:YAG laser (Er:YAG laser), erbium, and chromium:YSGG laser (Er, CR:YSGG laser) | Surgery, endodontics, and cavity preparation | 2940 nm, 2780 nm |
| Diode laser | Surgery, endodontics, and periodontics | 810–980 nm |
| $CO_2$ laser | Surgery | 10600 nm |

Three possible theories exist for bactericidal effects of NIR laser light in the literature [21–23]:

(i) direct heat absorption through the bacterium itself,

(ii) heating by absorption of the substrate in which the bacterium is located,

(iii) photodamage effect.

The commonly used lasers in dentistry are the neodymium:YAG laser with 1064 nm, the diode laser with 810–980 nm, erbium lasers with 2940 nm/2780 nm, and the $CO_2$ laser with 10600 nm. Table 1 shows their typical fields in dentistry.

Many attempts have been made to investigate the antimicrobial potential of lasers, with numerous studies showing that the emission of laser light has a bactericidal effect in a root canal [24–29].

This literature overview provides the current state of science about Nd:YAG and diode lasers (1064 nm, 810 nm, and 980 nm) in endodontics and their action spectra in periodontal tissue with determined power settings. A comparison of these effects should evaluate a preferable laser device as support for the best results in endodontic treatments.

The Department of Restorative Dentistry at RWTH Aachen University in Germany—headed by Professor Dr. Gutknecht—has already developed a treatment protocol that could support the classic endodontic therapy concept due to the laser-specific bactericidal effect.

The proper use of the laser as an adjunct in endodontic therapy with known standards is recommended for the best clinical benefits for the patient.

## 2. Materials and Methods

To compare the variety of studies, the following criteria were selected for an adequate comparison:

(i) comparable operational settings of the laser device (200/300/400 microns fiber, 1.5 W, 15 pps/cw),

(ii) similar experimental design,

(iii) at least 20 treated root canals,

(iv) prepared root canals to minimum ISO 30.

These parameters were chosen on account of the ability for reproduction and the actual state of knowledge by research results of the Conservative Dentistry Department, RWTH Aachen. The operational setting of 1.5 W and 15 pps showed acceptable clinical results. In these studies, the risk of possible damaging side effects was also clarified.

Table 2: Keywords used to research and their number of results on the website http://www.ncbi.nlm.nih.gov/pubmed.

| Search keyword | Results |
|---|---|
| Laser in dentistry | 6688 |
| Laser, endodontics | 795 |
| Diode laser, in dentistry | 614 |
| Nd:YAG-laser, in dentistry | 532 |
| Nd:YAG-laser, root canal | 160 |
| Nd:YAG-laser, endodontics | 143 |
| Diode laser, root canal | 100 |
| Diode laser, endodontics | 98 |
| Laser, root canal | 37 |

Some studies did not operate with contaminated teeth but rather with dentin cuts, inoculated agar plates, or animal teeth. Since these studies used at least similar parameters compared to what is mentioned above, they were also included in the general evaluation owing to the impact of the laser light on different microorganisms.

Furthermore, different variables such as the effect of the laser with respect to apical reinfection after successful root filling are listed separately or edited in Section 4, as long as they can contribute relevant information to the purpose of this review.

First, a PubMed online search was performed using specific keywords, which are listed in Table 2.

A manual search in the library of Conservative Dentistry of the RWTH Aachen was progressed, whereby the listed magazines were evaluated.

*English*

(i) Journal of Clinical Laser Medicine & Surgery,

(ii) Photomedicine and Laser Surgery,

(iii) The Journal of Oral Laser Applications,

(iv) Lasers in Medical Science.

*German*

(i) Zeitschrift für Laserzahnheilkunde,

(ii) Laserzahnmedizin Jahrbuch '11.

Most of the studies encountered in print media were also available online. The search was conducted from April 2011 until April 2016.

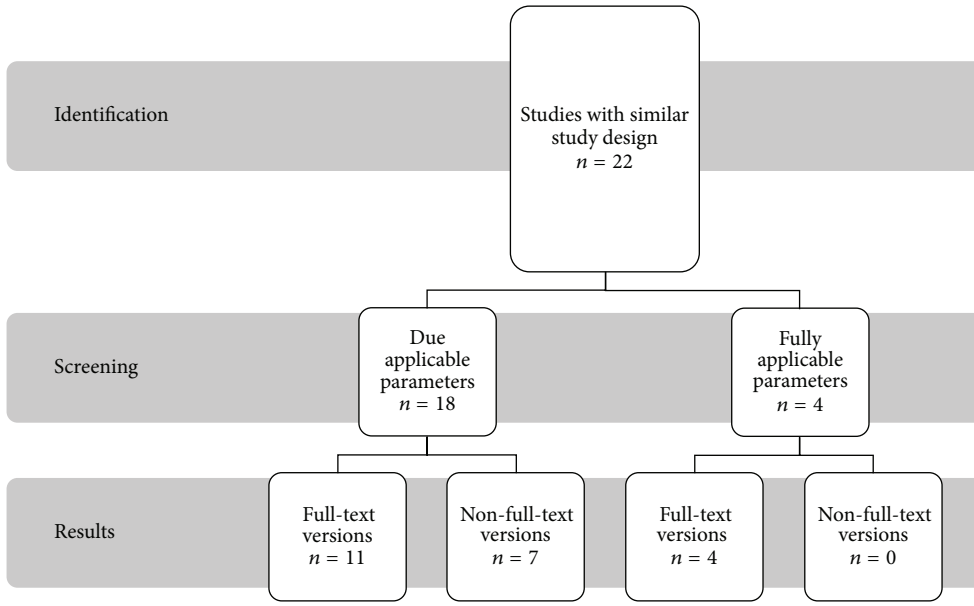



Figure 2: Presentation of search strategy for Nd:YAG laser 1064 nm.

Table 3: Overview of comparable studies for Nd:YAG 1064 nm.

| Year of publication | First author | Study design | Title |
|---|---|---|---|
| 1999 | Moritz [30] | In vitro | The bactericidal effect of Nd:YAG, Ho:YAG, and Er:YAG laser irradiation in the root canal: an in vitro comparison |
| 1997 | Moritz [26] | In vivo | Nd:YAG laser irradiation of infected root canals in combination with microbiological examinations |
| 1996 | Gutknecht [31] | In vivo | Long-term clinical evaluation of endodontically treated teeth by Nd:YAG lasers |
| 1996 | Gutknecht [32] | In vitro | Bactericidal effect of the Nd:YAG laser in in vitro root canals |

For Nd:YAG laser, a total of 22 studies fulfilled the inclusion criteria for the most part and researched with rateable scientific evidence plotted in Figure 2. Four studies provide the desired requirement, while eighteen studies partly fulfilled the criteria and are listed separately.

Figure 3 shows that seven studies were evaluated for 980 nm diode laser, of which only one study fully provides the desired requirements. Six studies partly fulfilled the criteria and are listed separately.

Proceeding strictly according to the required laser settings, only one study was found for diode laser 810 nm that fully complies with the requirements detailed in Figure 4. Excluded studies contain different laser settings, and lack of information regarding the laser fiber used or a substrate was irradiated rather than teeth, but listed in Section 4 for information value.

## 3. Results

*3.1. Studies on Nd:YAG Laser.* For the Nd:YAG laser, a total of four comparable studies were found, as shown in Table 3.

Moritz et al. showed that a setting of 1.5 W for Nd:YAG laser has the best results in terms of bactericidity with less risk of thermal damage to tissue [26, 30]. They reached a bacterial reduction of 99.16% for *E. coli* and *E. faecalis*. In spite of its massive cell wall, the highly heat-resistant *E. faecalis* was sufficiently reduced [30].

Moritz et al. achieved an almost complete elimination of bacteria in their in vivo study in 1997 with the Nd:YAG laser after two radiation treatments. In 50% of cases, they reached this result after the first radiation. The maximum log kill amounted to 4.22 for *Streptococcus* and 3.33 for *Staphylococcus*. In the control group, an antibacterial solution ($H_2O_2$) was used and only one log kill of a logarithm could be achieved. In this instance, the kind of the irrigation solution should also be considered. NaOCl leads to better results in combination with $H_2O_2$. Furthermore, they also noted that a sufficient elimination of bacteria in the entire root canal can be achieved by sufficiently long exposure and adequate management of the light fiber [26].

Gutknecht et al. showed a success rate in their longitudinal study of 82% and reached a germ reduction of 84% with Nd:YAG laser up to a depth of 1000 $\mu$m still [31]. In their study in 1996, Gutknecht et al. showed that between 97.91% and 99.9997% of bacteria *(E. faecalis)* were eliminated by laser radiation [32].

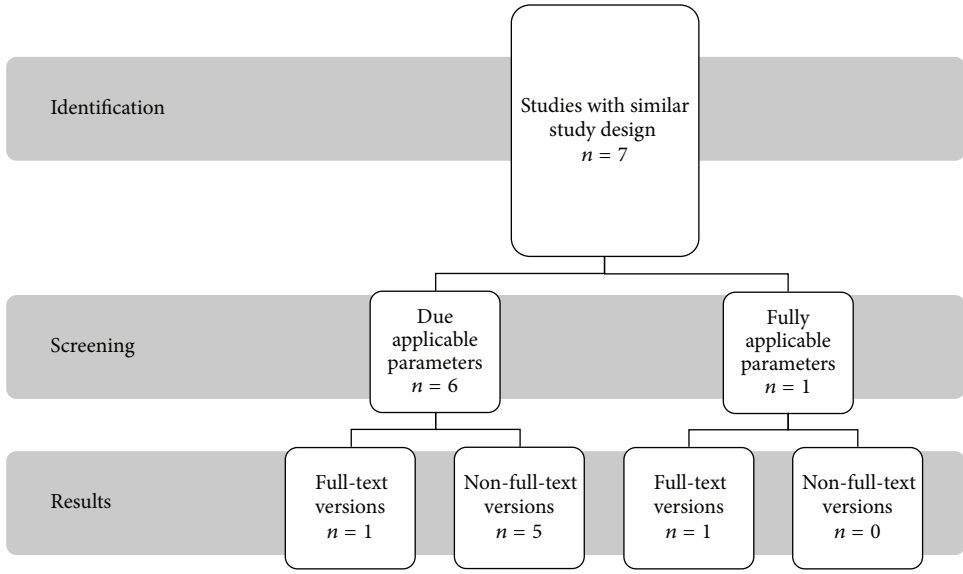



Figure 3: Presentation of search strategy for diode laser 980 nm.

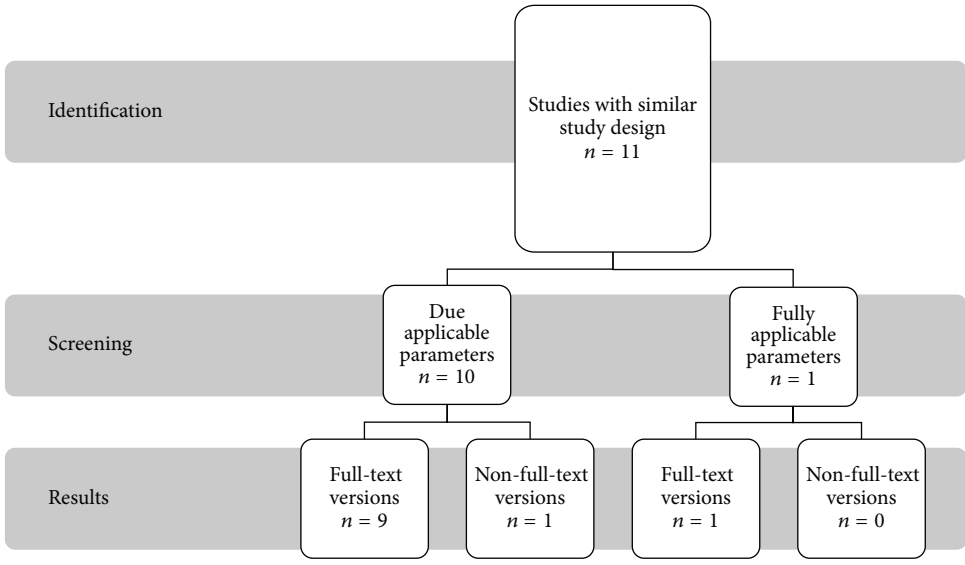



Figure 4: Presentation of search strategy for diode laser 810 nm.

Table 4: One study for the diode laser 810 nm matches all demanded criteria.

| Year of publication | First author | Study design | Title |
|---|---|---|---|
| 2012 | Beer [33] | Extracted teeth | Comparison of two diode lasers on bactericidity in root canals—an in vitro study |

Table 5: One study for the diode laser 980 nm matches all demanded criteria.

| Year of publication | First author | Study design | Title |
|---|---|---|---|
| 2006 | Schoop [34] | Dentin cuts | Innovative wavelengths in endodontic treatment |

*3.1.1. Studies on Diode Laser.* For each diode laser device (810 nm and 980 nm), only one study fulfilled the demanded parameters, as shown in Tables 4 and 5.

*3.2. Diode Laser 810 nm.* Beer et al. could achieve a bacterial reduction of 98.8% with the 810 nm diode laser in 2012, describing "the laser as modern state-of-the-art instrument for endodontics" [33]. Irradiation of the input cavity showed significantly better results.

Table 6: Listing of additional mentioned studies with different parameters as they occur in the text.

| Year of publication | First author | Title |
|---|---|---|
| 1983 | Eriksson [35] | Innovative wavelengths in endodontic treatment |
| 1998 | Farge [36] | In vitro study of a Nd:YAP laser in endodontic retreatment |
| 1999 | Lan [37] | Temperature elevation on the root surface during Nd:YAG laser irradiation in the root canal |
| 1997 | Ramsköld [38] | Thermal effects and antibacterial properties of energy levels required to sterilize stained root canals with an Nd:YAG laser |
| 1995 | Weller [39] | In vitro radicular temperatures produced by injectable thermoplasticized gutta-percha |
| 2001 | Mazaheri [40] | Temperaturentwicklung auf der wurzeloberfläche bei einer endodontischen behandlung mit einem diodenlaser |
| 2000 | Gutknecht [41] | Diode laser radiation and its bactericidal effect in root canal wall dentin |
| 1993 | Behrens [42] | Die transmission und absorption der temperatur und energie des Nd-YAG-lasers im dentin |
| 2013 | Sadik [43] | Effects of laser treatment on endodontic pathogen *Enterococcus faecalis*: a systematic review |
| 2004 | Gutknecht [44] | Irradiation of infected root canals with Nd:YAG lasers. A review |
| 1997 | Klinke [45] | Antibacterial effects of Nd:YAG laser irradiation within root canal dentin |
| 2011 | Pirnat [21] | Study of the direct bactericidal effect of Nd:YAG and diode laser parameters used in endodontics on pigmented and nonpigmented bacteria |
| 1999 | Neuman [22] | Characterization of photodamage to *Escherichia coli* in optical traps |
| 2008 | Mirsaidov [23] | Optimal optical trap for bacterial viability |
| 2012 | Meire [46] | In vitro inactivation of endodontic pathogens with Nd:YAG and Er:YAG lasers |
| 2007 | de Paz [47] | Redefining the persistent infection in root canals: possible role of biofilm communities |
| 1985 | Nair [48] | Root canal and periapical flora: a light and electron microscopy study |
| 1997 | Klinke [45] | Antibacterial effects of Nd:YAG laser irradiation within root canal dentin |
| 1996 | Odor [49] | Pattern of transmission of laser light in teeth |
| 1995 | Vaarkamp [50] | Propagation of light through human dental enamel and dentine |
| 1997 | Jalil [51] | Surface topography of enamel and dentine from primary teeth following infrared Nd-YAG laser irradiation: an in vitro study |
| 1994 | Hardee [52] | Evaluation of the antibacterial effects of intracanal Nd:YAG laser irradiation |
| 1997 | Moritz [29] | Irradiation of infected root canals with a diode laser in vivo: results of microbiological examinations |
| 1993 | Kales [54] | Review and forecast of laser markets |
| 2014 | Kanumuru [53] | Efficacy of $Ca(oH)_2$ against *E. faecalis* compared with three dental lasers on root canal dentin—an in vitro study |

*3.3. Diode Laser 980 nm.* In their study published in 2006, Schoop et al. also observed that above a setting of 1.5 W there are signs of changes in the surface and increased bactericidal effect with diode laser. The desired efficiency increases with the intensity of the laser [34].

## 4. Discussion

The comparison of the three laser systems showed that the applied formulas for calculating the actual bactericidal effect widely differ. Most studies choose different parameters of the laser device such as the intensity of radiation, exposure time, and the laser fiber used or they differ in purely practical approaches.

To investigate the actual effect of the laser on the respective microorganisms, laser fibers with a greater diameter were also used in the studies and are mentioned. In this context, clinical restrictions like heavy accessibility, strong curved root canals, or poor visibility should be eliminated. Thus, a lighter ability for reproduction could be guaranteed. These studies allow partial statements about a possible target of the selected settings to achieve the best possible bactericidal effect and are listed in Table 6.

### *4.1. Effects of Laser Light*

*4.1.1. Thermal Effects.* Across existing literature, there are relatively few studies dealing with periodontal tissue damage by overheating. In 1983, Eriksson and Albrektsson defined a heating of 47°C as critical limit for the survival of bone in rabbits [35]. Follow-up studies set a temperature increase of 10°C as the critical limit [36–39]. According to a thesis

by Mazaheri in 2001 at RWTH Aachen, the maximum average temperature (10 ms interval pause, 10 ms pulse length) remains in the irradiation of root canals with the diode laser with a setting of 3 W still below the critical limit when the optical fiber is performed permanently moving coronal and apical in a circular motion in the root canal [40]. Gutknecht et al. observed a bacterial reduction in a depth of 500 microns in the teeth of cattle at a setting of 3 W cw [41]. The temperature limit is exceeded at 4 W and prolonged irradiation for 15 seconds, resulting in thermal damage.

*4.1.2. Power Settings.* In this research, a value of 1.5 W for the diode and Nd:YAG laser has been set as an inclusion criterion. With this setting, a thermal damage is excluded within recommended handling for both laser devices and the bactericidal effects are acceptable [42]. A temperature on the root surface was observed after 45 sec. of 37°C at the recommended setting 15 pps and 1.5 W and after 90 sec. of 38°C.

In a systematic review of the current literature about the effectiveness of Nd:YAG laser on the pathogenic gram-positive bacteria *E. faecalis*, Sadik et al. showed that 1.5 W could allow an effective bacteria reduction [43].

*4.1.3. Effects of Laser Irradiated Root Surfaces.* Gutknecht described that an application of the laser below 1 W is less important in endodontics because neither is the smear layer completely removed nor are the dentinal tubules sealed. With settings of 1.25 W–1.5 W significant changes on the root canal surface were determined. The organic material was completely removed and the surface of the inorganic substance was merged, resulting in a partial or complete occlusion of dentinal tubules [44]. This fact is to be valued positively because a reinfection is less possible with close canals.

In 2008, Klinke et al. discussed the angle between the optical fiber of the laser and the dentinal wall [45]. The laser beam hits the wall primarily at a very acute angle, depending on the mobility of the fiber in the canal, the root canal curvature, and the exit window of the laser beam from the end of the fiber. In their study, the angle between the glass fiber and dentin surface was defined as 5°. The lesser elimination of bacteria compared to other studies could result from this aspect. Further studies in terms of this angle would be interesting. The actual surface of the dentin also plays a role in terms of bactericidal effect. Darker areas cause carbonization and require a higher absorption of laser energy. The result is a local temperature increase with a bactericidal effect, albeit within no transmission of laser energy into deeper layers of dentin.

Beer et al. investigated irradiating the opening cavity of a tooth before irradiating the root canal itself, resulting in a significant higher bactericidal effect [33]. Further studies would be interesting to explore this issue in greater depth.

*4.1.4. Effects on Microorganisms.* Pirnat et al. examined the direct effect of Nd:YAG (1064 nm) and diode laser (810 nm) on *P. gingivalis*, *E. coli*, and *E. faecalis* in 2011. They postulated two possible theories for the bactericidal effect of NIR laser light: the first refers to heating by absorption of the substrate in which the bacterium is located and the second refers to the direct absorption through the bacterium itself. In their attempt, external factors such as surrounding tissue or blood should not have an influence on the results. For this reason, they irradiated a sapphire substrate that is optically transparent for the NIR spectrum and concluded that both laser systems have a minor direct bactericidal effect on nonpigmented bacteria such as *E. coli* and *E. faecalis* [21]. However, such substrates significantly differ from the in vivo situation; for example, there is no oxygen in the bacterial microenvironment. This is necessary for the bacteria photodamage effect, although the mechanism of this degradation was not further understood [22, 23]. Future studies in this direction would be useful.

The gram-positive bacterium *E. faecalis* is more resistant in this study according to its cell wall structure compared with the gram-negative bacterium *E. coli*. The Nd:YAG laser could reduce 57% of the pigmented bacterium *P. gingivalis* and 37% could be ascertained for the diode laser. The most determining factor is believed to be the presence of the black pigment *protoporphyrin IX* in *P. gingivalis*, which absorbs the energy of the NIR light. Likewise, no growth was ascertained on the agar plates used. This fact shows that not only the bacterium itself but also its environment plays a key role for an effective endodontic laser therapy. Meire et al. irradiated bacteria inoculated agar plates *(Candida albicans, Enterococcus faecalis,* and *Propionibacterium acnes)* in a study published in 2012. The Er:YAG laser was predominant in this experiment compared to Nd:YAG laser [46]. However, the present thickness of the Er:YAG laser fiber limits an efficient transference of the light in the root canal.

The agar plates and the bacterial suspensions used in this study absorbed the laser light to a small extent. Furthermore, nonpigmented bacteria were used, which could explain the lesser effect of the Nd:YAG laser in this experiment. The different absorption of wavelengths in dentin has an effect on the depth of penetration. The Er:YAG laser had a lesser effect on the bacteria found in deeper dentinal tubules, whereas the Nd:YAG laser was significantly superior.

Meire et al. supported the statement made by Pirnat et al. that the Nd:YAG laser kills the bacteria probably by heating their environment. A comparison of studies covering the antimicrobial effect of laser light is not easy to realize because the statements about energy density or experimental conditions are often lacking. In a natural environment such as root canal wall dentin bacteria occur in a biofilm [47, 48], making them more responsive to laser light by high cell density and the presence of extracellular matrix. This fact could explain the poor action of the Nd:YAG laser on agar plates and bacterial suspensions. Different studies have shown that the bactericidal effect in the tooth is strengthened through enamel prisms and dentinal tubules as these act as a light guide [45, 49, 50]. However, additional in vivo studies are needed.

Meire et al. suppose that blood or blood products in a natural environment could lead to a raised number of *porphyrins* and *melanin pigments* in the bacteria in which

the bactericidal effect is improved by Nd:YAG laser. Another interesting aspect is the dentin, which was examined more closely in a study in 1997 [51]. Carious dentin absorbs 1064 nm more wavelength in comparison to healthy dentin, which increases the desired bactericidal effect.

Hardee et al. achieved a bacterial reduction of 99% of the test bacterium *Bacillus stearothermophilus* with Nd:YAG laser, in conjunction with a log kill of 2 in comparison to a log 6 population before irradiation. Usually this bacterium is not found in infected root canals. It was selected due to its high heat resistance because the bactericidal effect of Nd:YAG laser is assumed by heat [52].

The Department of Restorative Dentistry, RWTH Aachen, currently deals with the effect of ring-firing laser fibers in the root canals, which allows the laser light to not only emit in vertical direction. New possibilities concerning the bactericidal depth effect of diode lasers and Nd:YAG lasers could be achieved.

*4.2. Nd:YAG versus Diode Laser.* A direct comparison of the selected devices is currently not feasible in relation to exact similar demanded experimental setups.

In 1997, Moritz et al. described the diode laser (810 nm) and the Nd:YAG laser (1064 nm) in endodontic treatment as equally effective and they recommended further studies to evaluate the anaerobic bacteria [26].

In a study by Kanumuru and Subbaiah in 2014, the Nd:YAG laser was most effective in the elimination of *E. faecalis* compared to 980 nm and followed 810 nm diode laser [53].

Due to the accumulation of different aggressive and resistant bacteria in an infected root canal, the additional use of Nd:YAG and diode lasers in combination with conventional methods such as mechanical conditioning or rinsing fluids seems to hold a positive value, as can be demonstrated by this literature review.

*4.3. Nd:YAG Laser*

*Advantages*. The Nd:YAG laser has clear advantages in the depth effect compared with 810 nm and 980 nm diode laser. Far more studies about Nd:YAG can be found in the literature compared to both diode lasers in endodontics. It is effective against pigmented microorganisms.

Furthermore, it removes the smear layer in a root canal, which interferes with adequate disinfection using additional rinsing fluids. It also has a simultaneous additional bactericidal effect.

*Disadvantages*. Drawbacks include the relatively high cost and its size in comparison to the two diode lasers. They are easy to handle due to their small size and the device can be used without power supply in battery mode, which Nd:YAG laser is incapable of at present.

*4.4. Diode Lasers 810 nm and 980 nm.* Comparing the 810 nm with the 980 nm diode laser, both are equally favorable. Both are adequate funds in endodontic therapy and should be investigated in further detail. For 810 nm diode lasers, the majority of studies can be found in the literature, although the parameters are not exactly comparable.

According to a study of Kales in 1993, the diode laser determines 99% of the turnover on the whole market and is estimated at 25% by the buyers in comparison to all other laser devices [54].

*4.4.1. Variability of Reported Results.* Sadik et al. postulated that the various investigated laser systems of the past 30 years could not be compared with a meta-analysis since the results of the studies were not presented in a standardized manner. From this perspective, it would be desirable if future studies use a solid study design with the same basic parameters, such as the diameter of laser fiber, the same practical approach to the irradiation of the teeth (number of repetitions, pauses), pulse frequency (pps), and power (W) [43].

This statement is the final testimony and prime cause because this present literature review also does not lead to any clear result in terms of effectiveness brought against the bacteria in an infected root canal compared to the three lasers. There are too many different variable facts in the studies to make a statement about the more effective wavelength or the preferable device and the data situation is contradictory. The Nd:YAG laser is more frequently evaluated, although the comparability of the different study designs is also lacking. The various studies are difficult to measure, given that different parameters, fiber strengths, or handling methods are used.

At present, a statement based upon recommended guidelines is not really possible. When properly used, it emerges that disinfection by laser can increase the endodontic success with a very low risk of damaging side effects and with acceptable durability.

A recommended standardized procedure for the individual wavelengths is suggested, although further scientific studies would be desirable. Additional in vivo studies with Nd:YAG and diode lasers in endodontics are necessary. It should be considered internationally with the same procedure including a clear treatment outline. Generally established criteria such as the same fibers (diameter), the same settings of the laser parameters (power, pulse frequency), the same trace of radiation in practical implementation, and duration are essential to conduct a comparison about the antibacterial effects of endodontic treatment between the three laser devices. This would be desirable to define an evidence-based "gold standard."

## 5. Conclusions

In endodontics, Nd:YAG laser (1064 nm) and diode laser (810 nm and 980 nm) devices are used to remove bacteria in infected teeth. This literature overview aimed to compare and evaluate the advantages and disadvantages in using these laser devices with standardized settings.

The PubMed database was searched using precise keywords between April 2011 and April 2016. Likewise, print media from the Library of RWTH Aachen University were examined.

A total of 22 eligible studies were found regarding Nd:YAG laser 1064 nm. Four studies fulfilled all demanded criteria in this review for this laser device. Seven studies referring to the diode laser 980 nm were examined, although only one fulfilled all criteria. Eleven studies were found regarding the diode laser 810 nm, but also only one study could fulfill all necessary criteria.

The analysis of the selected studies showed that all three laser systems are able to successfully decimate bacteria that are present in infected teeth. Pigmented bacteria are efficiently better removed by the Nd:YAG laser. Moreover, in deeper dentin layers, Nd:YAG laser showed better results. Concerning handiness, size, and purchase price, the diode laser is preferable.

In summary, a direct comparison cannot be made between the selected laser devices due to different study designs, materials, and equipment. Prospective randomized trials are needed to further verify which laser system is to be preferred for the best results in endodontic therapy and evaluate an evidence-based and international guideline.

## Competing Interests

The authors declare that they have no conflict of interests.

## References


[1] S. Kakehashi, H. R. Stanley, and R. J. Fitzgerald, "The effects of surgical exposures of dental pulps in germ-free and conventional laboratory rats," *Oral Surgery, Oral Medicine, Oral Pathology*, vol. 20, no. 3, pp. 340–349, 1965.

[2] G. Sundqvist, "Taxonomy, ecology, and pathogenicity of the root canal flora," *Oral Surgery, Oral Medicine, Oral Pathology*, vol. 78, no. 4, pp. 522–530, 1994.

[3] B. Tsatsas, A. Tzamouranis, and F. Mitsis, "A bacteriological examination of root canals before filling," *Journal of the British Endodontic Society*, vol. 7, no. 2, pp. 78–80, 1974.

[4] S. E. Gharbia and H. N. Shah, "Hydrolytic enzymes liberated by black-pigmented gram-negative anaerobes," *FEMS Immunology and Medical Microbiology*, vol. 6, no. 2-3, pp. 139–145, 1993.

[5] P. N. R. Nair, U. Sjögren, G. Krey, K.-E. Kahnberg, and G. Sundqvist, "Intraradicular bacteria and fungi in root-filled, asymptomatic human teeth with therapy-resistant periapical lesions: a long-term light and electron microscopic follow-up study," *Journal of Endodontics*, vol. 16, no. 12, pp. 580–588, 1990.

[6] F. D. Ostrander, M. C. Crowley, and J. Dowson, "A clinical study of the treatment of root canal and periapical infections with penicillin," *Journal of Dental Research*, vol. 26, no. 6, pp. 403–407, 1947.

[7] R. W. Hession, "Long-term evaluation of endodontic treatment: anatomy, instrumentation, obturation—the endodontic practice triad," *International Endodontic Journal*, vol. 14, no. 3, pp. 179–184, 1981.

[8] M. Shih, F. J. Marshall, and S. Rosen, "The bactericidal efficiency of sodium hypochlorite as an endodontic irrigant," *Oral Surgery, Oral Medicine, Oral Pathology*, vol. 29, no. 4, pp. 613–619, 1970.

[9] N. Gutknecht, "Lasereinsatz in der Endodontie—Voraussetzungen für den Therapieerfolg," in *Laserzahnmedizin Jahrbuch*, vol. 11, pp. 57–61, Oemus, Aachen, Germany, 2011.

[10] M. Hülsmann and E. Schäfer, "Good clinical practice," in *Die Wurzelkanalbehandlung. Stellungnahme des Endodontie-Beirats der DGZ*, DGZMK-Stellungnahme, 2007.

[11] A. Vahdaty, T. R. Pitt Ford, and R. F. Wilson, "Efficacy of chlorhexidine in disinfecting dentinal tubules in vitro," *Endodontics & Dental Traumatology*, vol. 9, no. 6, pp. 243–248, 1993.

[12] D. R. Drake, A. H. Wiemann, E. M. Rivera, and R. E. Walton, "Bacterial retention in canal walls in vitro: effect of smear layer," *Journal of Endodontics*, vol. 20, no. 2, pp. 78–82, 1994.

[13] D. Clark-Holke, D. Drake, R. Walton, E. Rivera, and J. M. Guthmiller, "Bacterial penetration through canals of endodontically treated teeth in the presence or absence of the smear layer," *Journal of Dentistry*, vol. 31, no. 4, pp. 275–281, 2003.

[14] R. Michiels, T. E. M. Vergauwen, A. Mavridou, M. Meire, M. De Bruyne, and R. J. G. De Moor, "Investigation of coronal leakage of root fillings after smear-layer removal with EDTA or Nd:YAG lasing through capillary-flow porometry," *Photomedicine and Laser Surgery*, vol. 28, supplement 2, pp. S43–S50, 2010.

[15] C. Yesilsoy, E. Whitaker, D. Cleveland, E. Phillips, and M. Trope, "Antimicrobial and toxic effects of established and potential root canal irrigants," *Journal of Endodontics*, vol. 21, no. 10, pp. 513–515, 1995.

[16] D. A. Spratt, J. Pratten, M. Wilson, and K. Gulabivala, "An in vitro evaluation of the antimicrobial efficacy of irrigants on biofilms of root canal isolates," *International Endodontic Journal*, vol. 34, no. 4, pp. 300–307, 2001.

[17] M. Samiei, S. M. V. Pakdel, S. Rikhtegaran, S. Shakoei, D. Ebrahimpour, and P. Taghavi, "Scanning electron microscopy comparison of the cleaning efficacy of a root canal system by Nd:YAG laser and rotary instruments," *Microscopy and Microanalysis*, vol. 20, no. 4, pp. 1240–1245, 2014.

[18] J. R. F. Archilla, M. S. N. A. Moreira, S. P. H. Miyagi, A. C. Bombana, N. Gutknecht, and M. M. Marques, "Single session of Nd:YAG laser intracanal irradiation neutralizes endotoxin in dental root dentin," *Journal of Biomedical Optics*, vol. 17, no. 11, Article ID 118002, 2012.

[19] J. A. Weichman and F. M. Johnson, "Laser use in endodontics. A preliminary investigation," *Oral Surgery, Oral Medicine, Oral Pathology*, vol. 31, no. 3, pp. 416–420, 1971.

[20] N. Gutknecht, *Lasertherapie in der Zahnärztlichen Praxis*, Quintessenz, Berlin, Germany, 1999.

[21] S. Pirnat, M. Lukac, and A. Ihan, "Study of the direct bactericidal effect of Nd:YAG and diode laser parameters used in endodontics on pigmented and nonpigmented bacteria," *Lasers in Medical Science*, vol. 26, no. 6, pp. 755–761, 2011.

[22] K. C. Neuman, E. H. Chadd, G. F. Liou, K. Bergman, and S. M. Block, "Characterization of photodamage to *Escherichia coli* in optical traps," *Biophysical Journal*, vol. 77, no. 5, pp. 2856–2863, 1999.

[23] U. Mirsaidov, W. Timp, K. Timp, M. Mir, P. Matsudaira, and G. Timp, "Optimal optical trap for bacterial viability," *Physical Review E—Statistical, Nonlinear, and Soft Matter Physics*, vol. 78, no. 2, Article ID 021910, 2008.

[24] R. Franzen, N. Gutknecht, S. Falken, N. Heussen, and J. Meister, "Bactericidal effect of a Nd:YAG laser on *Enterococcus faecalis* at pulse durations of 15 and 25 ms in dentine depths of 500 and 1,000 $\mu$m," *Lasers in Medical Science*, vol. 26, no. 1, pp. 95–101, 2011.

[25] N. Gutknecht, R. Franzen, M. Schippers, and F. Lampert, "Bactericidal effect of a 980-nm diode laser in the root canal

wall dentin of bovine teeth," *Journal of Clinical Laser Medicine and Surgery*, vol. 22, no. 1, pp. 9–13, 2004.

[26] A. Moritz, O. Doertbudak, N. Gutknecht, K. Goharkhay, U. Schoop, and W. Sperr, "Nd:YAG laser irradiation of infected root canals in combination with microbiological examinations," *Journal of the American Dental Association*, vol. 128, no. 11, pp. 1525–1530, 1997.

[27] U. Schoop, W. Kluger, A. Moritz, N. Nedjelik, A. Georgopoulos, and W. Sperr, "Bactericidal effect of different laser systems in the deep layers of dentin," *Lasers in Surgery and Medicine*, vol. 35, no. 2, pp. 111–116, 2004.

[28] Y. Yasuda, T. Kawamorita, H. Yamaguchi, and T. Saito, "Bactericidal effect of Nd:YAG and Er:YAG lasers in experimentally infected curved root canals," *Photomedicine and Laser Surgery*, vol. 28, supplement 2, pp. S75–S78, 2010.

[29] A. Moritz, N. Gutknecht, U. Schoop, K. Goharkhay, O. Doertbudak, and W. Sperr, "Irradiation of infected root canals with a diode laser in vivo: results of microbiological examinations," *Lasers in Surgery and Medicine*, vol. 21, no. 3, pp. 221–226, 1997.

[30] A. Moritz, U. Schoop, K. Goharkhay et al., "The bactericidal effect of Nd:YAG, Ho:YAG, and Er:YAG laser irradiation in the root canal: an in vitro comparison," *Journal of Clinical Laser Medicine and Surgery*, vol. 17, no. 4, pp. 161–164, 1999.

[31] N. Gutknecht, F. Kaiser, A. Hassan, and F. Lampert, "Long-term clinical evaluation of endodontically treated teeth by Nd:YAG lasers," *Journal of Clinical Laser Medicine and Surgery*, vol. 14, no. 1, pp. 7–11, 1996.

[32] N. Gutknecht, A. Moritz, G. Conrads, T. Sievert, and F. Lampert, "Bactericidal effect of the Nd:YAG laser in in vitro root canals," *Journal of Clinical Laser Medicine and Surgery*, vol. 14, no. 2, pp. 77–80, 1996.

[33] F. Beer, A. Buchmair, J. Wernisch, A. Georgopoulos, and A. Moritz, "Comparison of two diode lasers on bactericidity in root canals-an in vitro study," *Lasers in Medical Science*, vol. 27, no. 2, pp. 361–364, 2012.

[34] U. Schoop, W. Kluger, S. Dervisbegovic et al., "Innovative wavelengths in endodontic treatment," *Lasers in Surgery and Medicine*, vol. 38, no. 6, pp. 624–630, 2006.

[35] A. R. Eriksson and T. Albrektsson, "Temperature threshold levels for heat-induced bone tissue injury: a vital-microscopic study in the rabbit," *The Journal of Prosthetic Dentistry*, vol. 50, no. 1, pp. 101–107, 1983.

[36] P. Farge, P. Nahas, and P. Bonin, "In vitro study of a Nd:YAP laser in endodontic retreatment," *Journal of Endodontics*, vol. 24, no. 5, pp. 359–363, 1998.

[37] W.-H. Lan, "Temperature elevation on the root surface during Nd:YAG laser irradiation in the root canal," *Journal of Endodontics*, vol. 25, no. 3, pp. 155–156, 1999.

[38] L. O. Ramsköld, C. D. Fong, and T. Strömberg, "Thermal effects and antibacterial properties of energy levels required to sterilize stained root canals with an Nd:YAG laser," *Journal of Endodontics*, vol. 23, no. 2, pp. 96–100, 1997.

[39] R. N. Weller and K. A. Koch, "In vitro radicular temperatures produced by injectable thermoplasticized gutta-percha," *International Endodontic Journal*, vol. 28, no. 2, pp. 86–90, 1995.

[40] P. Mazaheri, *Temperaturentwicklung auf der Wurzeloberfläche bei einer endodonitischen Behandlung mit einem Diodenlaser*, Mainz, 2001.

[41] N. Gutknecht, D. Van Gogswaardt, G. Conrads, C. Apel, C. Schubert, and F. Lampert, "Diode laser radiation and its bactericidal effect in root canal wall dentin," *Journal of Clinical Laser Medicine and Surgery*, vol. 18, no. 2, pp. 57–60, 2000.

[42] V. G. Behrens, N. Gutknecht, and R. Renziehausen, "Die transmission und absorption der temperatur und energie des Nd-YAG-lasers im dentin," *ZWR*, vol. 102, no. 9, pp. 629–634, 1993.

[43] B. Sadik, S. Arikan, N. Beldüz, Y. Yaşa, D. Karasoy, and M. Çehreli, "Effects of laser treatment on endodontic pathogen *Enterococcus faecalis*: a systematic review," *Photomedicine and Laser Surgery*, vol. 31, no. 5, pp. 192–200, 2013.

[44] N. Gutknecht, "Irradiation of infected root canals with Nd:YAG lasers. A review," *LaserZahnheilkunde*, vol. 4, no. 4, pp. 219–226, 2004.

[45] T. Klinke, W. Klimm, and N. Gutknecht, "Antibacterial effects of Nd:YAG laser irradiation within root canal dentin," *Journal of Clinical Laser Medicine and Surgery*, vol. 15, no. 1, pp. 29–31, 1997.

[46] M. A. Meire, T. Coenye, H. J. Nelis, and R. J. G. De Moor, "In vitro inactivation of endodontic pathogens with Nd:YAG and Er:YAG lasers," *Lasers in Medical Science*, vol. 27, no. 4, pp. 695–701, 2012.

[47] L. C. de Paz, "Redefining the persistent infection in root canals: possible role of biofilm communities," *Journal of Endodontics*, vol. 33, no. 6, pp. 652–662, 2007.

[48] P. N. Nair and H. U. Luder, "Root canal and periapical flora: a light and electron microscopy study," *Schweizerische Monatsschrift fur Zahnmedizin*, vol. 95, no. 10, pp. 992–1003, 1985.

[49] T. M. Odor, T. F. Watson, T. R. Pitt Ford, and F. Mcdonald, "Pattern of transmission of laser light in teeth," *International Endodontic Journal*, vol. 29, no. 4, pp. 228–234, 1996.

[50] J. Vaarkamp, J. J. ten Bosch, and E. H. Verdonschot, "Propagation of light through human dental enamel and dentine," *Caries Research*, vol. 29, no. 1, pp. 8–13, 1995.

[51] L. A. Jalil, R. Labella, and G. J. Pearson, "Surface topography of enamel and dentine from primary teeth following infrared Nd-YAG laser irradiation: an in vitro study," *Lasers in Medical Science*, vol. 12, no. 1, pp. 61–67, 1997.

[52] M. W. Hardee, L. J. Miserendino, W. Kos, and H. Walia, "Evaluation of the antibacterial effects of intracanal Nd:YAG laser irradiation," *Journal of Endodontics*, vol. 20, no. 8, pp. 377–380, 1994.

[53] N. R. Kanumuru and R. Subbaiah, "Bacterial efficacy of $Ca(oH)_2$ against *E. faecalis* compared with three dental lasers on root canal dentin—an in vitro study," *Journal of Clinical and Diagnostic Research*, vol. 8, no. 11, pp. ZC135–ZC137, 2014.

[54] D. Kales, "Review and forecast of laser markets," *Laser Focus World*, vol. 29, pp. 72–73, 1993.

Hindawi Publishing Corporation
BioMed Research International
Volume 2016, Article ID 1027525, 14 pages
http://dx.doi.org/10.1155/2016/1027525

*Review Article*

# MicroRNAs as Salivary Markers for Periodontal Diseases: A New Diagnostic Approach?

**Gerhard Schmalz,[1] Simin Li,[1] Ralph Burkhardt,[2] Sven Rinke,[3] Felix Krause,[1] Rainer Haak,[1] and Dirk Ziebolz[1]**

[1]*Department of Cariology, Endodontology and Periodontology, University of Leipzig, 04109 Leipzig, Germany*
[2]*Institute of Laboratory Medicine, Clinical Chemistry, and Molecular Diagnostics, University of Leipzig, 04109 Leipzig, Germany*
[3]*Department of Prosthodontics, University Medical Center Goettingen, Goettingen, Germany*

Correspondence should be addressed to Dirk Ziebolz; dirk.ziebolz@medizin.uni-leipzig.de

Received 19 January 2016; Accepted 20 March 2016

Academic Editor: Kenji Yoshida



The aim of this review is to discuss current findings regarding the roles of miRNAs in periodontal diseases and the potential use of saliva as a diagnostic medium for corresponding miRNA investigations. For periodontal disease, investigations have been restricted to tissue samples and five miRNAs, that is, miR-142-3p, miR-146a, miR-155, miR-203, and miR-223, were repeatedly validated in vivo and in vitro by different validation methods. Particularly noticeable are the small sample sizes, different internal controls, and different case definitions of periodontitis in in vivo studies. Beside of that, the validated miRNAs are associated with inflammation and therefore with various diseases. Furthermore, several studies successfully explored the use of salivary miRNA species for the diagnosis of oral cancer. Different cancer types were investigated and heterogeneous methodology was used; moreover, no overlap of results was found. In conclusion, five miRNAs have consistently been reported for periodontitis; however, their disease specificity, detectability, and expression in saliva and their importance as noninvasive markers are questionable. In principle, a salivary miRNA diagnostic method seems feasible. However, standardized criteria and protocols for preanalytics, measurements, and analysis should be established to obtain comparable results across different studies.

## 1. Introduction

MicroRNAs (miRNAs) are endogenous ~22 nt RNAs that play important regulatory roles in animals and plants by targeting mRNAs for cleavage or translational repression [1]. Many multifarious investigations regarding miRNAs have demonstrated their essential roles in physiological and pathological processes in the immune response [2]. Additionally, miRNAs likely contribute to development and progression of systemic diseases, such as cancer [3] and vascular disease [4]. Consequently, there is considerable interest in use of miRNAs as not only diagnostic markers but also potential therapeutic targets for various diseases [5]. In particular, the roles of miRNAs in oral cancer and precancer have been investigated. MiRNA expression appears to differ between healthy tissue and squamous cell carcinoma tissues of the oral cavity, which is discussed in an existing review [6]. Furthermore, some investigations have linked precancerous lesions and their risk of becoming malignant to changes in miRNA expression [7].

Furthermore, miRNAs are associated with bacterial infections [8] and, thus, are most likely associated with infectious diseases of oral cavity, for example, dental caries, endodontic infections, and periodontitis. Kim et al. (2015), however, already give an overview of current findings regarding dental disease, including periodontal disease and oral cancer. Kim et al. (2015) also mentioned limitations of current diagnostic and potential benefit of salivary miRNA diagnostic [9]. This is the reason to discuss the roles of miRNAs in periodontal diseases. As of now, only a few in vitro and in vivo investigations regarding these roles are available. A recent review article demonstrated potential roles of miRNAs during periodontal inflammation, showing the considerable role of them in

periodontitis [10]. Thereby, different roles and mechanisms are shown and a very comprehensive overview on molecular pathways is given; however, methodology was only discussed between selected studies.

As mentioned above, an interesting approach is the analysis of miRNA species in saliva. High quality miRNAs were shown to be detectable in saliva [11], and the possibility of their usage in oral cancer detection appears to have a high potential for future diagnostics [12]. This noninvasive approach could be relevant for further diseases of the oral cavity. Nevertheless, the potential usage of miRNA as salivary markers for periodontal diseases was already not discussed yet. As shown for oral cancer, using miRNA for noninvasive diagnostics seems feasible [12] and could therefore serve as a salivary marker for periodontitis. The potential is high, so maybe recent indispensable invasive diagnostics or X-ray could become avoided by salivary miRNA diagnostic in the future and an early diagnosis might help to prevent bone and, in conclusion, tooth loss.

Nevertheless, despite of the huge potential, many different results are available, both for salivary oral cancer diagnostics and for analysis of periodontal tissue. Accordingly, it is questionable if the reasons are methodological differences between the studies or maybe if the usage of miRNAs in general is unsuitable.

Therefore, this review article shows current results of in vitro and in vivo studies regarding periodontal diseases as well as results of selected current studies dealing with salivary miRNAs for oral cancer detection. The aim was to detect promising future perspectives, but also current challenges regarding this issue. In particular, methodological aspects are focused to detect potential limitations of miRNAs as noninvasive markers for periodontitis.

## 2. Methods

*2.1. Search Strategy.* Our literature research was performed using the online database PubMed (http://www.ncbi.nlm.nih.gov/pubmed). To obtain a broad overview of the available theme-specific publications, we used different keywords for our search. We used "miRNA AND periodontitis" as keywords to obtain results for periodontal diseases. We used "miRNA AND saliva AND oral disease" and "miRNA AND saliva AND cancer" as keywords to obtain relevant articles for noninvasive possibilities, that is, the use of saliva to investigate miRNAs in diseases of the oral cavity. Then, the reference lists of relevant articles were searched for further results. To interpret the results, relevant publications were searched and analyzed critically. Neither a meta-analysis nor other statistical comparisons of existing data were performed.

*2.2. Selection Criteria.* Only current full-length articles in English language were included. The results were verified for their relevance. The following selection criteria were defined: miRNA investigations for in vitro and in vivo studies examining periodontal disease. Additionally, in vivo human investigations regarding salivary miRNA analysis in oral cancer and precancer were included.

*2.3. Selection Process.* Following the literature search, all references were limited to relevant publications. In vivo periodontal disease investigations that did not address periodontal tissue were excluded. Salivary diagnostic studies that did not explicitly concentrate on miRNAs were excluded, and only investigations for oral cancer and precancer were included.

## 3. Results

### *3.1. Periodontal Diseases*

*3.1.1. In Vitro.* Eight in vitro studies, which detected different miRNAs, were included for periodontal disease (Table 1). Nahid et al. (2011) examined the expression of cytokines associated with inflammation and differences in expression of cytokines and miRNA during infection with live and heat-killed bacteria in THP-1 monocytes. They demonstrated that expression of miR-146a is associated with infections caused by periodontal pathogenic bacteria in vitro [13]. Another study indicated that miR-146a is significantly overexpressed in THP-1 cells after stimulation with LPS from *P.g.* [14]. Furthermore, miR-146a was shown to be a negative regulator of TLR-NF$\kappa$B pathway in human periodontal ligament cells after *P. gingivalis* LPS stimulation, therefore being involved in inflammatory response [15]. Moreover, expression of miR-146a and miR-146b-5p in human gingival fibroblasts after stimulation with *P.g.* LPS was also investigated in a study by Xie et al. (2013). They demonstrated that expression of these miRNAs significantly increased upon LPS stimulation and concluded that miR-146 could work as a negative regulator of inflammation in periodontal disease [16]. Differential expression of miR-146a and miR-155 in dental pulp and in gingival and periodontal fibroblasts after stimulation with LPS from *Escherichia coli* was also reported. The increased expression of miR-146a was the highest in gingival fibroblasts and decreased expression of miR-155 was only significant in gingival fibroblasts [17]. Naqvi et al. (2014) showed different expressions of miR-146a. In this in vitro study THP-1 macrophages that were stimulated with LPS from *Aggregatibacter actinomycetemcomitans*, *P.g.*, and also *P.g.* grown on cigarette smoke extract were examined. The different LPS caused both some identical and also varied expressions of miRNAs in human macrophages. Moreover, LPS from *P.g.* grown on cigarette smoke extract compared to LPS from unaffected *P.g.* caused differential miRNA expression, particularly that of miR-29b in human macrophages [18].

Results reported in other two studies were more heterogenous: Ouhara et al. (2014) demonstrated upregulated miR-584 in human gingival epithelial cells after stimulation with *P.g.* [19]. Furthermore, Moffat and Lamont (2011) focused on miR-203 expression in gingival epithelial cells stimulated with *P.g.* In this study, 14 miRNAs displayed significant changes in expression after exposure to *P.g.*; of these miRNAs, miR-203 was examined in detail and showed higher expression in tissues after *P.g.* stimulation [20].

*3.1.2. In Vivo Animal.* Nahid et al. (2011) investigated expression of miR-146a, miR-132, and miR-155 in spleens and

Table 1: Comparison of methods from current investigations regarding miRNAs in periodontal disease.

| Author and year | Object of investigation | RNA extraction method | Detection method for miRNA profile | Validation of specific miRNAs | Number of study participants | Internal control | Statistical analysis | Investigated and/or deregulated miRNAs* |
|---|---|---|---|---|---|---|---|---|
| In vitro human | | | | | | | | |
| Moffatt and Lamont 2011 [20] | Gingiva epithelial cells | TRIzol (Invitrogen), miRNeasy kit (Qiagen) | Microarray (LC Sciences) | qRT-PCR TaqMan (Applied Biosystems) | — | RNU-48 | ANOVA, *t*-test | **miR-203** |
| Nahid et al. 2011 [13] | Human THP-1 monocytes | mirVana miRNA isolation kit (Ambion) | — | TaqMan microRNA assay (Applied Biosystems) | — | RNU44 | One-way analysis of variance followed by the two-sided, unpaired Student's *t*-test | **miR-146a** **miR-155** miR-132 |
| Honda et al. 2012 [14] | THP-1 cells and THP-1-derived macrophages | TRIzol reagent (Invitrogen) | Agilent human miRNA microarrays (Release 12.0) (Agilent technologies) | TaqMan microRNA Assay (Applied Biosystems) | — | RNU44 | Unpaired *t*-test, ANOVA-Williams test | **miR-146a** **miR-155** |
| Xie et al. 2013 [16] | Human gingival fibroblasts | TRIzol reagent (Invitrogen) | miRNA microarray analyses (Kangchen Bio-Tech) | qRT-PCR analysis (SYBR Green qPCR Master Mix PA-112, SABiosciences, Qiagen) | — | U6 small nuclear RNA | Student's *t*-test | **miR-146a** miR-146b-5p |
| Sipert et al. 2014 [17] | Human fibroblasts from dental pulps, gingivae, and periodontal ligaments | TRIzol (Invitrogen) | NCode miRNA rapid labeling system (Cat. # MIRLSRPD-20) (Invitrogen) Ncode Multi-Species miRNA microarray kit V2, (Invitrogen) | Taqman miRNA assays, (Applied Biosystems) | — | U6B | Two-way ANOVA followed by Bonferroni post hoc test | **miR-146a** **miR-155** |
| Naqvi et al. 2014 [18] | Human THP-1-differentiated macrophages | miRNeasy kit (Qiagen) | NanoString nCounter miRNA assay (NanoString Technologies) | Quantitative real-time PCR EvaGreen Master Mix (Biotium) | — | RNU6B | Student's *t*-test (two-tailed) | miR-29b miR-32 **miR-146a** miR-891 |
| Ouhara et al. 2014 [19] | Simian virus 40 antigen immortalized gingival epithelial cell line, OBA-9 | mirVana miRNA Isolation Kit (Applied Biosystem) | miRCURY LNA microRNA Array, v.16.0 (Exiqo) | TaqMan MicroRNA Assays System (Applied Biosystems) | — | RNU48 | Student's *t*-test, Tukey honestly significant difference | miR-584 |
| Jiang et al. 2015 [15] | Human periodontal ligament cells | TRIzol reagent (Invitrogen) | — | Quantitative RT-PCR analysis (RT SYBR® Green qPCR Master Mixes PA-112, SABiosciences, Qiagen) | — | U6 small nuclear RNA | ANOVA and Student-Newman-Keuls test | **miR-146a** |

Table 1: Continued.

| Author and year | Object of investigation | RNA extraction method | Detection method for miRNA profile | Validation of specific miRNAs | Number of study participants | Internal control | Statistical analysis | Investigated and/or deregulated miRNAs* |
|---|---|---|---|---|---|---|---|---|
| In vivo animal | | | | | | | | |
| Nahid et al. 2011 [13] | Maxillae and spleens from ApoE$^{-/-}$ mice | mirVana miRNA isolation kit (Ambion) | — | TaqMan microRNA assay (Applied Biosystems) | — | snoRNU202 | One-way analysis of variance followed by the two-sided, unpaired Student's $t$-test | **miR-146a**<br>**miR-155**<br>miR-132 |
| In vivo human | | | | | | | | |
| Lee et al. 2011 [21] | Gingival tissue | mirVana™ miRNA Isolation kit (Ambion) | RT$^2$ miRNA PCR array system (SABiosciences) | TaqMan miRNA assays (Applied Biosystems) | n.i. | RNU44 | Student's $t$-test | miR-181b<br>miR-19b<br>miR-23a<br>miR-30a<br>miR-let7a<br>miR-301a |
| Xie et al. 2011 [22] | Gingival tissue | TRIzol reagent (Invitrogen) | miRNA microarray (Kangchen Bio-Tech) | Quantitative RT-PCR analysis (RT SYBR Green qPCR Master Mixes PA-112, SABiosciences, Qiagen) | 10 periodontitis patients<br>10 healthy subjects | U6 small nuclear RNA | Unpaired Student's $t$-test | miR-126<br>miR-20a<br>miR-142-3p<br>miR-19a<br>let-7f<br>**miR-203**<br>miR-17<br>**miR-223**<br>miR-146b<br>**miR-146a**<br>**miR-155**<br>miR-205 |
| Stoecklin-Wasmer et al. 2012 [24] | gingival tissue | TRIzol (Invitrogen) RNeasy (Qiagen,)* | Microarray** | qRT-PCR | 198 gingival tissue samples, 158 diseased and 40 healthy samples from 86 patients with periodontitis | n.i. | R (the R Development Core Team, 2005) and bioconductor statistical frameworks | miR-451<br>**miR-223**<br>miR-486-5p<br>miR-1246<br>miR-1260<br>miR-141 |

Table 1: Continued.

| Author and year | Object of investigation | RNA extraction method | Detection method for miRNA profile | Validation of specific miRNAs | Number of study participants | Internal control | Statistical analysis | Investigated and/or deregulated miRNAs* |
|---|---|---|---|---|---|---|---|---|
| Perri et al. 2012 [26] | Gingival tissue | TissueLyser LT and miRNeasy Mini Kit (Qiagen) | Quantitative microRNA PCR array miRNA PCR Array (SABiosciences) | — | 10 nonobese patients and 10 obese patients each group with 5 periodontally healthy sites and 5 chronic periodontitis sites | SNORD48 and RNU6-2 | ANOVA and nonpaired Student's *t*-test | **miR-142-3p** miR-15a miR-30e miR-30d miR-22 miR-130a miR-106b miR-103 miR-185 miR-210 miR-18a |
| Ogata et al. 2014 [23] | Gingival tissue | miRNeasy Mini Kit (Qiagen) | Human miRNA microarray 8 × 15K kit (Agilent Technologies) | Real-time PCR SYBR Advantage qPCR Premix (Clontec) | 3 chronic periodontitis patients and 3 edentulous residual ridges | U6 | One-way ANOVA | miR-150 miR-200b **miR-223** miR-144 miR-379 miR-222 |
| Kalea et al. 2015 [27] | Gingival tissue | mirVana miRNA Isolation Kit (Ambion) | Affymetrix GeneChip miRNA 3.0 arrays (Affymetrix) | TaqMan MicroRNA expression assays (Applied Biosystems) | 36 eligible individuals | U6snoRNA and RNU6B | Partek 6.6 and 1-way analysis of variance, R-statistical environment | miR-4721 miR-557 miR-196a miR-323a-3p miR-200b-5p miR-188-5p |
| Motedayyen et al. 2015 [25] | Gingival tissue | mirVana miRNA isolation kit (Ambion) | — | Real-time PCR (TaqMan Universal Master Mix II, no UNG, and hsa-miRNA146a kits, Applied Biosystems) | 10 healthy controls, 20 chronic periodontitis patients | n.i. | Student's *t*-test or Mann-Whitney *U* test; Pearson test or Spearman test | **miR-146a** |

RNA extraction and isolation methods are listed. The proof of quality and quantity and quality assurance are shown as important quality criteria to draw conclusions regarding the reproducibility and standardization of the investigations. Furthermore, methods for miRNA profiling and miRNA validation are listed. In addition, the number of study participants is shown to draw conclusions regarding the validity of the results. n.i.: no information, *only miRNAs which were validated by RT-PCR were considered, and **Stoecklin-Wasmer et al. refer to an earlier report by Demmer et al. 2008 [62]. MiRNAs which were reported in more than one study are highlighted in bold type.

Table 2: Criteria for patient selection.

| Author and year | PD | | CAL | | BOP | | Radiographic bone loss | |
|---|---|---|---|---|---|---|---|---|
| | Healthy | Periodontitis | Healthy | Periodontitis | Healthy | Periodontitis | Healthy | Periodontitis |
| Stoecklin-Wasmer et al. 2012 [24] | ≤4 mm | >4 mm | ≤2 mm | ≥3 mm | negative | positive | n.i. | n.i. |
| Xie et al. 2011 [22] | <3 mm | ≥5 mm | <1 mm | ≥3 mm | n.i. | * | No | Yes |
| Lee et al. 2011 [21] | ≤3 mm | >5 mm | none | >3 mm | ** | n.i. | No | Yes |
| Perri et al. 2012 [26] | ≤4 mm | >5 mm | n.i. | n.i. | Negative | Positive | No | Yes |
| Ogata et al. 2014 [23] | n.i. | ≥6 mm | n.i. | >6 mm | n.i. | Positive | n.i. | n.i. |
| Motedayyen et al. 2015 [25] | <3 mm | n.i. | <3 mm | n.i. | n.i. | n.i. | No | n.i. |
| Kalea et al. 2015 [27] | n.i. | >5 mm | n.i. | *** | n.i. | n.i. | n.i. | *** |

PD: pocket depth; CAL: clinical attachment loss; BOP: bleeding on probing; n.i.: no information. *GI > 1; **BOP in whole gingiva < 10%, ***bone loss > 30%.

maxillae of ApoE$^{-/-}$ mice after infection with periodontal pathogenic bacteria *P.g.*, *Treponema denticola* and *Tannerella forsythia*. MiR-146a primarily showed a significant increase in both maxillae and spleens, while miR-132 and miR-155 showed only minor changes [13].

*3.1.3. In Vivo Human.* Seven in vivo studies investigating differential expression of miRNAs in human tissue samples were included (Table 1). In particular, Lee et al. (2011) compared the expression of inflammatory miRNAs in healthy and inflamed periodontal tissue. Microarray analysis indicated that expression of six miRNAs was upregulated more than eightfold and that expression of 22 miRNAs increased more than fourfold in periodontal disease tissue compared with healthy tissue. Of these miRNAs, six could be validated by qRT-PCR [21]. A similar investigation considered miRNA profiles of healthy and inflamed gingival tissues. Microarray analysis of healthy and inflamed gingival tissues indicated that expression of five miRNAs was upregulated more than fivefold, 85 miRNAs, twofold to fivefold. Moreover, expression of 34 miRNAs was downregulated twofold to fivefold in inflamed tissue. 12 miRNAs were validated by qRT-PCR [22]. A study of Japanese patients identified 17 upregulated and 22 downregulated miRNAs; six were selected for validation [23]. A further investigation also detected differential miRNA expression by microarray analysis, wherein 91 upregulated and 68 downregulated miRNAs were found. Six miRNAs were validated, and potential target genes were investigated [24]. Furthermore, Motedayyen et al. (2015) showed a positive relationship between miR-146a and clinical parameters in patients with chronic periodontitis [25]. Thus, many miRNAs with differential expression as detected by microarray analysis have not been validated by qRT-PCR; these miRNAs will not be discussed (Table 1).

Additionally, two studies investigated the roles of miRNAs in periodontal disease in association with systemic factors and disease; thus, differential expression of miRNAs between obese periodontitis patients and nonobese periodontitis patients was examined. However, different, nonoverlapping miRNAs were reported in each investigation [26, 27].

Notably, criteria for patient selection and for classification of healthy and diseased patients were similar across all studies. However, differences exist between investigations in defining periodontal health and periodontitis (Table 2). Additionally the number of study participants for in vivo investigations varied enormously between studies with ten patients or less for each group in most cases (Table 1). Likewise, methodological approach was similar across studies: microarray based technology was often used to screen and identify miRNAs with differential expression, and qRT-PCR was used for subsequent validation of top-hits. However, detailed methods were different; thus, for example, different miRNA isolation methods were used. Also differences in statistical analysis are conspicuous, especially internal controls that distinguish between the studies (Table 1).

*3.2. MiRNA Detection in Saliva as a Noninvasive Diagnostic Tool.* Nine investigations were included that illustrate the potential of saliva as noninvasive diagnostic tool for miRNA analyzes. We focused on salivary miRNA species analysis as a diagnostic method for oral cancer and precancer detection, because no results of appropriate dental and periodontal disease investigations could be found. Six of included studies examined oral squamous cell carcinoma, one precancerous lesions, one esophageal cancer, and one tumor of parotid gland. Park et al. (2009) showed presence of miRNA species in whole saliva and in saliva supernatants and described differential expression of miR-125a and miR-200a between cases and controls. Besides that stability of exogenous (miR-124a) and endogenous (miR-191) miRNA in saliva was also examined in this study, and endogenous miRNA was shown to have higher stability than exogenous miRNA [28]. Another investigation found that miR-9, miR-191, and miR-134 appeared to be potential markers for noninvasive diagnosis of oral squamous cell carcinoma using saliva. Additionally, this study describes a reliable isolation method for miRNAs from even small volumes of saliva [29]. In a study by Hung et al. (2016) salivary miRNAs, specifically miR-31 and miR-21 were investigated in oral premalignant disorders, particularly showing miR-31 to be a sufficient marker for high risk disorders and malignant transformation [30]. Also Liu et al. (2012) examined miR-31, and its expression in saliva was compared with its expression in plasma; thereby, a correlation could be shown. These authors also observed a decrease in expression of miR-31 after surgical resection of primary tumor [31]. Similarly, Duz et al. (2016) reduced miR-139-5p expression in patients with tongue carcinoma and normalization after surgical treatment [32].

In a study investigating expression of salivary miRNAs in premalignant lesions of oral cavity, a correlation was found between deregulated miRNA expression in tissue and saliva, although miRNA concentrations in saliva were lower [33]. A further investigation compared expression of miRNA in saliva between patients with oral squamous cell carcinoma, patients with oral squamous cell carcinoma in remission, patients with oral lichen planus, and a healthy control group. In addition to differences in miRNA expression between the groups, miR-27b was significantly overexpressed in saliva of patients with oral squamous cell carcinoma. Furthermore, completely different profiles of miRNAs could be found in cancerous tissues compared to biological fluids [34]. Additionally, another study found miRNA-184 to have the potential to distinguish between OSCC and potentially malignant disorders [35].

In addition, altered miRNA expression in saliva was also verified for esophageal cancer [36] and parotid gland tumors [37]. Their methodologies are shown in Table 3. In principle, methods of measurement (microarray, qRT-PCR) are similar between the studies. Remarkable differences could be found regarding form of saliva, extraction methods, statistical analysis, and internal controls. The number of study participants was already higher than for periodontitis. However, heterogeneous groups sizes with partly seven or eight patients each group [33, 34] and partly 50 patients and more each group [28, 29] were investigated (Table 3).

## 4. Discussion

Several studies investigating putative role of miRNAs in periodontal diseases have been performed; however, spectrum of miRNAs identified was substantially heterogeneous. Both in vitro and in vivo investigations were performed for periodontal disease, and five miRNAs, that is, miR-142-3p, miR-146a, miR-155, miR-203, and miR-223, were validated in more than one study. Accordingly, we can conclude that these miRNAs may play important role and thus could become potential markers for periodontal disease. However, majority of identified miRNA species differed significantly across studies (Table 1).

MiR-146a and miR-155 were dominant in in vitro studies. Otherwise, these miRNAs have also been identified in other inflammatory diseases and various cancers. MiR-146a seems to play a role in multifarious diseases and its function in bacterial infections has already been discussed [38]. MiR-155 has also been mentioned [39]. LPS may have a substantial effect on the expression of miR-146a and miR-155 [40]. Consequently, their altered expression during periodontal disease is not surprising because bacterial virulence factors such as LPS are relevant in pathogenesis of periodontitis [41]. These miRNAs are associated with many other inflammatory diseases, which might be indicative of a limited specificity. Additionally, miR-142-3p is associated with inflammation and LPS exposure [42, 43]. MiR-203 may also play an important role in inflammation and correlate with LPS exposure [44]. Interestingly, miR-203 expression is associated with immune reaction of skin [45, 46]. MiR-203 expression in keratinocytes is important; therefore, this miRNA may also be involved in gingivitis and periodontitis as determined by its effect on gingival keratinocytes. Finally, miR-223 also plays an important role in inflammation and LPS exposure [47]. Accordingly, correlations between these five miRNAs and periodontal disease are possible. Interestingly, these miRNAs appear to play a role in oral cancer. Thus, their roles in periodontal disease should be further examined (Table 4) as it might indicate a link between periodontitis and head and neck squamous cell carcinoma (HNSCC). Figure 1 shows a summary of the interactions between periodontitis and HNSCC [48], and some potentially involved miRNAs. In this context, miR-29b, which showed an in vitro correlation with LPS from *P.g.* grown on cigarette smoke extract [18], has also been examined for oral cancer [49]. These findings raise the question if these miRNAs could serve as diagnostic markers for periodontal diseases or simply reflect an increased inflammatory state associated with various diseases.

Another problem is the great diversity of results. Although many miRNAs were found, convergence between the investigations, particularly in vivo, is small, resulting in conflicting conclusions. Reasons for differences between investigations of periodontal disease may differ. On the one hand, small variety in patient selection and different clinical procedures potentially causes these differences (Table 2). For future studies an evident graduation, for example, Page and Eke 2007, should be used for standardization [50]. On the other hand, variations in methods used for miRNA detection could play a crucial role (Table 3). A comparison of methods used suggests that miRNA detection procedures were quite similar. However, many differences in detailed procedures are evident. Using microarray and quantitative RT-PCR for analyzing and validating miRNAs are essential components of most investigations. However, detailed procedures for methods primarily differ, so several arrays from different manufacturing companies were performed. Importantly, differences in all of chosen criteria can be found between the investigations. Besides variations in statistical analyses it is important that internal controls differed in most cases.

Another aspect that could be crucial for the varying results is the small number of study participants. One study even investigated only three healthy and three diseased individuals [23]. A large group of 86 patients was selected only once [24]. The use of small sample sizes increases vulnerability to a range of errors and biases and may be a major reason for heterogeneous study results and potential false-positive findings. Similar issues were faced in early human genetic association studies, where many of reported genotype-phenotype associations could not be replicated in subsequent studies [51, 52]. Today, replication of initial findings in second cohort is considered essential for establishing the credibility of a genotype-phenotype association [53] and similar approaches could be adopted for studies reporting associations between miRNAs and diseases. Clearly, complex reason for differences in results exists. Different methods, clinical criteria, small groups of patients, and small specificity of miRNAs for periodontal disease could be causal complex.

In summary, comparability of results for the roles of miRNAs in periodontal disease is questionable. Potential

Table 3: Comparison of methods for salivary miRNA diagnosis.

| Author and year | Number of study participants | Form of saliva | miRNA extraction method | Detection of miRNA profile | Validation | Internal control | Statistical analysis | Potential marker for carcinoma |
|---|---|---|---|---|---|---|---|---|
| Park et al. 2009 [28] | 50 oral squamous cell carcinoma (OSCC) patients and 50 healthy matched control subjects | Unstimulated whole saliva and saliva supernatant | mirVana™ miRNA Isolation Kit (Ambion) DNA-free™ (Ambion) | RT-preamp-qPCR (Applied Biosystems) | (i) RT-preamp-qPCR (Applied Biosystems) (ii) Four-plex RT-preamp-qPCR for miR-142-3p, miR-200a, miR-125a, and miR-93. | U6 snRNA | Mann-Whitney $U$ test | miR-125a miR-200a |
| Liu et al. 2012 [31] | 45 patients with OSCC and 10 patient with oral verrucous leukoplakia 24 healthy individuals | Unstimulated saliva supernatant | mirVana PARIS Isolation kit (Ambion) DNase digestion | TaqMan miRNA assay system (Applied Biosystems) | TaqMan miRNA assay system (Applied Biosystems) | miR-16 | Mann-Whitney test, Wilcoxon matched pairs test, and linear regression analysis, receiver-operating characteristics ROC analysis | miR-31 |
| Yang et al. 2013 [33] | 8 progressing LGD leukoplakias 7 nonprogressing LGD leukoplakias 7 healthy volunteers | Unstimulated saliva | RNeasy Micro Kit (Qiagen) | TaqMan® low density array (TLDA) qRT-PCR system (Applied Biosystems) were used for global miR expression analysis in tissue samples | TaqMan MicroRNA Assay (Applied Biosystems) | RNU6 | Random variance $t$-test, Benjamini-Hochberg false discovery rate (FDR) method, Mann-Whitney $U$ test, Student's $t$-tests (two-sided) | miR-10b, miR-145, miR-99b, miR-708, miR-181c, miR-30e, miR-660, miR-197 |
| Xie et al. 2013 [36] | 39 patients with esophageal cancer and 19 healthy controls | Stimulated whole saliva and supernatant | mirVana PARIS Kit (Ambion) | 7 whole saliva samples for EC group and 3 for healthy group for Agilent microarray 11.0 (Agilent Technologies) | RT-qPCR SYBR Premix Ex Taq (TaKaRa) | miR-16 | Mann-Whitney $U$ test or the Kruskall-Wallis $H$ test, $\chi^2$ test, receiver-operating characteristics ROC curves, Spearman's correlation test | miR-10b, miR-144, miR-451 (in whole saliva) miR-10b*, miR-144, miR-21, miR-451 (in saliva supernatant) |
| Matse et al. 2013 [37] | 38 patients with malignant and 29 with benign parotid gland tumors | Whole saliva and supernatant | mirVana Paris kit (Ambion) DNase I (Qiagen) | TaqMan Human MicroRNA Cards (Applied Biosystems) TaqMan MicroRNA assays (Applied Biosystems) | TaqMan microRNA assays (Applied Biosystems) | U6 snRNA | Wilcoxon rank-sum test, ROC curves | hsa-miR-374, hsa-miR-222, hsa-miR-15b, hsa-let-7g, hsa-miR-132, mmu-miR-140-5p |

Table 3: Continued.

| Author and year | Number of study participants | Form of saliva | miRNA extraction method | Detection of miRNA profile | Validation | Internal control | Statistical analysis | Potential marker for carcinoma |
|---|---|---|---|---|---|---|---|---|
| Momen-Heravi et al. 2014 [34] | 9 OSCC patients before treatment, 8 patients with OSCC in remission, 8 patients with OLP, and 9 healthy controls | Unstimulated whole saliva | RNeasy kit (Qiagen) | multiplexed NanoString nCounter miRNA expression assay (NanoString Technologies) | TaqMan MicroRNA assay (Applied Biosystems) | miRNA-191 | 1-way analysis of variance, followed by a 2-tailed Mann-Whitney *U* test or Student's *t*-test, ROC curves analysis | miR-27b |
| Salazar et al. 2014 [29] | 5 HNSCC patients, 5 healthy controls for expression analysis 56 HNSCC patients, 56 healthy controls for validation | Unstimulated whole saliva | QIAzol lysis reagent (Qiagen) NucleoSpin miRNA kit (Macherey-Nagel) | miScript™ miRNA PCR arrays (Qiagen) | RT-qPCR miScript SYBR green PCR master mix (Qiagen) | SNORD96A | Mann Withney *U*-test, ROC curves, Wilcoxon rank sum test, Bonferroni method | miR-9, miR-134, miR-191 |
| Zahran et al. 2015 [35] | 20 healthy controls, 40 potentially malignant disorders, 20 OSCC, 20 recurrent aphthous stomatitis | Unstimulated saliva supernatant | miRNeasy serum/plasma extraction kit (Qiagen) | — | RT-qPCR SYBR green PCR kit (Qiagen) | SNORD68 | One-way ANOVA, *F*-test, Dunnett *t*-test, Scheffe's multiple comparison, two-tailed tests | miR-21, miR-184, miR-145 |
| Duz et al. 2016 [32] | 50 saliva samples from 25 TSCC patients (once prior to and once after surgical treatment) | Unstimulated saliva supernatant | mirVana PARIS kit (Ambion) | 8 samples (4 TSCC patients 4 healthy control) using Agilent 8 × 60K human V19 miRNA microarrays | TaqMan MicroRNA assay (Applied Biosystems) | RNU6b | Two-sided Student's *t*-test, Receiver operating characteristic (ROC) curves, 95% confidence interval (CI) | miR-139-5p |
| Hung et al. 2016 [30] | 20 patients with oral potentially malignant disorders, 24 healthy individuals | Unstimulated saliva supernatant | mirVana PARIS isolation kit (Ambion) | — | TaqMan microRNA assay (Applied Biosystems) | miR-16 | Unpaired test, ROC curves, Kaplan-Meier method, log-rank test, Cox proportional hazard model | miR-21 miR-31 |

RNA extraction and isolation methods are listed. The proof of quality and quantity and quality assurance are shown as important quality criteria to draw conclusions regarding the reproducibility and standardization of the investigations. Furthermore, methods for miRNA profiling and miRNA validation are listed. In addition, the number of study participants is provided to draw conclusions regarding the validity of the results. The form of saliva is also listed.

Table 4: Comparison of miRNas frequently mentioned in connection with periodontal disease with results from selected oral cancer and precancer investigations.

| | Kozaki et al. 2008 [63] | Park et al. 2009 [28] | Cervigne et al. 2009 [64] | Scapoli et al. 2010 [65] | Lajer et al. 2011 [66] | Lundegard et al. 2015 [56] |
|---|---|---|---|---|---|---|
| miR-146a | X | | X | X | X | |
| miR-155 | | | X | X | X | |
| miR-203 | X | | | | | X |
| miR-142-3p | | X | X | | | |
| miR-223 | | | X | | X | |

Each miRNA that was validated in the context of periodontal disease was also mentioned in investigations for cancer and precancer of the oral cavity. This table provides a small exemplary overview. Consequently, even more studies with similar results are not shown here.

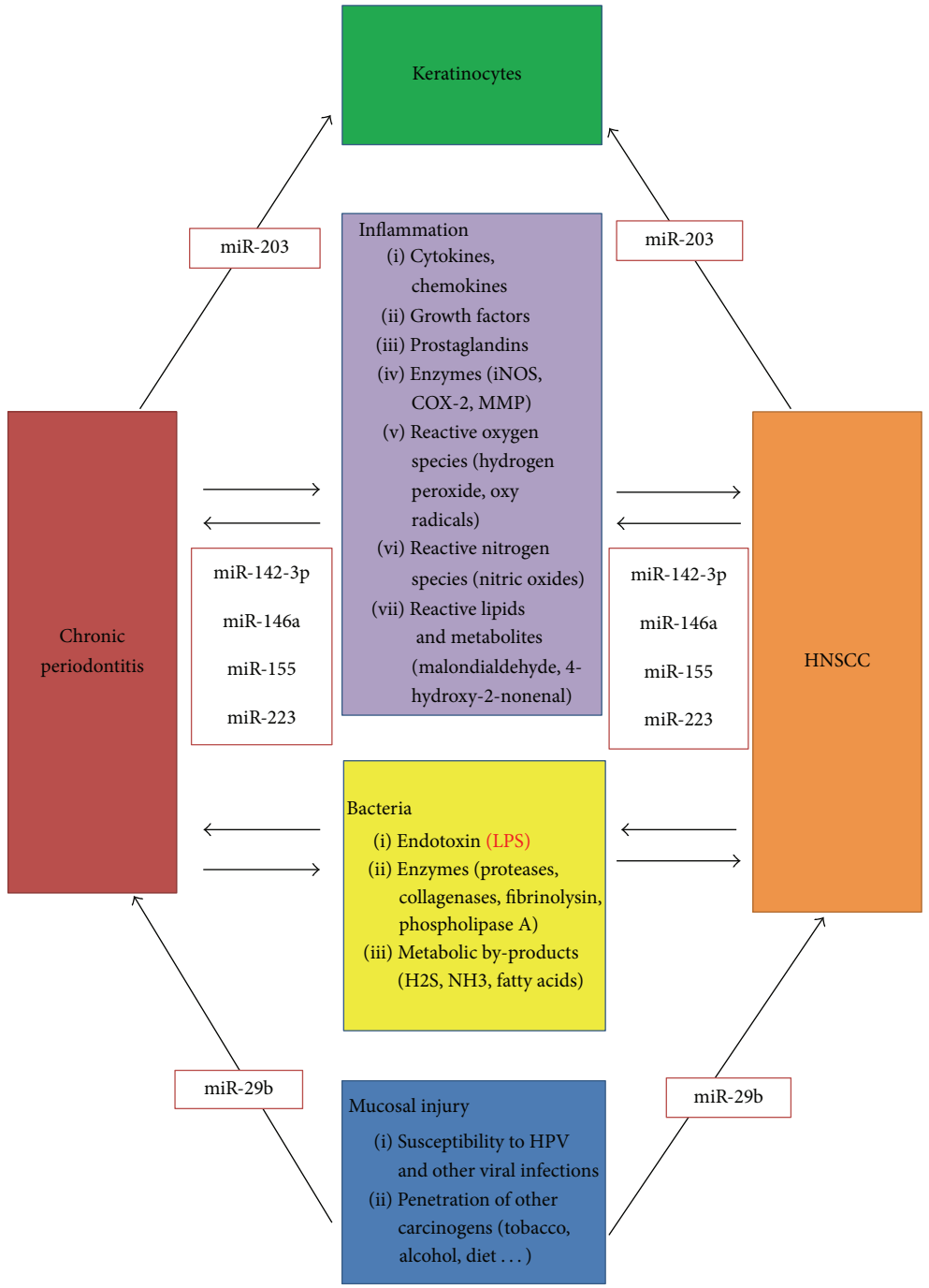



Figure 1: Model for the relationship among chronic periodontitis, HNSCC (modif. Han et al. 2014 [48]) and potential miRNAs that may be involved in the corresponding processes.

candidate miRNAs as promising markers of periodontal disease do not appear to be specific for periodontitis.

Use of salivary miRNAs as noninvasive diagnostic markers has been studied in context of oral cancer, precancer, esophageal cancer, and parotid gland cancer so far. Again, reported results of these studies were quite divergent with little overlap in identified miRNA species. Importantly, these investigations used similar methods for miRNA detection in periodontal tissue and saliva; however, detailed methods are quite different (Tables 1 and 3).

In principle, detectability of miRNAs in saliva seems certainly possible, so, for example, a current study was able to detect noncoding RNAs in saliva: 127 to 418 miRNAs could be detected in each sample of human cell free saliva, with miR-223-3p being most abundant [54]. Interestingly, miR-223 is also found in periodontal tissue. Different studies have examined saliva to identify miRNAs as potential markers for oral cancer and precancer and report remarkably diverse results (Table 3). Strangely, basic principles of investigations were similar, but different internal controls, methods for miRNA isolation, statistical analysis, and forms of saliva (stimulated/unstimulated, whole saliva/supernatant) were chosen. Notably, number of study participants is already higher in these investigations than in studies of periodontitis, but also heterogeneous with partly small group sizes (Tables 1 and 3). In addition, form of saliva may play an important role, especially whether stimulated or unstimulated saliva is used may be relevant as well as exact procedure for obtaining saliva and criteria for patient selection. Furthermore, lack of stability of exogenous miRNAs in saliva may result in quick changes of miRNA concentrations from bacteria and inflammatory reactions [28]. Potential concentrations of miRNAs in body fluids in exosomes could also affect their detectability in human saliva samples [55]. Beside of that, however, it was mentioned that there are vesicle-free noncoding RNAs in saliva [54]. Furthermore, it is questionable whether identified miRNAs from tissue investigations are also potential markers for salivary diagnostic methods. The finding of completely different miRNA expression between cancerous tissue and body fluids [34] could be similar for periodontitis.

Detailed procedures of miRNA extraction and detection could also be relevant. In a recent study by Lundegard et al. (2015) expression of miR-203 was examined in whole saliva using two different PCR methods. The study concluded that detecting low levels of miRNA in saliva is difficult; more efficient extraction methods and more sensitive PCR techniques are necessary to use saliva as a reproducible source of miRNAs [56]. Because of differences in methods used, it is questionable if investigations are comparable at all. Some miRNAs may have variable expression between different microarray, PCR, and preparation methods [57]. Reproducibility and standardization of procedure appear relevant. Validated protocols such as those already described [58] may help to standardize the procedures. This would allow better reproducibility and provide more meaningful and more comparable results. This is supported by results of Hung et al. and Liu et al. both showing miR-31 to be a potential marker for malignant tumor [30, 31]. As shown in Table 3, both studies used same form of saliva, extraction, and validation methods as well as internal controls (Table 3). Accordingly, use of saliva for diagnosis of periodontal disease under condition of uniform methods seems conceivable.

However, the mechanism of miRNA infiltration in saliva during periodontal diseases must be considered. Thereby, exosomes might play a key role in miRNA transport from different cells into saliva [59]. In periodontal tissue, the junctional epithelial layers might be of highest relevance in this context. The junctional epithelium contains only few desmosomes and has therefore widened intercellular spaces [60]. Accordingly, this epithelium allows transport of several molecules between tissue and gingival crevicular fluid (GCF) [61]. Additionally, an enlarged permeability with an increase of GCF flow is observed during gingival and periodontal inflammation [61]. Consequently, high amounts of miRNA might pass the junctional epithelium, arriving GCF and thus saliva.

If comparable results can be achieved, further investigations will be of interest. For example, changes in miRNA expression after surgical resection of a tumor, as already described [3, 32], could become clinically useful by demonstrating improved periodontal conditions after therapy as well. Therefore, it could be assessed whether periodontitis therapy was successful by analyzing expression of specific miRNAs. Additionally, correlation between periodontitis and systemic diseases could be illustrated by altered miRNA expression [26, 27]. Although this topic seems to have great potential to provide further insights regarding oral disease, a critical view is needed. Currently, we have insufficient knowledge regarding oral disease and the roles of miRNAs in pathological processes. At present it is impossible to provide a clear statement regarding real relevance and possibilities of miRNA analysis. Nevertheless, using miRNAs to understand oral diseases, particularly periodontitis, and potential use of miRNAs as noninvasive markers or as therapeutic targets could be a great approach, which justifies basic research in any case. In conclusion, we can confirm unequivocally that salivary miRNA diagnosis for periodontal disease is a revolutionary idea. However, considering that additional investigations and standardized methods are required, possibilities of exploiting potential could be estimated only in the future.

## 5. Conclusion

Besides similar methods regarding miRNA extraction, profiling, and validation, there are methodical differences between studies, especially in internal controls and sample size, resulting in heterogeneous results. In principle, salivary miRNA diagnostic methods seem feasible. However, in our opinion, standardized criteria and protocols should be established and followed exactly to obtain comparable results. Five miRNAs related to inflammation are available, which may be used as potential markers for periodontitis. However, their detectability and expression in saliva and, accordingly, their importance as noninvasive markers are questionable.

## Competing Interests

The authors declare that they have no conflict of interests.

## Acknowledgments

The authors acknowledge support from the German Research Foundation (DFG) and Universität Leipzig within the program of Open Access Publishing.

## References

[1] D. P. Bartel, "MicroRNAs: genomics, biogenesis, mechanism, and function," *Cell*, vol. 116, no. 2, pp. 281–297, 2004.

[2] R. M. O'Connell, D. S. Rao, A. A. Chaudhuri, and D. Baltimore, "Physiological and pathological roles for microRNAs in the immune system," *Nature Reviews Immunology*, vol. 10, no. 2, pp. 111–122, 2010.

[3] J. Hao, S. Zhao, Y. Zhang et al., "Emerging role of MicroRNAs in cancer and cancer stem cells," *Journal of Cellular Biochemistry*, vol. 115, no. 4, pp. 605–610, 2014.

[4] S. Qin and C. Zhang, "MicroRNAs in vascular disease," *Journal of Cardiovascular Pharmacology*, vol. 57, no. 1, pp. 8–12, 2011.

[5] R. Maqbool and M. U. Hussain, "MicroRNAs and human diseases: diagnostic and therapeutic potential," *Cell and Tissue Research*, vol. 358, no. 1, pp. 1–15, 2014.

[6] D. Chen, R. J. Cabay, Y. Jin et al., "MicroRNA deregulations in head and neck squamous cell carcinomas," *Journal of Oral & Maxillofacial Research*, vol. 4, no. 1, article e2, 2013.

[7] R. Roy, N. De Sarkar, S. Ghose et al., "Association between risk of oral precancer and genetic variations in microRNA and related processing genes," *Journal of Biomedical Science*, vol. 21, article 48, 2014.

[8] C. Staedel and F. Darfeuille, "MicroRNAs and bacterial infection," *Cellular Microbiology*, vol. 15, no. 9, pp. 1496–1507, 2013.

[9] S. H. Kim, S. Y. Lee, Y. M. Lee, and Y. K. Lee, "MicroRNAs as biomarkers for dental diseases," *Singapore Dental Journal*, vol. 36, pp. 18–22, 2015.

[10] M. Kebschull and P. N. Papapanou, "Mini but mighty: microRNAs in the pathobiology of periodontal disease," *Periodontology 2000*, vol. 69, no. 1, pp. 201–220, 2015.

[11] R. S. Patel, A. Jakymiw, B. Yao et al., "High resolution of microRNA signatures in human whole saliva," *Archives of Oral Biology*, vol. 56, no. 12, pp. 1506–1513, 2011.

[12] X. Lin, H.-C. Lo, D. T. W. Wong, and X. Xiao, "Noncoding RNAs in human saliva as potential disease biomarkers," *Frontiers in Genetics*, vol. 6, article 175, 2015.

[13] M. A. Nahid, M. Rivera, A. Lucas, E. K. L. Chan, and L. Kesavalu, "Polymicrobial infection with periodontal pathogens specifically enhances microRNA miR-146a in ApoE-/- mice during experimental periodontal disease," *Infection and Immunity*, vol. 79, no. 4, pp. 1597–1605, 2011.

[14] T. Honda, N. Takahashi, S. Miyauchi, and K. Yamazaki, "Porphyromonas gingivalis lipopolysaccharide induces miR-146a without altering the production of inflammatory cytokines," *Biochemical and Biophysical Research Communications*, vol. 420, no. 4, pp. 918–925, 2012.

[15] S.-Y. Jiang, D. Xue, Y.-F. Xie et al., "The negative feedback regulation of microRNA-146a in human periodontal ligament cells after Porphyromonas gingivalis lipopolysaccharide stimulation," *Inflammation Research*, vol. 64, no. 6, pp. 441–451, 2015.

[16] Y. F. Xie, R. Shu, S. Y. Jiang, D. L. Liu, J. Ni, and X. L. Zhang, "MicroRNA-146 inhibits pro-inflammatory cytokine secretion through IL-1 receptor-associated kinase 1 in human gingival fibroblasts," *Journal of Inflammation*, vol. 10, article 20, 2013.

[17] C. R. Sipert, A. C. Morandini, T. J. Dionísio, A. J. Trachtenberg, W. P. Kuo, and C. F. Santos, "MicroRNA-146a and microRNA-155 show tissue-dependent expression in dental pulp, gingival and periodontal ligament fibroblasts *in vitro*," *Journal of Oral Science*, vol. 56, no. 2, pp. 157–164, 2014.

[18] A. R. Naqvi, J. B. Fordham, A. Khan, and S. Nares, "MicroRNAs responsive to Aggregatibacter actinomycetemcomitans and Porphyromonas gingivalis LPS modulate expression of genes regulating innate immunity in human macrophages," *Innate Immunity*, vol. 20, no. 5, pp. 540–551, 2014.

[19] K. Ouhara, I. J. Savitri, T. Fujita et al., "MiR-584 expressed in human gingival epithelial cells is induced by porphyromonas gingivalis stimulation and regulates interleukin-8 production via lactoferrin receptor," *Journal of Periodontology*, vol. 85, no. 6, pp. e198–e204, 2014.

[20] C. E. Moffatt and R. J. Lamont, "Porphyromonas gingivalis induction of microRNA-203 expression controls suppressor of cytokine signaling 3 in gingival epithelial cells," *Infection and Immunity*, vol. 79, no. 7, pp. 2632–2637, 2011.

[21] Y. H. Lee, H. S. Na, S. Y. Jeong, S. H. Jeong, H. R. Park, and J. Chung, "Comparison of inflammatory microRNA expression in healthy and periodontitis tissues," *Biocell*, vol. 35, no. 2, pp. 43–49, 2011.

[22] Y. F. Xie, R. Shu, S. Y. Jiang, D. L. Liu, and X. L. Zhang, "Comparison of microRNA profiles of human periodontal diseased and healthy gingival tissues," *International Journal of Oral Science*, vol. 3, no. 3, pp. 125–134, 2011.

[23] Y. Ogata, S. Matsui, A. Kato, L. Zhou, Y. Nakayama, and H. Taka, "MicroRNA expression in inflamed and noninflamed gingival tissues from Japanese patients," *Journal of Oral Science*, vol. 56, no. 4, pp. 253–260, 2014.

[24] C. Stoecklin-Wasmer, P. Guarnieri, R. Celenti, R. T. Demmer, M. Kebschull, and P. N. Papapanou, "MicroRNAs and their target genes in gingival tissues," *Journal of Dental Research*, vol. 91, no. 10, pp. 934–940, 2012.

[25] H. Motedayyen, S. Ghotloo, M. Saffari, M. Sattari, and R. Amid, "Evaluation of MicroRNA-146a and its targets in gingival tissues of patients with chronic periodontitis," *Journal of Periodontology*, vol. 86, no. 12, pp. 1380–1385, 2015.

[26] R. Perri, S. Nares, S. Zhang, S. P. Barros, and S. Offenbacher, "MicroRNA modulation in obesity and periodontitis," *Journal of Dental Research*, vol. 91, no. 1, pp. 33–38, 2012.

[27] A. Z. Kalea, R. Hoteit, J. Suvan et al., "Upregulation of gingival tissue miR-200b in obese periodontitis subjects," *Journal of Dental Research*, vol. 94, pp. 59–69, 2015.

[28] N. J. Park, H. Zhou, D. Elashoff et al., "Salivary microRNA: discovery, characterization, and clinical utility for oral cancer detection," *Clinical Cancer Research*, vol. 15, no. 17, pp. 5473–5477, 2009.

[29] C. Salazar, R. Nagadia, P. Pandit et al., "A novel saliva-based microRNA biomarker panel to detect head and neck cancers," *Cellular Oncology*, vol. 37, no. 5, pp. 331–338, 2014.

[30] K.-F. Hung, C.-J. Liu, P.-C. Chiu et al., "MicroRNA-31 upregulation predicts increased risk of progression of oral potentially malignant disorder," *Oral Oncology*, vol. 53, pp. 42–47, 2016.

[31] C.-J. Liu, S.-C. Lin, C.-C. Yang, H.-W. Cheng, and K.-W. Chang, "Exploiting salivary miR-31 as a clinical biomarker of oral squamous cell carcinoma," *Head and Neck*, vol. 34, no. 2, pp. 219–224, 2012.

[32] M. B. Duz, O. F. Karatas, E. Guzel et al., "Identification of miR-139-5p as a saliva biomarker for tongue squamous cell carcinoma: A Pilot Study," *Cellular Oncology*, vol. 39, no. 2, pp. 187–193, 2016.

[33] Y. Yang, Y. X. Li, X. Yang, L. Jiang, Z. J. Zhou, and Y. Q. Zhu, "Progress risk assessment of oral premalignant lesions with saliva miRNA analysis," *BMC Cancer*, vol. 13, article 129, 2013.

[34] F. Momen-Heravi, A. J. Trachtenberg, W. P. Kuo, and Y. S. Cheng, "Genomewide study of salivary MicroRNAs for detection of oral cancer," *Journal of Dental Research*, vol. 93, supplement 7, pp. 86S–93S, 2014.

[35] F. Zahran, D. Ghalwash, O. Shaker, K. Al-Johani, and C. Scully, "Salivary microRNAs in oral cancer," *Oral Diseases*, vol. 21, no. 6, pp. 739–747, 2015.

[36] Z. Xie, G. Chen, X. Zhang et al., "Salivary microRNAs as promising biomarkers for detection of esophageal cancer," *PLoS ONE*, vol. 8, no. 4, Article ID e57502, 2013.

[37] J. H. Matse, J. Yoshizawa, X. Wang et al., "Discovery and prevalidation of salivary extracellular microRNA biomarkers panel for the noninvasive detection of benign and malignant parotid gland tumors," *Clinical Cancer Research*, vol. 19, no. 11, pp. 3032–3038, 2013.

[38] Z. Liu, B. Xiao, B. Tang et al., "Up-regulated microRNA-146a negatively modulate *Helicobacter pylori*-induced inflammatory response in human gastric epithelial cells," *Microbes and Infection*, vol. 12, no. 11, pp. 854–863, 2010.

[39] L. N. Schulte, A. J. Westermann, and J. Vogel, "Differential activation and functional specialization of miR-146 and miR-155 in innate immune sensing," *Nucleic Acids Research*, vol. 41, no. 1, pp. 542–553, 2013.

[40] Y. Cheng, W. Kuang, Y. Hao et al., "Downregulation of miR-27a* and miR-532-5p and upregulation of mir-146a and mir-155 in LPS-induced RAW264.7 Macrophage cells," *Inflammation*, vol. 35, no. 4, pp. 1308–1313, 2012.

[41] D. Mayrand and S. C. Holt, "Biology of asaccharolytic black-pigmented *Bacteroides* species," *Microbiological Reviews*, vol. 52, no. 1, pp. 134–152, 1988.

[42] S. A. Moschos, A. E. Williams, M. M. Perry, M. A. Birrell, M. G. Belvisi, and M. A. Lindsay, "Expression profiling in vivo demonstrates rapid changes in lung microRNA levels following lipopolysaccharide-induced inflammation but not in the anti-inflammatory action of glucocorticoids," *BMC Genomics*, vol. 8, article 240, 2007.

[43] G. Xu, Z. Zhang, J. Wei et al., "MicroR-142-3p down-regulates IRAK-1 in response to Mycobacterium bovis BCG infection in macrophages," *Tuberculosis*, vol. 93, no. 6, pp. 606–611, 2013.

[44] X.-F. Ke, J. Fang, X.-N. Wu, and C.-H. Yu, "MicroRNA-203 accelerates apoptosis in LPS-stimulated alveolar epithelial cells by targeting PIK3CA," *Biochemical and Biophysical Research Communications*, vol. 450, no. 4, pp. 1297–1303, 2014.

[45] M. N. Primo, R. O. Bak, B. Schibler, and J. G. Mikkelsen, "Regulation of pro-inflammatory cytokines TNF$\alpha$ and IL24 by microRNA-203 in primary keratinocytes," *Cytokine*, vol. 60, no. 3, pp. 741–748, 2012.

[46] T. Wei, N. Xu, F. Meisgen, M. Ståhle, E. Sonkoly, and A. Pivarcsi, "Interleukin-8 is regulated by miR-203 at the posttranscriptional level in primary human keratinocytes," *European Journal of Dermatology*, 2013.

[47] M. Haneklaus, M. Gerlic, L. A. J. O'Neill, and S. L. Masters, "MiR-223: infection, inflammation and cancer," *Journal of Internal Medicine*, vol. 274, no. 3, pp. 215–226, 2013.

[48] Y. W. Han, W. Houcken, B. G. Loos, H. A. Schenkein, and M. Tezal, "Periodontal disease, atherosclerosis, adverse pregnancy outcomes, and head-and-neck cancer," *Advances in Dental Research*, vol. 26, no. 1, pp. 47–55, 2014.

[49] C. N. Yang, Y. T. Deng, J. Y. Tang et al., "MicroRNA-29b regulates migration in oral squamous cell carcinoma and its clinical significance," *Oral Oncology*, vol. 51, no. 2, pp. 170–177, 2016.

[50] R. C. Page and P. I. Eke, "Case definitions for use in population-based surveillance of periodontitis," *Journal of Periodontology*, vol. 78, no. 7, pp. 1387–1399, 2007.

[51] J. N. Hirschhorn, K. Lohmueller, E. Byrne, and K. Hirschhorn, "A comprehensive review of genetic association studies," *Genetics in Medicine*, vol. 4, no. 2, pp. 45–61, 2002.

[52] A. T. Hattersley and M. I. McCarthy, "What makes a good genetic association study?" *The Lancet*, vol. 366, no. 9493, pp. 1315–1323, 2005.

[53] S. J. Chanock, T. Manolio, M. Boehnke et al., "Replicating genotype-phenotype associations," *Nature*, vol. 447, no. 7145, pp. 655–660, 2007.

[54] J. H. Bahn, Q. Zhang, F. Li et al., "The landscape of microRNA, piwi-interacting RNA, and circular RNA in human saliva," *Clinical Chemistry*, vol. 61, no. 1, pp. 221–230, 2015.

[55] A. Gallo, M. Tandon, I. Alevizos, and G. G. Illei, "The majority of microRNAs detectable in serum and saliva is concentrated in exosomes," *PLoS ONE*, vol. 7, no. 3, article e30679, 2012.

[56] M. Lundegard, K. Nylander, and K. Danielsson, "Difficulties detecting miRNA-203 in human whole saliva by the use of PCR," *Medicina Oral, Patologia Oral y Cirugia Bucal*, vol. 20, no. 2, pp. e130–e134, 2015.

[57] R. A. Ach, H. Wang, and B. Curry, "Measuring microRNAs: comparisons of microarray and quantitative PCR measurements, and of different total RNA prep methods," *BMC Biotechnology*, vol. 8, article 69, 2008.

[58] J. M. Yoshizawa and D. T. W. Wong, "Salivary microRNAs and oral cancer detection," *Methods in Molecular Biology*, vol. 936, pp. 313–324, 2013.

[59] V. Palanisamy, S. Sharma, A. Deshpande, H. Zhou, J. Gimzewski, and D. T. Wong, "Nanostructural and transcriptomic analyses of human saliva derived exosomes," *PLoS ONE*, vol. 5, article e8577, 2010.

[60] S. Hatakeyama, T. Yaegashi, Y. Oikawa et al., "Expression pattern of adhesion molecules in junctional epithelium differs from that in other gingival epithelia," *Journal of Periodontal Research*, vol. 41, no. 4, pp. 322–328, 2006.

[61] D. D. Bosshardt and N. P. Lang, "The junctional epithelium: from health to disease," *Journal of Dental Research*, vol. 84, no. 1, pp. 9–20, 2005.

[62] R. T. Demmer, J. H. Behle, D. L. Wolf et al., "Transcriptomes in healthy and diseased gingival tissues," *Journal of Periodontology*, vol. 79, no. 11, pp. 2112–2124, 2008.

[63] K. Kozaki, I. Imoto, S. Mogi, K. Omura, and J. Inazawa, "Exploration of tumor-suppressive microRNAs silenced by DNA hypermethylation in oral cancer," *Cancer Research*, vol. 68, no. 7, pp. 2094–2105, 2008.

[64] N. K. Cervigne, P. P. Reis, J. Machado et al., "Identification of a microRNA signature associated with progression of leukoplakia to oral carcinoma," *Human Molecular Genetics*, vol. 18, no. 24, pp. 4818–4829, 2009.

[65] L. Scapoli, A. Palmieri, L. Lo Muzio et al., "MicroRNA expression profiling of oral carcinoma identifies new markers of tumor

progression," *International Journal of Immunopathology and Pharmacology*, vol. 23, no. 4, pp. 1229–1234, 2010.

[66] C. B. Lajer, F. C. Nielsen, L. Friis-Hansen et al., "Different miRNA signatures of oral and pharyngeal squamous cell carcinomas: a prospective translational study," *British Journal of Cancer*, vol. 104, no. 5, pp. 830–840, 2011.

Hindawi Publishing Corporation
BioMed Research International
Volume 2016, Article ID 2509757, 6 pages
http://dx.doi.org/10.1155/2016/2509757

*Research Article*

# A Comparative Study of Microleakage on Dental Surfaces Bonded with Three Self-Etch Adhesive Systems Treated with the Er:YAG Laser and Bur

**Youssef Sanhadji El Haddar,[1] Sibel Cetik,[1,2] Babak Bahrami,[1] and Ramin Atash[1]**

[1]*Department of Stomatology and Dentistry, Erasmus Hospital, Université Libre de Bruxelles, 1070 Brussels, Belgium*
[2]*Laboratory of Physiology and Pharmaceutics, Faculty of Medicine, Université Libre de Bruxelles, 1070 Brussels, Belgium*

Correspondence should be addressed to Sibel Cetik; sibel.cetik@ulb.ac.be

Received 26 October 2015; Revised 27 January 2016; Accepted 1 February 2016

Academic Editor: Toni Zeinoun



*Aim*. This study sought to compare the microleakage of three adhesive systems in the context of Erbium-YAG laser and diamond bur cavity procedures. Cavities were restored with composite resin. *Materials and Methods*. Standardized Class V cavities were performed in 72 extracted human teeth by means of diamond burs or Er-YAG laser. The samples were randomly divided into six groups of 12, testing three adhesive systems (Clearfil $s^3$ Bond Plus, Xeno® Select, and Futurabond U) for each method used. Cavities were restored with composite resin before thermocycling (methylene blue 2%, 24 h). The slices were prepared using a microtome. Optical microscope photography was employed to measure the penetration. *Results*. No statistically significant differences in microleakage were found in the use of bur or laser, nor between adhesive systems. Only statistically significant values were observed comparing enamel with cervical walls ($p < 0.001$). *Conclusion*. It can be concluded that the Er:YAG laser is as efficient as diamond bur concerning microleakage values in adhesive restoration procedures, thus constituting an alternative tool for tooth preparation.

## 1. Introduction

The Er-YAG is currently the best adapted laser for dental applications, due to its wavelength coinciding with the water and hydroxyapatite peaks of absorption, thus conferring the ability to be very well absorbed in the dental tissues it targets, while also causing only limited penetration [1]. The transmitted energy has a thermomechanical effect on the water contained in the enamel and dentin.

As there is more water contained in decayed dentinal tissue than in healthy dentinal tissue, the treatment is more efficient on the decayed dentin, enabling selective tissue ablation. These observations are in line with the current dentistry approach of restorative dentistry, which protects the dental structure integrity by using the least invasive means possible. In this respect, Er:YAG lasers represent an ideal tool for modern dentistry. However, several parameters must still be studied, particularly in terms of the efficiency of the adhesive systems used on the surfaces undergoing these techniques.

The results presented in the literature on this matter are, in fact, highly divergent. Some studies have focused on the morphostructural analysis of the dental tissue following laser ablation as demonstrating an architecture in favor of bonding [2], whereas others have argued the contrary [3, 4].

We focused on one of the principal determining parameters of bonding quality: microleakage. Studies on this subject also present numerous contradictions. Some authors have reported unacceptably high microleakage values [5–8] with lasers, though their results are questionable, due to the use of excessive energy values (>300 mJ) during treatment. In contrast, other authors have reported the lack of significant differences between burs and lasers [9–14], whereas others have asserted that better waterproof values can be obtained with lasers compared to burs [15, 16].

Our current study thus sought to help clarify this question of microleakage from adhesive systems used on dental surfaces treated with Erbium-YAG laser.

## 2. Materials and Methods

*2.1. Sample Selection.* We included 72 extracted human wisdom teeth, all without crown changes, in this *in vitro* study. Osseous and gingival tissues as well as any residual calculus were resected by means of gouge plier and ultrasound. They were then cleaned and preserved in a physiological salt solution (0.9% NaCl) at room temperature prior to the experimental phase, in accordance with the recommendations of the International Organization for Standardization (ISO) [17].

*2.2. Cavity Preparations.* All samples were prepared by creating class V cavities using either a diamond bur or laser device.

The countersunk cavities were created by means of a diamond bur (Komet; 012 flat-end chuck cylinder) at high speed with abundant water spraying. These were performed in every 5th cavity in order to maintain an optimal ablation capacity and limit heating.

The laser-created cavities were prepared by means of an Er:YAG device (Fidelis Plus III, Fotona, Slovenia) with an R14 handpiece, on an articulated arm. The device parameters were chosen according to the manufacturer's recommendations: 300 mJ/pulses in 30 Hz, with a power of 9 Watts [18] for the enamel; 200 mJ/pulse in 20 Hz with a power of 4 Watts 20 for the dentine; duration of impulse: 100 $\mu$s (very short pulse) [19].

The system includes application of a cooling spray and enabled us to perform ablation without causing thermal damage to surrounding tissues. The radiation was delivered perpendicularly to the dental surfaces, maintaining a distance of approximately 6 mm during the operation.

Each cavity was created half in the enamel and half in cement. The chosen dimensions were as follows: 1.5 mm deep, 4 mm in length in the mesiodistal direction, and 2 mm in height. By using a periodontal probe (PCP UNC 15, Hu-Friedy, Chicago) during the procedures, we were able to scrupulously adhere to the previously defined dimensions.

*2.3. Composite Resin Bonding.* All the samples prepared in the laser ($n = 36$) and bur ($n = 36$) categories were divided into three groups ($n = 12$ in each) in order to test the microleakage of three different self-etching adhesive systems (Clearfil $s^3$ Bond Plus, Kuraray, Japan/Xeno Select, Dentsply, United States/Futurabond U, VOCO, Germany). Each of these systems was applied following the manufacturer's instructions. All the cavities were then restored with a composite resin (Filtek™ Supreme, 3M, United States), polymerized for 20 sec with an LED lamp (Elipar™ S10, 3M, United States, maximum intensity = 1200 MW/$cm^2$), and polished by means of abrasive discs of decreasing size (Sof-Lex™, 3M, United States).

The roots were then partially sectioned, with a color code attributed to each of the six groups in order to enable differentiation of the samples after 24 h thermocycling. The apexes were sealed with wax (Cavex Set Up Regular, Cavex, Netherlands). A colored varnish corresponding to each group was coated on the teeth in order to cover them completely, preserving the restorations and 1 mm of dental tissue around them. This was to prevent any excessive infiltration of the coloring agent, which would have made the results unfit for exact interpretation (see Table 1).

Table 1: Color code chosen for the different sample groups.

| Curettage method | Used adhesive system | Color of the varnish |
|---|---|---|
| Laser Er:YAG | Clearfil $s^3$ Bond Plus | Blue |
| | Xeno Select | Yellow |
| | Futurabond U | Red |
| Fraise | Clearfil $s^3$ Bond Plus | Green |
| | Xeno Select | Pink |
| | Futurabond U | Orange |

The filling then underwent an ageing process, boosted by thermocycling (2500 cycles of dumping in a bath of 0°C then of 50°C at 30 sec per bath), and then plunged into 2% methylene blue for 24 hours. Those samples were then abundantly rinsed to eliminate any excess of coloring agent.

*2.4. Cylinder Shaping.* The objective of this step was to block the cavities in a suitable position for performing histological cuts. For that purpose, the teeth had to be fixed to zinc-plated blind with one eye nut (diameter M6) to be correctly positioned to be screwed to the microtome for making the cuts. The shaping process was as follows:

(i) Sanding the nuts to increase the retention of the envelopment resin.

(ii) Cutting 20 mL plastic irrigation syringes of 20 mL (Terumo®, Japan) with a manual saw to serve as a mold.

(iii) Sample orientation to obtain a cut of the cavity in the sagittal plan and adhesion of these to the nut with wax (Cavex Set Up Regular, Cavex, Netherlands).

(iv) Positioning of the tooth/nut system in the center of the mold on a Vaseline-coated glass plate.

(v) Coating with some transparent polymethyl methacrylate resin (Orthocryl, Dentaurum, Germany).

(vi) Resin cylinder polymerization in a pressure cooker ($T^\circ \sim 50^\circ$C; $p^\circ = 2$ bars) for 15 min.

*2.5. Histological Slice Procedure.* After demolding, cylinders were fixed to a microtome (Leitz on 1600, Solms, Germany) through the nut. First, cuts were made at the cavity level in order to set up the section plane within the zone of interest. After securing one side with a small amount of cyanoacrylate glue, a second cut was made to obtain 700 $\mu$m histological slices.

Slices were then submitted to microscopic analysis to assess the degree of penetration of the coloring agent in the enamel and cementum walls. A score of 0–3 was attributed as shown in Table 2.

Figure 1 provides a visual description of the scoring.

In order to guarantee assessment objectivity, a double-blind analysis was performed, in which every blade saw was assigned a random number (1–72) determined by a randomization algorithm. All samples could, thus, be studied without

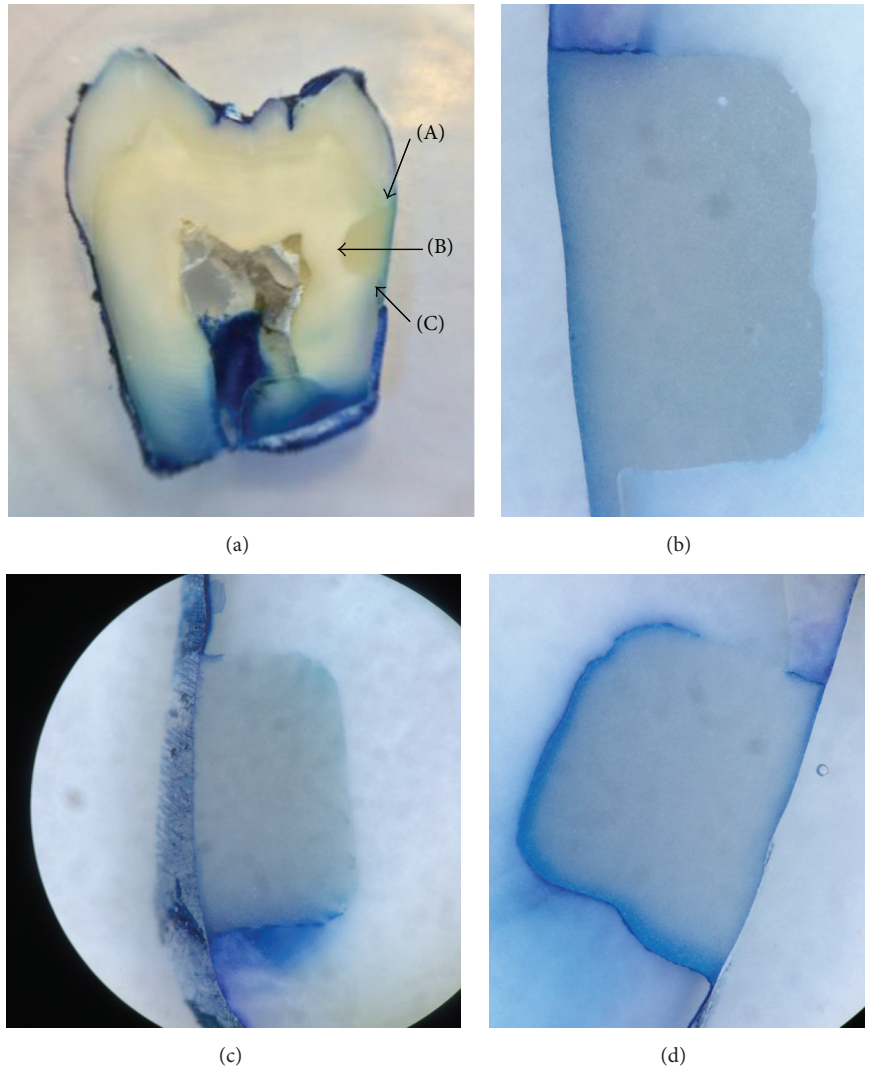



Figure 1: Illustration of the scoring system (a) macroscopic view; score 0: no infiltration. "A" represents the enamel wall, "B" the pulpal wall, and "C" the cement wall. (b) microscopic view; score 1: infiltration (here: enamel infiltration) inferior to the half of the wall length. (c) microscopic view; score 2: infiltration (here: cement infiltration) superior to the half of the wall length, without penetrating the pulpal wall. (d) microscopic view; score 3: infiltration (here: cement infiltration) with pulpal infiltration.

Table 2: Criteria used to score the infiltration.

| Score | Location of the infiltration |
|---|---|
| 0 | No infiltration |
| 1 | Half wall |
| 2 | Infiltration from half the wall to the whole wall without penetrating the pulp wall |
| 3 | Pulp wall |

knowing the group to which they belonged. Three examiners then analyzed all samples and scored them according to the above-mentioned methodology.

In the cases where a difference between investigators' observations was noted, the observations were discussed until a consensus was reached. The results were then listed in a contingency table. The collected information was rearranged to allow for comparison of waterproof quality between

(i) enamel and cement walls,

(ii) bur curettage and laser,

(iii) the various adhesive systems used.

These results were submitted to statistical analysis, using chi-squared test.

## 3. Results

Tables 3–5 display the results of the microleakage scores reported in this study. These were submitted to chi-squared tests in order to estimate whether there was a difference in waterproof quality between the enamel and cement, between

Table 3: Scoring for enamel infiltration with laser (a) and bur (b) techniques.

(a)

| | Score 0 | Score 1 | Score 2 | Score 3 |
|---|---|---|---|---|
| Clearfil s$^3$ Bond Plus | 3 | 8 | 0 | 1 |
| Xeno Select | 2 | 10 | 0 | 0 |
| Futurabond U | 6 | 4 | 1 | 1 |

(b)

| | Score 0 | Score 1 | Score 2 | Score 3 |
|---|---|---|---|---|
| Clearfil s$^3$ Bond Plus | 1 | 9 | 2 | 0 |
| Xeno Select | 0 | 10 | 1 | 1 |
| Futurabond U | 1 | 9 | 1 | 1 |

Table 4: Scoring for the cement infiltration with laser (a) and bur (b) techniques.

(a)

| | Score 0 | Score 1 | Score 2 | Score 3 |
|---|---|---|---|---|
| Clearfil s$^3$ Bond Plus | 1 | 0 | 0 | 11 |
| Xeno Select | 0 | 3 | 1 | 8 |
| Futurabond U | 1 | 1 | 0 | 10 |

(b)

| | Score 0 | Score 1 | Score 2 | Score 3 |
|---|---|---|---|---|
| Clearfil s$^3$ Bond Plus | 1 | 2 | 1 | 8 |
| Xeno Select | 1 | 1 | 1 | 9 |
| Futurabond U | 2 | 0 | 0 | 10 |

curettage with laser and with a bur, and finally between the different adhesive systems.

(I) A highly significant difference ($p = 0.001$) was found between the infiltration of coloring agent in the enamel and the dentine, all groups considered.

(II) A statistically significant difference ($0.01 < p < 0.05$) was found concerning the infiltration of the coloring agent in the enamel between the bur and laser techniques, regardless of adhesive system type.

(III) Concerning the cement, no statistically significant difference was noted between the laser and bur ($p > 0.05$).

(IV) There was no statistically significant difference between the various adhesive systems used ($p > 0.05$).

## 4. Discussion

In this study, samples from all groups exhibited less microleakage at the cervical wall, in line with reports published by several authors [8, 20, 21]. This can be accounted for by the fact that adhesion to the dentin is more technical and dependent upon substract bonding to the enamel. On the other hand, careful observation of the enamel walls revealed infiltration limited to the enamel-dentin junction in most samples. This observation aligns with those made by Setien et al., who also observed infiltration in the enamel in samples without previous etching [22]. Ceballos et al. obtained similar results and described infiltration of 90.7% of the enamel when only lasers were used [7].

Table 5: (a) Values of the difference in waterproof quality between enamel and cement methods. "A" represents the enamel wall and "B" the cement wall. (b) Study of waterproof difference between laser (A, C) and bur (B, D) techniques in the enamel and cement. (c) Scores obtained for the adhesive systems.

(a)

| | Score 0 | Score 1 | Score 2 | Score 3 |
|---|---|---|---|---|
| A | 13 | 50 | 5 | 4 |
| B | 6 | 7 | 3 | 56 |

(b)

| | Score 0 | Score 1 | Score 2 | Score 3 |
|---|---|---|---|---|
| A | 11 | 22 | 1 | 2 |
| B | 2 | 28 | 4 | 2 |
| | Score 0 | Score 1 | Score 2 | Score 3 |
| C | 2 | 4 | 1 | 29 |
| D | 4 | 3 | 2 | 27 |

(c)

| | Score 0 | Score 1 | Score 2 | Score 3 |
|---|---|---|---|---|
| Clearfil s$^3$ Bond Plus | 6 | 19 | 3 | 20 |
| Xeno Select | 3 | 24 | 3 | 18 |
| Futurabond U | 10 | 14 | 2 | 22 |

The same authors observed infiltration of coloring agent in all groups, which reached the pulp wall in most cases, an observation also made during our study. Concerning the difference of microleakage between the laser curettage method and that using a bur, no statistically significant difference was observed, in line with numerous conclusions found in literature [7, 9, 23, 24].

However, several authors assert that less microleakage occurs using lasers if preliminary etching is performed [5, 6, 25–27]. In contrast, other recent authors propose better adhesion and bonding strength with the laser [28–30] and those are considered even better when the enamel is etched [31]. The results of our study demonstrated that, at the enamel level, the coloring agent penetrated less in the laser group samples. One explanation for this was that we obtained dental surfaces without fragments, with "smear layer" or oil forming a microretentive surface during the laser procedure, as shown in some studies or environmental scanning electron microscope (ESEM) analysis [32, 33]. Our results should nevertheless be interpreted with caution, being, on the one hand, not highly significant and, on the other hand, referring to surfaces curetted with the bur that underwent no acid processing. Finally, none of the tested adhesive systems demonstrated a superior performance to any other, irrespective of sample group. Literature reports that Clearfil s$^3$ Bond presented the highest microtensile bond strength to dentin in

both laser-irradiated and bur-cut cavity preparation methods in studies involving self-etch adhesive systems [34].

## 5. Conclusion

Based on our results, and within the limits of this study, we conclude that, in terms of microleakage, there is no difference between the bur technique of cavity preparation and that using an Er:YAG laser. The laser can be used as an alternative to the bur for the cavity preparation. Furthermore, none of the tested adhesive systems proved superior to any other.

## Competing Interests

The authors declare that they have no competing interests.

## Acknowledgments

The authors are thankful to the Belgian University Foundation for its support regarding the publication of the present study.

## References

[1] J. Diaci and B. Gaspirc, "Comparison of Er:YAG and Er,Cr:YSGG lasers used in dentistry," *Journal of Laser Health Academy*, vol. 1, pp. 1–13, 2002.

[2] M. Curti, J.-P. Rocca, M.-F. Bertrand, and S. Nammour, "Morpho-structural aspects of Er:YAG-prepared class V cavities," *Journal of Clinical Laser Medicine and Surgery*, vol. 22, no. 2, pp. 119–123, 2004.

[3] M. T. Schein, J. S. Bocangel, G. E. C. Nogueira, and P. A. L. Schein, "SEM evaluation of the interaction pattern between dentin and resin after cavity preparation using ER:YAG laser," *Journal of Dentistry*, vol. 31, no. 2, pp. 127–135, 2003.

[4] C. S. Delfino, W. C. Souza-Zaroni, S. A. M. Corona, J. Djalma Pecora, and R. G. Palma-Dibb, "Effect of Er:YAG laser energy on the morphology of enamel/adhesive system interface," *Applied Surface Science*, vol. 252, no. 24, pp. 8476–8481, 2006.

[5] V. Armengol, A. Jean, B. Enkel, M. Assoumou, and H. Hamel, "Microleakage of class V composite restorations following Er:YAG and Nd:YAP laser irradiation compared to acid-etch: an in vitro study," *Lasers in Medical Science*, vol. 17, no. 2, pp. 93–100, 2002.

[6] M. C. Borsatto, S. A. M. Corona, R. G. P. Dibb, R. P. Ramos, and J. D. Pécora, "Microleakage of a resin sealant after acid-etching, Er:YAG laser irradiation and air-abrasion of pits and fissures," *Journal of Clinical Laser Medicine and Surgery*, vol. 19, no. 2, pp. 83–87, 2001.

[7] L. Ceballos, R. Osorio, M. Toledano, and G. W. Marshall, "Microleakage of composite restorations after acid or Er-YAG laser cavity treatments," *Dental Materials*, vol. 17, no. 4, pp. 340–346, 2001.

[8] S. A. M. Corona, M. C. Borsatto, J. D. Pecora, R. A. S. De Sá Rocha, T. S. Ramos, and R. G. Palma-Dibb, "Assessing microleakage of different class V restorations after Er:YAG laser and bur preparation," *Journal of Oral Rehabilitation*, vol. 30, no. 10, pp. 1008–1014, 2003.

[9] M. Hossain, Y. Yamada, Y. Nakamura, Y. Murakami, Y. Tamaki, and K. Matsumoto, "A study on surface roughness and microleakage test in cavities prepared by Er:YAG laser irradiation and etched bur cavities," *Lasers in Medical Science*, vol. 18, no. 1, pp. 25–31, 2003.

[10] M. F. R. Khan, K. Yonaga, Y. Kimura, A. Funato, and K. Matsumoto, "Study of microleakage at class I cavities prepared by Er:YAG laser using three types of restorative materials," *Journal of Clinical Laser Medicine and Surgery*, vol. 16, no. 6, pp. 305–308, 1998.

[11] W. Niu, J. N. Eto, Y. Kimura, F. H. Takeda, and K. Matsumoto, "A study on microleakage after resin filling of class V cavities prepared by Er:YAG laser," *Journal of Clinical Laser Medicine and Surgery*, vol. 16, no. 4, pp. 227–231, 1998.

[12] B. C. Quo, J. L. Drummond, A. Koerber, S. Fadavi, and I. Punwani, "Glass ionomer microleakage from preparations by an Er/YAG laser or a high-speed handpiece," *Journal of Dentistry*, vol. 30, no. 4, pp. 141–146, 2002.

[13] Y. Shigetani, Y. Tate, A. Okamoto, M. Iwaku, and N. Abu-Bakr, "A study of cavity preparation by Er:YAG laser. Effects on the marginal leakage of composite resin restoration," *Dental Materials Journal*, vol. 21, no. 3, pp. 238–249, 2002.

[14] R. F. Z. Lizarelli, P. C. G. Silva, S. T. Porto Neto, and V. S. Bagnato, "Study of microleakage at class V cavities prepared by Er:YAG laser using rewetting surface treatment," *Journal of Clinical Laser Medicine and Surgery*, vol. 22, no. 1, pp. 51–55, 2004.

[15] E. M. Roebuck, C. J. Whitters, and W. P. Saunders, "The influence of three Erbium:YAG laser energies on the in vitro microleakage of Class V compomer resin restorations," *International Journal of Paediatric Dentistry*, vol. 11, no. 1, pp. 49–56, 2001.

[16] E. K. Kohara, M. Hossain, Y. Kimura, K. Matsumoto, M. Inoue, and R. Sasa, "Morphological and microleakage studies of the cavities prepared by Er:YAG laser irradiation in primary teeth," *Journal of Clinical Laser Medicine and Surgery*, vol. 20, no. 3, pp. 141–147, 2002.

[17] ISO, *Technical Specification. Dental Materials—Testing of Adhesion to Tooth Structure*, International Organization for Standardization ISO/TS 11405, 2nd edition, 2003.

[18] R. Hibst, "Lasers for caries removal and cavity preparation: state of the art and future directions," *Journal of Oral Laser Applications*, vol. 2, pp. 203–212, 2002.

[19] R. S. Navarro, S. Gouw-Soares, A. Cassoni, P. Haypek, D. M. Zezell, and C. de Paula Eduardo, "The influence of erbium:yttrium-aluminum-garnet laser ablation with variable pulse width on morphology and microleakage of composite restorations," *Lasers in Medical Science*, vol. 25, no. 6, pp. 881–889, 2010.

[20] S. A. Corona, M. Borsatto, R. G. Dibb, R. P. Ramos, A. Brugnera, and J. D. Pécora, "Microleakage of class V resin composite restorations after bur, air-abrasion or Er:YAG laser preparation," *Operative Dentistry*, vol. 26, no. 5, pp. 491–497, 2001.

[21] M. F. Bertrand, *Préparations cavitaires au laser Er:YAG et collage d'une résine composite [Ph.D. thesis]*, Université de Nice-Sophia Antipolis, 2002.

[22] V. J. Setien, D. S. Cobb, G. E. Denehy, and M. A. Vargas, "Cavity preparation devices: effect on microleakage of Class V resin-based composite restorations," *American Journal of Dentistry*, vol. 14, no. 3, pp. 157–162, 2001.

[23] C. R. G. Torres, M. A. Máximo de Araujo, S. C. Teixeira, R. Máximo de Araújo, and M. F. R. Lima Huhtala, "Cavity preparation with Er:YAG laser or rotary instruments: investigation

of the composite resin restorations interface," *World Journal of Dentistry*, vol. 2, p. 83, 2011.

[24] A. C. C. Aranha, M. L. Turbino, G. L. Powell, and C. De Paula Eduardo, "Assessing microleakage of class V resin composite restorations after Er:YAG laser and bur preparation," *Lasers in Surgery and Medicine*, vol. 37, no. 2, pp. 172–177, 2005.

[25] G. R. Palma Dibb, S. A. Milori Corona, M. C. Borsatto, K. C. Ferreira, R. Pereira Ramos, and J. Djalma Pécora, "Assessing microleakage on class V composite resin restorations after Er:YAG laser preparation varying the adhesive systems," *Journal of Clinical Laser Medicine and Surgery*, vol. 20, no. 3, pp. 129–133, 2002.

[26] M.-F. Bertrand, G. Semez, E. Leforestier, M. Muller-Bolla, S. Nammour, and J.-P. Rocca, "Er:YAG laser cavity preparation and composite resin bonding with a single-component adhesive system: Relationship between shear bond strength and microleakage," *Lasers in Surgery and Medicine*, vol. 38, no. 6, pp. 615–623, 2006.

[27] A. Davari, M. Sadeghi, and H. Bakhshi, "Shear bond strength of an etch-and-rinse adhesive to Er:YAG laser- and/or phosphoric acid-treated dentin," *Journal of Dental Research, Dental Clinics, Dental Prospects*, vol. 7, no. 2, pp. 67–73, 2013.

[28] T. M. O. Ramos, T. M. O. Ramos-Oliveira, P. M. O. de Freitas et al., "Effects of Er:YAG and Er,Cr:YSGG laser irradiation on the adhesion to eroded dentin," *Lasers in Medical Science*, vol. 30, no. 1, pp. 17–26, 2015.

[29] N. Brulat, C. Fornaini, J.-P. Rocca, and E. Darque-Ceretti, "Role of surface tension and roughness on the wettability of Er:YAG laser irradiated dentin: *in vitro* study," *Laser Therapy*, vol. 22, no. 3, pp. 187–194, 2013.

[30] B. Bahrami, N. Askari, M. Tielemans et al., "Effect of low fluency dentin conditioning on tensile bond strength of composite bonded to Er:YAG laser-prepared dentin: a preliminary study," *Lasers in Medical Science*, vol. 26, no. 2, pp. 187–191, 2011.

[31] F. Öznurhan and A. Ölmez, "Morphological analysis of the resin-dentin interface in cavities prepared with Er,Cr:YSGG Laser or bur in primary teeth," *Photomedicine and Laser Surgery*, vol. 31, no. 8, pp. 386–391, 2013.

[32] C. Camerlingo, M. Lepore, G. M. Gaeta et al., "Er:YAG laser treatments on dentine surface: Micro-Raman spectroscopy and SEM analysis," *Journal of Dentistry*, vol. 32, no. 5, pp. 399–405, 2004.

[33] R. Khatavkar and V. Hegde, "Analyse de surface du mordançage au laser Erbium: YAG comparé au mordançage à l'acide Observations ESEM, étude *in vitro*," *Laser Magazine*, vol. 1, pp. 43–45, 2012.

[34] G. E. Akin, S. Herguner-Siso, M. Ozcan, O. Ozel-Bektas, and H. Akin, "Bond strengths of one-step self-etch adhesives to laser-irradiated and bur-cut dentin after water storage and thermocycling," *Photomedicine and Laser Surgery*, vol. 30, no. 4, pp. 214–221, 2012.

Hindawi Publishing Corporation
BioMed Research International
Volume 2016, Article ID 6321906, 8 pages
http://dx.doi.org/10.1155/2016/6321906

*Clinical Study*

# Clinical, Radiographic and Microbiological Evaluation of High Level Laser Therapy, a New Photodynamic Therapy Protocol, in Peri-Implantitis Treatment; a Pilot Experience

**Gianluigi Caccianiga,[1] Gerard Rey,[2] Marco Baldoni,[1] and Alessio Paiusco[1]**

[1]*Department of Surgery and Translational Medicine, University of Milano-Bicocca, Milan, Italy*
[2]*University of Paris Diderot, Paris, France*

Correspondence should be addressed to Gianluigi Caccianiga; gianluigi.caccianiga@unimib.it

Received 2 December 2015; Accepted 26 January 2016

Academic Editor: Samir Nammour



*Aim*. Endosseous implants are widely used to replace missing teeth but mucositis and peri-implantitis are the most frequent long-term complications related with dental implants. Removing all bacterial deposits on contaminated implant surface is very difficult due to implant surface morphology. The aim of this study was to evaluate the bactericidal potential of photodynamic therapy by using a new high level laser irradiation protocol associated with hydrogen peroxide in peri-implantitis. *Materials and Methods*. 10 patients affected by peri-implantitis were selected for this study. Medical history, photographic documentation, periodontal examination, and periapical radiographs were collected at baseline and 6 months after surgery. Microbiological analysis was performed with PCR Real Time. Each patient underwent nonsurgical periodontal therapy and surgery combined with photodynamic therapy according to High Level Laser Therapy protocol. *Results*. All peri-implant pockets were treated successfully, without having any complication and not showing significant differences in results. All clinical parameters showed an improvement, with a decrease of Plaque Index (average decrease of 65%, range 23–86%), bleeding on probing (average decrease of 66%, range 26–80%), and probing depth (average decrease of 1,6 mm, range 0,46–2,6 mm). Periapical radiographs at 6 months after surgery showed a complete radiographic filling of peri-implant defect around implants treated. Results showed a decrease of total bacterial count and of all bacterial species, except for *Eikenella corrodens*, 6 months after surgery. *Conclusion*. Photodynamic therapy using HLLT appears to be a good adjunct to surgical treatment of peri-implantitis.

## 1. Introduction

Endosseous implants have become widely accepted treatment options for the replacement of missing teeth; the increasing use of implants has led clinicians to observe a higher frequency of peri-implant pathologies [1]. Mucositis and peri-implantitis, defined as inflammatory processes in the tissues surrounding an implant, are the most frequent long-term complications related with dental implants [2].

Peri-implantitis is a bacterially induced inflammatory reaction that results in loss of supporting bone around an implant in function, which may eventually lead to loss of the implant fixture (implant failure). Peri-implant mucositis is a reversible inflammatory process in the soft tissues surrounding a functioning implant, while peri-implantitis is an inflammation of peri-implant tissues accompanied with changes in the level of crestal bone and with the presence of bleeding on probing and/or suppuration, with or without concomitant deepening of peri-implant pockets [3, 4].

A recent study, investigating 1,497 participants and 6,283 implants, estimated for the frequency of peri-implant mucositis included 63.4% of participants and 30.7% of implants, and those of peri-implantitis were 18.8% of participants and 9.6% of implants [5].

The presence of microorganisms is fundamental for the development of peri-implant disease [6]. Within weeks after the installation of titanium implants, subgingival microflora associated with periodontitis is established. Bacterial colonization and maturation of biofilms depend on a favourable ecological environment and lead to shifts in the composition

and behaviour of the endogenous microbiota that may become intolerable for host tissues [7, 8]. A recent study investigated the microbial signatures of the peri-implant microbiome in health and disease using 16S pyrosequencing [9]. Peri-implant biofilms demonstrated significantly lower diversity than subgingival biofilms in both health and disease; however, several species, including previously unsuspected and unknown organisms, were unique to this niche. The peri-implant microbiome differs significantly from the periodontal community in both health and disease. Peri-implantitis is a microbially heterogeneous infection with predominantly Gram-negative species and is less complex than periodontitis.

Therapies currently recommended for the treatment of peri-implantitis are primarily based on scientific evidence resulting from periodontal disease treatment [10]. Biofilm removal from implant surfaces is the primary goal in the treatment of peri-implant disease [11, 12].

Therapies such as antibiotics, antiseptics, and laser treatments have been proposed as additional therapeutic options in nonsurgical treatment of peri-implantitis and mucositis [13]. Also different surgical procedures, sometimes associated with laser irradiation, have been employed to obtain healing and/or regeneration of defects in patients with peri-implantitis [14].

Cumulative Interceptive Supportive Therapy (CIST), proposed by Lang and Lindhe [15], is a cumulative protocol including four subsequent therapeutic phases, which increase antimicrobial potential depending on lesion extent and severity.

Surgical therapy is first-choice treatment for peri-implantitis because of lesion and compromised implant surface complexity [16].

Surgery main goal is to create access for debridement and decontamination of contaminated implant surface. Biofilm and calcified deposits must be removed in order to allow healing and reduce the risk for disease future progression [17, 18].

Mechanical instrumentation should be followed by chemical decontamination of the implant surface. Different solutions have been used, including citric acid, chloramines, tetracycline, chlorhexidine, hydrogen peroxide, and sodium chloride. No method was superior to the other [19].

Studies from literature show that regenerative surgical therapy of peri-implantitis presents some controversial issues, such as the real possibility to obtain decontamination of implant surface, regeneration of lost bone tissue, and reosteointegration of implant surface [20, 21].

Lasers were introduced into medicine in 1964 [22] and are now successfully widely employed in dentistry for treatment of different pathologies. Recently, an increasing number of studies evaluating the efficacy of photodynamic therapy for periodontal diseases treatment have been published [23, 24].

Photodynamic therapy (PDT) can be defined as eradication of target cells by reactive oxygen species produced by means of a photosensitizing compound and light of an appropriate wavelength. It could provide an alternative for targeting microbes directly at the site of infection, thus overcoming the problems associated with antimicrobials. Photodynamic action describes a process in which light, after being absorbed by dyes, sensitizes organisms for visible light induced cell damage [25].

At the beginning of the last century, researchers found that microbes became susceptible to visible light mixed with a photosensitizing compound. Raab et al. first showed the killing of protozoa *Paramecium caudatum* in the presence of acridine orange when irradiated with light in the visible range of spectrum. This combination of two nontoxic elements, dye and light, in an oxygenated environment induces damage and total destruction of microorganisms. In 1904, Von Tappeiner and Jodlbauer coined the term photodynamic to describe oxygen-dependent chemical reactions induced by photosensitization which could inactivate bacteria [26].

PDT involves three components: photosensitizer, light, and oxygen. When a photosensitizer is irradiated with light of specific wavelength it undergoes a transition from a low-energy ground state to an excited singlet state. Subsequently, the photosensitizer may decay back to its ground state, with emission of fluorescence, or may undergo a transition to a higher-energy triplet state. The triplet state can react with endogenous oxygen to produce singlet oxygen and other radical species, causing a rapid and selective destruction of the target tissue.

PDT produces cytotoxic effects on subcellular organelles and molecules. Its effects are targeted on mitochondria, lysosomes, cell membranes, and nuclei of tumor cells. Photosensitizer induces apoptosis in mitochondria and necrosis in lysosomes and cell membranes.

The aim of this study was to evaluate the bactericidal potential of photodynamic therapy by using a new high level laser irradiation protocol associated with hydrogen peroxide in peri-implantitis.

## 2. Materials and Methods

*2.1. Study Population.* We selected 10 patients for this study affected by peri-implantitis.

Patient selection was guided by precise inclusion and exclusion criteria:

(i) Age between 35 and 70 years old.

(ii) Presence of peri-implantitis which did not undergo surgical treatment in the last 12 months. At least peri-implant pockets >4 mm with bleeding on probing.

(iii) Nonsmoking history.

(iv) Absence of allergies.

(v) Absence of uncontrolled systemic disease.

(vi) Absence of antibiotic therapy in the last 6 months.

(vii) Absence of pregnancy or lactating.

(viii) Absence of abuse of alcohol or drugs.

(ix) Acceptance of the surgical intervention by signing an informed consensus.

We decide not to impose restriction about the gender of the patients (male or female).

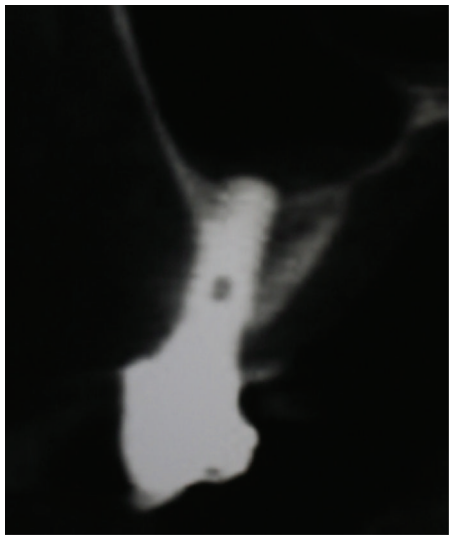


Figure 1: Initial radiograph.

*2.2. Clinical, Radiographic, and Microbiological Parameters.* The initial treatment consisted of a medical history, photographic documentation, periodontal examination, and periapical radiographs (Figure 1).

Data were collected at baseline and 6 months after surgery.

For each patient periodontal charting was performed, assessing probing depth, Plaque Index, and bleeding on probing. Microbiological analysis was performed with PCR Real Time, using paper tips to withdraw gingival fluid in periimplant pockets before and after treatment.

*2.3. Presurgical Procedures.* One week before surgery each patient underwent nonsurgical periodontal therapy combined with photodynamic therapy according to High Level Laser Therapy protocol.

Scaling and root planing of all periodontal and periimplant pockets was performed using Gracey curettes and ultrasonic instruments combined with Betadine (5 : 1 ratio) irrigation and air powder abrasive device with sodium bicarbonate powder.

*2.4. High Level Laser Therapy Protocol.* Photodynamic therapy was applied using Oxylaser solution (hydrogen peroxide stabilized with glycerophosphoric complex) and high power diode laser with the following parameters:

(i) Power: 2.5 W.

(ii) Frequency: 10.0 kHz.

(iii) T-on 20 $\mu$s, T-off 80 $\mu$s.

(iv) Mean power: 0.5 W.

(v) 60 seconds per site.

(vi) Fiber: 400 microns.

Oxylaser solution was irrigated in each periodontal and peri-implant pocket, that emerging from gingival sulcus was aspirated, and remaining part was left in site for two minutes.

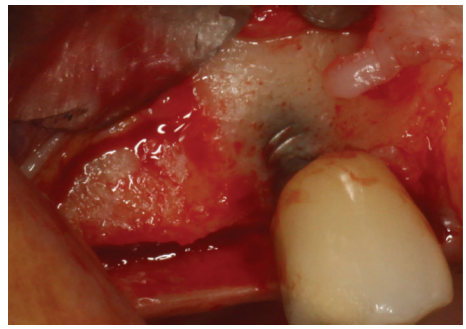


Figure 2: Peri-implant defect.

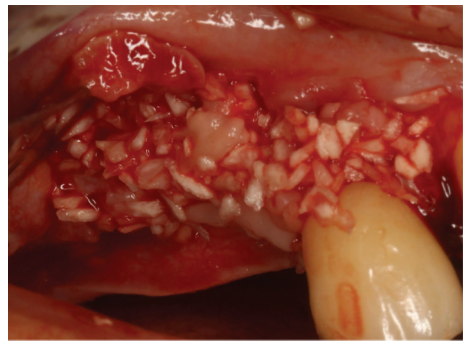


Figure 3: Bone graft after degranulation and HLLT.

Laser fiber was introduced within the pocket, reaching the bottom and radiating subgingival tissues with a movement back and forth 60 seconds for each single pocket.

*2.5. Surgical Procedures.* Surgical procedures were performed under local anesthesia. Intrasulcular incisions were performed and a full thickness mucoperiosteal flap was elevated to expose both the labial and palatal aspects of periimplant defect (Figure 2). Granulation tissue was curetted and removed by using Gracey curettes and ultrasonic instruments combined with Betadine (5 : 1 ratio) irrigation and air powder abrasive device with sodium bicarbonate powder. High level laser irradiation was applied on implant surface 60 seconds for each single pocket and debridement procedures were repeated until complete cleaning of the implant surface. After bone grafting (Figure 3) full thickness buccal and lingual flaps were repositioned and sutured (Figure 4), giving a first internal mattress suture to remove flap tensions.

*2.6. Follow-Up.* Sutures were removed 15 days after surgery and High Level Laser Therapy was performed to allow further decontamination. Every 20 days for 3 months patients underwent HLLT. 6 months after surgery clinical, radiographic, and microbiological data were collected (Figures 5–8).

## 3. Results

Initially 12 patients were considered for this study, but 2 were excluded due to the following reasons: 1 patient had

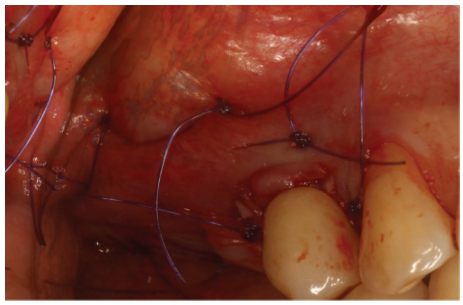


Figure 4: Sutures.

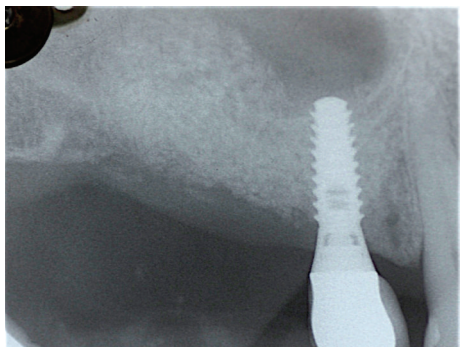


Figure 5: Radiograph 6 months after surgery.

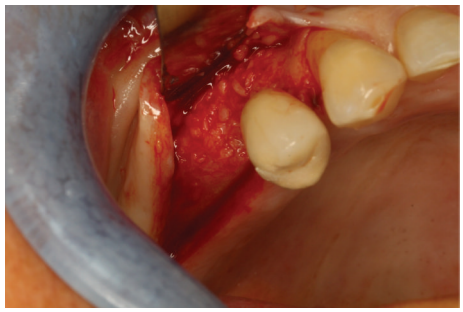


Figure 6: Reentry surgery for implant placement showing new bone formation on implant treated.

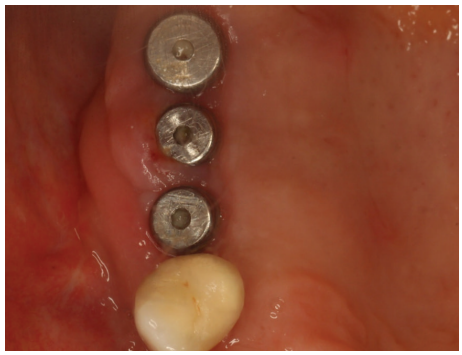


Figure 7: Implant placement in regenerated bone.

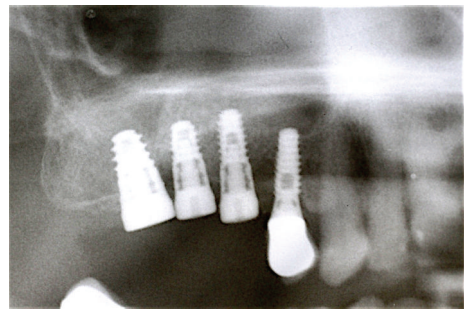


Figure 8: Radiographic evaluation after implant placement.

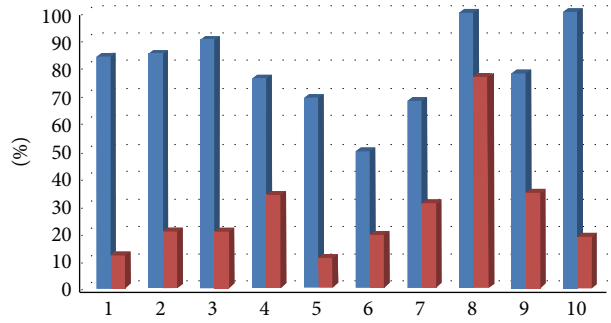



Figure 9: Plaque Index at baseline and 6 months after therapy.

uncontrolled diabetes mellitus and 1 patient did not follow hygiene instructions.

All 10 patients included in the study (4 males and 6 females; average age 48,6 years; range between 35 and 63 years) agreed to undergo surgery and High Level Laser Therapy.

Implants treated in this study were

(i) 4 Nobel implants with TiUnite surface,

(ii) 3 Straumann implants with SLA surface (one represented in the case report),

(iii) 1 Straumann implant with SLActive surface,

(iv) 2 Zimmer implants with MTX surface.

All peri-implant pockets were treated successfully, without having any complication and not showing significant differences in results.

All clinical parameters showed an improvement, with a decrease of Plaque Index (average decrease of 65%, range 23–86%, Figure 9), bleeding on probing (average decrease of 66%, range 26–80%, Figure 10), and probing depth (average decrease of 1,6 mm, range 0,46–2,6 mm, Figure 11).

Periapical radiographs at 6 months after surgery showed a complete radiographic filling of peri-implant defect around implants treated.

Microbiological analysis was carried out on different bacterial species, including *Aggregatibacter actinomycetemcomitans* (Aa), *Porphyromonas gingivalis* (Pg), *Tannerella forsythia* (Tf), *Treponema denticola* (Td), *Fusobacterium nucleatum* (Fn), *Campylobacter rectus* (Cr), and *Eikenella corrodens* (Ec) and on total bacterial count.

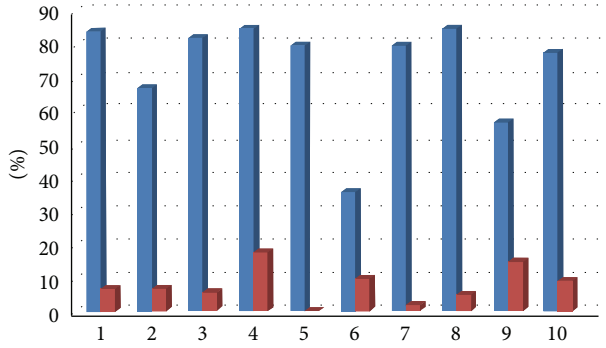



Figure 10: Bleeding on probing at baseline and 6 months after therapy.

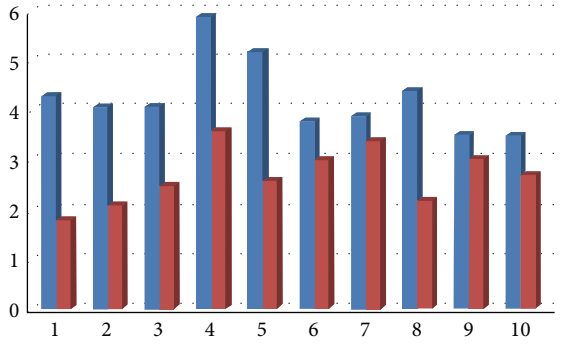



Figure 11: Probing depth at baseline and 6 months after therapy.

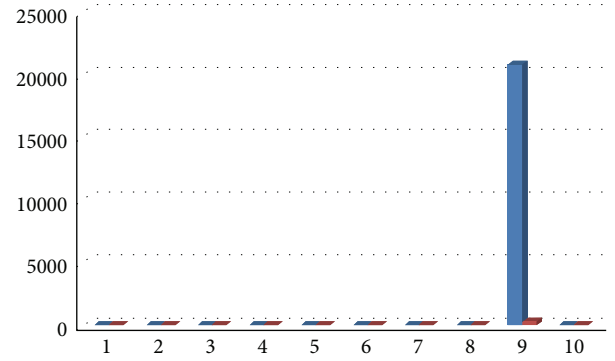



Figure 12: Aa: microbiological analysis at baseline and 6 months after surgery.

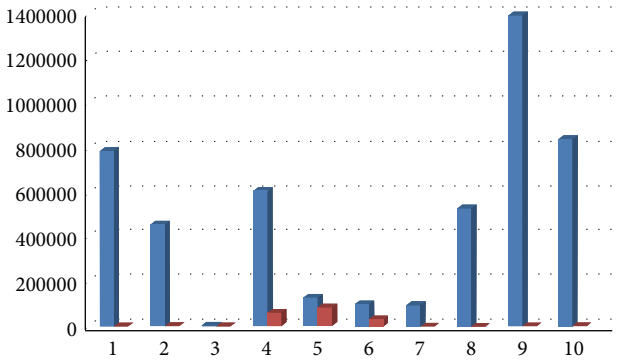



Figure 13: Pg: microbiological analysis at baseline and 6 months after surgery.

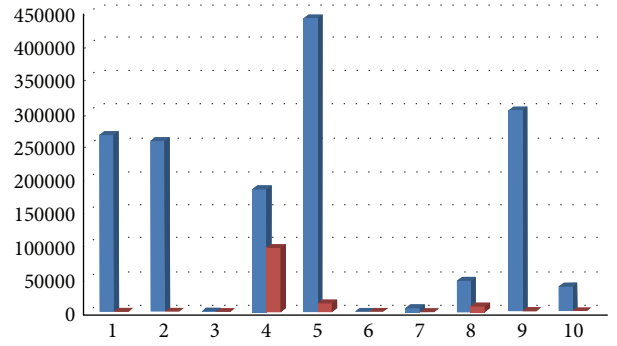



Figure 14: Tf: microbiological analysis at baseline and 6 months after surgery.

Results showed a decrease of total bacterial count and of all bacterial species, except for Ec, 6 months after surgery, with a medium decrease of 98,70% for Aa (Figure 12), 89% for Pg (range 100%–34,55%, Figure 13), 92% for Tf (range 100%–34,55%, Figure 14), 88% for Td (range 100%–34,55%, Figure 15), 85,68% for Fn (range 100%–34,55%, Figure 16), 89,64% for Cr (range 100%–34,55%, Figure 17), and 85,27% for total bacterial count (range 100%–34,55%, Figure 19). Ec showed a medium increase of 38,64% (range 100%–491,07%, Figure 18).

## 4. Discussion

Peri-implant surfaces exposed to peri-implantitis, particularly rough ones, promote plaque accumulation and defect evolution both in the dog [2] and in humans [27] but, if decontaminated, may regain original osteophilic ability.

The prerequisite for obtaining reosteointegration of a rough implant surface exposed by bone loss is deep decontamination of bacterial biofilm.

This can be realized with mechanical instrumentation, antiseptics, pharmacological, or photodynamic devices, considering that the primary aim is the removal of toxins and bacteria without permanence of antiseptics or alteration of implant morphological and osteophilic characteristics.

Mechanical treatment alone is not able to remove all the biofilm due to implant morphology and roughness, so it should be integrated with antiseptic or pharmacological devices.

The use of a simple system as the combination of CHX and saline solution at 0.2% could be sufficient to decontaminate implant surface as shown by Singh [28] in a study on monkeys in which researchers have achieved 39–46% of reosteointegration with this surface treatment through regenerative techniques (autogenous bone + ePTFE).

Even Kolonidis et al. [29] have obtained implant surface reosteointegration in a dog model after treatment with citric acid or $H_2O_2$ or saline solution.

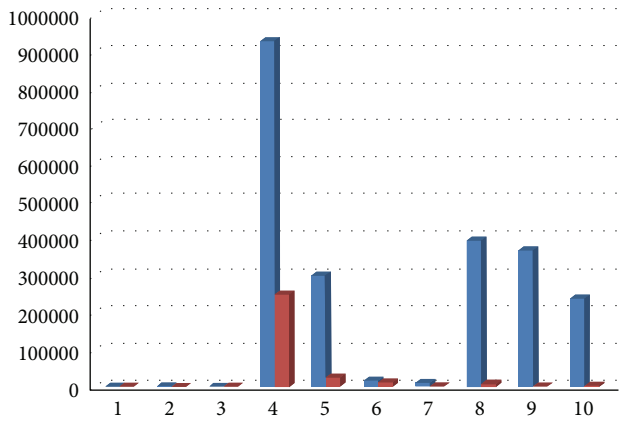



Figure 15: Td: microbiological analysis at baseline and 6 months after surgery.

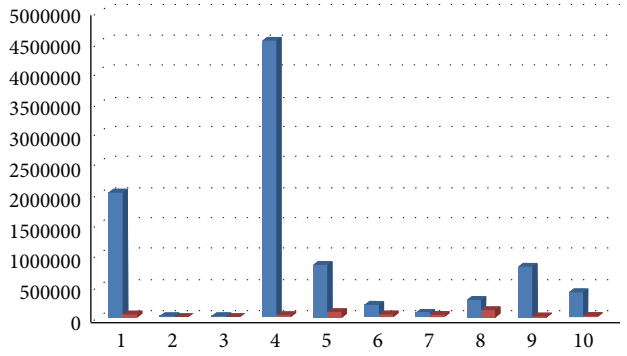



Figure 16: Fn: microbiological analysis at baseline and 6 months after surgery.

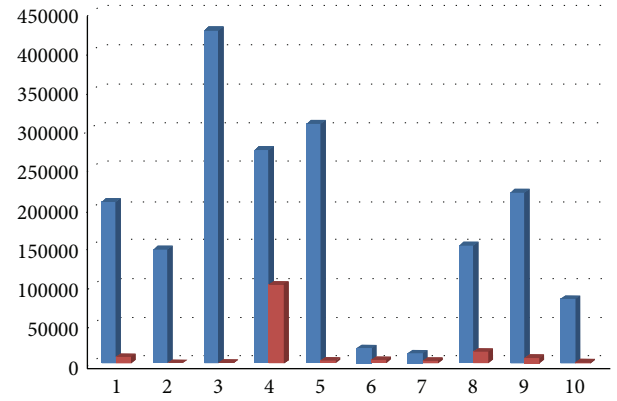



Figure 17: Cr: microbiological analysis at baseline and 6 months after surgery.

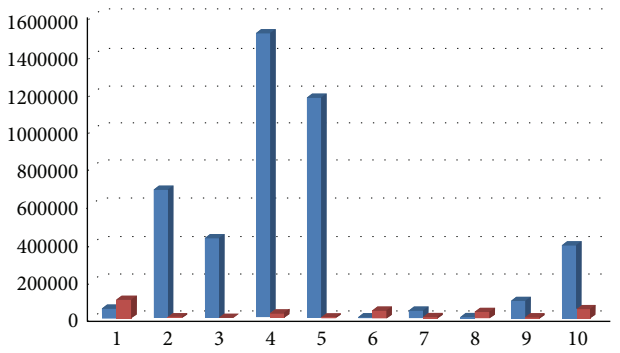



Figure 18: Ec: microbiological analysis at baseline and 6 months after surgery.

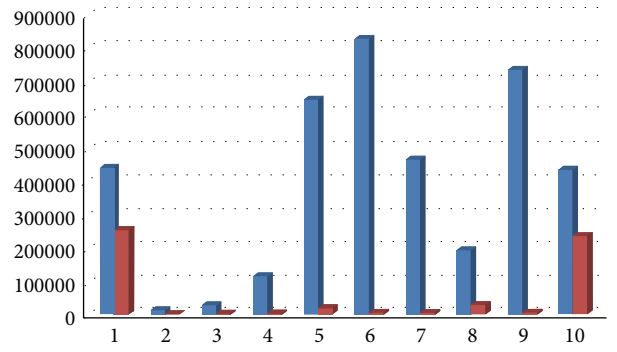



Figure 19: Total microbial count: microbiological analysis at baseline and 6 months after surgery.

However, complete decontamination of a rough implant surface is very difficult to achieve.

A recent study attempted to assess the cleaning potential of three different instrumentation methods commonly used for implant surface decontamination in vitro, using a bone defect-simulating model. None of the cleaning procedures performed, including Gracey curette, an ultrasonic device, and an air powder abrasive device with glycine powder, was able to perfectly clean implant surface [30].

A treatment option to achieve this fundamental goal could be represented by photodynamic therapy, in particular by High Level Laser Therapy technology.

The HLLT technology is a therapy based on the combination of a penetrating laser with a modified and stabilized $H_2O_2$ solution.

Several in vitro studies showed bactericidal activity of laser irradiation combined with hydrogen peroxide on numerous bacterial species.

A comparative study on the effects of laser alone and combined with $H_2O_2$ showed these results [31–34]:

(i) Laser used alone produces poor results in the elimination of bacterial species involved in periodontal disease.

(ii) $H_2O_2$ used alone produces little effects in microorganisms elimination.

(iii) Laser combined with hydrogen peroxide shows an antibacterial action much more effective on most of the microorganisms involved in periodontal disease.

Laser energy activates the modified $H_2O_2$ solution, releasing free radicals and singlet oxygen that have antibacterial activity on Gram-positive and Gram-negative periodontal pathogens. The photochemical effect of this photodynamic therapy consists of activation of a photosensitizer (in this

case hydrogen peroxide), with a monochromatic beam, as the laser beam characterized by a single wavelength. The interaction between this photosensitizer and the laser produces photochemical reactions in which the energy acceptor is oxygen. The stabilized hydrogen peroxide contains oxygen, and its presence allows the reactions of photoactivation and production of singlet oxygen. The singlet oxygen is an oxygen free radical that determines bacterial cells death (destruction of bacterial membrane, degradation of lysosomal membrane, alteration of mitochondrial function, and denaturation of DNA molecules).

Results showed a decrease of total bacterial count and of all bacterial species, except for *Eikenella corrodens*. Analyzing microbiological results regarding *Ec* we found that 7 patients had a medium decrease of 94,42% (range 85,26%–100%) and only 3 patients had a medium increase of 347,95% (range 73,47%–491,07%). In vitro studies we published in the last years, evaluating the efficacy of this protocol on different bacterial species, suggested that HLLT protocol is able to deplete all bacteria examined. Therefore recolonization of treated peri-implant pockets in these 3 patients by *Eikenella corrodens* is more likely than a persistence in the pocket of this bacterial species. Recolonization could be related to different factors, especially poor oral hygiene (confirmed in these 3 patients).

It is important to understand that this laser works at high power peaks (to kill bacteria), at reduced values of average power (below 0.8 watts), and with a very high frequency. All this is allowed by the fact that this laser works in microseconds and not in milliseconds, greatly increasing the frequency. The strong increase in the frequency (in the study consisting of 20 microseconds to 80 microseconds of T-on and T-off) allows the use of very high peak power (2.5 W) while maintaining an average power below the 0.8 watts, without having any thermal effect.

Summarizing the HLLT it is characterized by

(i) high peak power (2.5 watts): allowing the destruction of microorganisms (decontaminating effect),

(ii) reduced average power (0.5 watts) and timing of application reduced: reducing high thermal effects that are harmful to the tissues, resulting in only mild thermal effects (increased vasodilation), which increases blood flow to the site of intervention promoting healing and regeneration (increased intake of growth factors, oxygen, inflammatory, and stem cells),

(iii) high frequency (10,000 Hz): important activation and release of singlet oxygen (10,000 times per second) that increase the antibacterial activity,

(iv) maximum depth of penetration: with HLLT the photosensitizer used is oxygen-rich and transparent, increasing laser penetration depth compared to chromophores,

(v) elimination of silver compounds by $H_2O_2$ and stabilization with glycerol-phosphate that has biostimulating effects.

The proposed protocol does not rely only on photodynamic therapy but combines all the chemical and mechanical actions of the conventional nonsurgical therapy (sonic and curette instrumentation).

Peri-implant treatment relies on different types of action:

(i) Mechanical action (scaling with sonic instruments and/or curettes).

(ii) Chemical action (sonic irrigation with Betadine, in solution 1/5).

(iii) Mechanical and chemical action of air flow with high abrasive bicarbonate powder.

(iv) Physical action (photodynamic therapy): effective in eliminating even the most aggressive bacteria.

The combination of these three phases during therapy allows a deep disinfection on any implant surface.

In HLLT laser is set so as to avoid significant thermal effects, which does not modify the implant surface. The decontamination is performed with both nonsurgical and surgical protocol, with the combined use of sonic, chemical, physical, and photodynamic devices.

## 5. Conclusions

The majority of analyzed studies show modest beneficial effects of pulsed lasers in comparison to conventional therapies (with manual and/or sonic instrumentation) in the initial treatment of patients with peri-implantitis. Photodynamic therapy using HLLT, supported by a biological rationale and by preliminary results obtained with this study, appears to be a good adjunct to surgical treatment of peri-implantitis; the efficacy of the proposed protocol highlights the need to act on the site as less traumatically as possible but in an effective way in order to improve the bacterial and inflammatory condition.

Reduced periodontal inflammation, with a decrease in probing depth and bleeding on probing, and the massive reduction of bacteria, particularly aggressive pathogens often found in affected sites, are suggestive of the potential effectiveness of this protocol for the treatment of peri-implant disease.

## Competing Interests

The authors declare that they have no competing interests.

## References

[1] G. Alsaadi, M. Quirynen, A. Komárek, and D. van Steenberghe, "Impact of local and systemic factors on the incidence of late oral implant loss," *Clinical Oral Implants Research*, vol. 19, no. 7, pp. 670–676, 2008.

[2] T. Berglundh and J. Lindhe, "Dimension of the periimplant mucosa," *Journal of Clinical Periodontology*, vol. 23, no. 10, pp. 971–973, 1996.

[3] T. Albrektsson and F. Isidor, "Consensus report of session IV," in *Proceedings of the 1st European Workshop on Periodontology*, N. P. Lang and T. Karring, Eds., pp. 365–369, 1994.

[4] N. P. Lang and T. Berglundh, "Periimplant diseases: where are we now?—consensus of the Seventh European Workshop on Periodontology," *Journal of Clinical Periodontology*, vol. 38, supplement 11, pp. 178–181, 2011.

[5] M. A. Atieh, N. H. M. Alsabeeha, C. M. Faggion Jr., and W. J. Duncan, "The frequency of peri-implant diseases: a systematic review and meta-analysis," *Journal of Periodontology*, vol. 84, no. 11, pp. 1586–1598, 2013.

[6] A. Mombelli, M. A. van Oosten, E. Schurch Jr., and N. P. Land, "The microbiota associated with successful or failing osseointegrated titanium implants," *Oral Microbiology and Immunology*, vol. 2, no. 4, pp. 145–151, 1987.

[7] S. Mohamed, I. Polyzois, S. Renvert, and N. Claffey, "Effect of surface contamination on osseointegration of dental implants surrounded by circumferential bone defects," *Clinical Oral Implants Research*, vol. 21, no. 5, pp. 513–519, 2010.

[8] C. Fransson, J. Wennström, C. Tomasi, and T. Berglundh, "Extent of peri-implantitis-associated bone loss," *Journal of Clinical Periodontology*, vol. 36, no. 4, pp. 357–363, 2009.

[9] P. S. Kumar, M. R. Mason, M. R. Brooker, and K. O'Brien, "Pyrosequencing reveals unique microbial signatures associated with healthy and failing dental implants," *Journal of Clinical Periodontology*, vol. 39, no. 5, pp. 425–433, 2012.

[10] I. Ericsson, T. Berglundh, C. Marinello, B. Liljenberg, and J. Lindhe, "Long-standing plaque and gingivitis at implants and teeth in the dog," *Clinical Oral Implants Research*, vol. 3, no. 3, pp. 99–103, 1992.

[11] I. K. Karoussis, G. E. Salvi, L. J. A. Heitz-Mayfield, U. Brägger, C. H. F. Hämmerle, and N. P. Lang, "Long-term implant prognosis in patients with and without a history of chronic periodontitis: a 10-year prospective cohort study of the ITI® Dental Implant System," *Clinical Oral Implants Research*, vol. 14, no. 3, pp. 329–339, 2003.

[12] N. P. Lang, T. Berglundh, L. J. Heitz-Mayfield, B. E. Pjetursson, G. E. Salvi, and M. Sanz, "Consensus statements and recommended clinical procedures regarding implant survival and complications," *The International Journal of Oral & Maxillofacial Implants*, vol. 19, supplement, pp. 150–154, 2004.

[13] A. Mombelli and N. P. Lang, "Antimicrobial treatment of peri-implant infections," *Clinical Oral Implants Research*, vol. 3, no. 4, pp. 162–168, 1992.

[14] R. Haas, M. Baron, O. Dörtbudak, and G. Watzek, "Lethal photosensitization, autogenous bone, and e-PTFE membrane for the treatment of peri-implantitis: preliminary results," *The International Journal of Oral & Maxillofacial Implants*, vol. 15, no. 3, pp. 374–382, 2000.

[15] N. P. Lang and J. Lindhe, Eds., *Clinical Periodontology and Implant Dentistry*, John Wiley & Sons, New York, NY, USA, 2015.

[16] E. S. Karring, A. Stavropoulos, B. Ellegaard, and T. Karring, "Treatment of peri-implantitis by the Vector® system," *Clinical Oral Implants Research*, vol. 16, no. 3, pp. 288–293, 2005.

[17] A. M. Roos-Jansåker, C. Lindahl, H. Renvert, and S. Renvert, "Nine- to fourteen-year follow-up of implant treatment. Part I: implant loss and associations to various factors," *Journal of Clinical Periodontology*, vol. 33, no. 4, pp. 283–289, 2006.

[18] L. G. Persson, M. G. Araújo, T. Berglundh, K. Gröndahl, and J. Lindhe, "Resolution of peri-implantitis following treatment. An experimental study in the dog," *Clinical Oral Implants Research*, vol. 10, no. 3, pp. 195–203, 1999.

[19] N. Claffey, E. Clarke, I. Polyzois, and S. Renvert, "Surgical treatment of peri-implantitis," *Journal of Clinical Periodontology*, vol. 35, supplement 8, pp. 316–332, 2008.

[20] F. H. Nociti Jr., M. Â. N. Machado, C. M. Stefani, E. A. Sallum, and A. W. Sallum, "Absorbable versus nonabsorbable membranes and bone grafts in the treatment of ligature-induced peri-implantitis defects in dogs," *Clinical Oral Implants Research*, vol. 12, no. 2, pp. 115–120, 2001.

[21] F. H. Nociti Jr., R. G. Caffesse, E. A. Sallum, M. A. Machado, C. M. Stefani, and A. W. Sallum, "Clinical study of guided bone regeneration and/or bone grafts in the treatment of ligature-induced peri-implantitis defects in dogs," *Brazilian Dental Journal*, vol. 12, no. 2, pp. 127–131, 2001.

[22] L. Goldman, P. Hornby, R. Meyer, and B. Goldman, "Impact of the laser on dental caries," *Nature*, vol. 203, no. 4943, p. 417, 1964.

[23] P. Meisel and T. Kocher, "Photodynamic therapy for periodontal diseases: state of the art," *Journal of Photochemistry and Photobiology B: Biology*, vol. 79, no. 2, pp. 159–170, 2005.

[24] A. Azarpazhooh, P. S. Shah, H. C. Tenenbaum, and M. B. Goldberg, "The effect of photodynamic therapy for periodontitis: a systematic review and meta-analysis," *Journal of Periodontology*, vol. 81, no. 1, pp. 4–14, 2010.

[25] B. W. Sigusch, A. Pfitzner, V. Albrecht, and E. Glockmann, "Efficacy of photodynamic therapy on inflammatory signs and two selected periodontopathogenic species in a beagle dog model," *Journal of Periodontology*, vol. 76, no. 7, pp. 1100–1105, 2005.

[26] H. Von Tappeiner and A. Jodlbauer, "Uber die wirkung der photodynamischen (fluorescierenden) stoffe auf protozoen und enzyme," *Deutsches Archiv für Klinische Medizin*, vol. 80, pp. 427–487, 1904.

[27] V. Baelum and B. Ellegaard, "Implant survival in periodontally compromised patients," *Journal of Periodontology*, vol. 75, no. 10, pp. 1404–1412, 2004.

[28] P. Singh, "Understanding peri-implantitis: a strategic review," *Journal of Oral Implantology*, vol. 37, no. 5, pp. 622–626, 2011.

[29] S. G. Kolonidis, S. Renvert, C. H. F. Hämmerle, N. P. Lang, D. Harris, and N. Claffey, "Osseointegration on implant surfaces previously contaminated with plaque. An experimental study in the dog," *Clinical Oral Implants Research*, vol. 14, no. 4, pp. 373–380, 2003.

[30] P. Sahrmann, V. Ronay, D. Hofer, T. Attin, R. E. Jung, and P. R. Schmidlin, "In vitro cleaning potential of three different implant debridement methods," *Clinical Oral Implants Research*, vol. 26, no. 3, pp. 314–319, 2015.

[31] G. Caccianiga, G. Rey, T. Fumagalli, A. Cambini, G. Denotti, and M. S. Giacomello, "Photodynamic therapy (association diode laser/hydrogen peroxide): evaluation of bactericidal effects on periodontopathy bacteria: an in vitro study," *European Journal of Inflammation*, vol. 10, no. 2, supplement, pp. 101–106, 2012.

[32] G. Caccianiga, A. Baldini, A. Baldoni, and G. Tredici, "Application of laser in periodontology: microbiological evaluation with PCR-real time," in *Mediterranean Dental Implant Congress*, P. N. Bochlogyros, Ed., Medimond International Proceedings, 2004.

[33] G. Caccianiga, A. Cambini, G. Rey, A. Paiusco, T. Fumagalli, and M. S. Giacomello, "The use of laser diodes superpulses in implantology," *European Journal of Inflammation*, vol. 10, no. 2, supplement, pp. 97–100, 2012.

[34] G. Caccianiga, E. Urso, R. Monguzzi, K. Gallo, and G. Rey, "Efecto bactericida del láser de diodo en periodoncia," *Avances en Periodoncia e Implantologia Oral*, vol. 19, no. 3, pp. 131–140, 2007.

Hindawi Publishing Corporation
BioMed Research International
Volume 2016, Article ID 4741516, 5 pages
http://dx.doi.org/10.1155/2016/4741516

*Research Article*

# Safety Irradiation Parameters of Nd:YAP Laser Beam for Endodontic Treatments: An In Vitro Study

**A. Namour,[1] S. Geerts,[2] T. Zeinoun,[3] R. De Moor,[4] and S. Nammour[1]**

[1]*EMDOLA, Department of Dental Science, University of Liège, Quai G. Kurth, P.O. Box 45, 4020 Liège, Belgium*
[2]*Division of Conservative and Adhesive Dentistry, Department of Dentistry, University of Liège, Quai G. Kurth, P.O. Box 45, 4020 Liège, Belgium*
[3]*Department of Oral and Maxillofacial Surgery, Faculty of Dentistry, Lebanese University, Hadath Campus, Beirut, Lebanon*
[4]*Department of Operative Dentistry and Endodontology, Ghent Dental Laser Center, Dental School, Ghent University, 9000 Ghent, Belgium*

Correspondence should be addressed to A. Namour; amaurynamour@gmail.com

Received 9 January 2016; Revised 4 April 2016; Accepted 16 May 2016

Academic Editor: Matthias Stope



*Objective*. Nd:YAP laser has several potentialities of clinical applications in endodontics. The aim of our study is to determine the safety range of irradiation parameters during endodontic application of Nd:YAP laser that can be used without damaging and overheating the periodontal tissue. *Material and Methods*. Twenty-seven caries-free single-rooted extracted human teeth were used. Crowns were sectioned to obtain 11 mm root canal length. Temperature increases at root surfaces were measured by a thermocouple during Nd:YAP laser irradiation of root canals at different energy densities. Canal irradiation was accomplished with a circular and retrograde movement from the apex until the cervical part of the canal during 10 seconds with an axial speed of 1 mm/s. Each irradiation was done in a canal irrigated continuously with 2.25% NaOCl solution. *Results*. Periodontal temperature increase depends on the value of energy density. Means and standard deviations of temperature increases at root surfaces were below 10°C (safe threshold level) when the average energy densities delivered per second were equal to or below 4981 J/cm$^2$ and 9554 J/cm$^2$, respectively, for irradiations using a fiber diameter of 320 $\mu$m and 200 $\mu$m. *Conclusions*. Within the limitations of this study and under specific irradiation conditions, Nd:YAP laser beam may be considered harmless for periodontal tissues during endodontic applications.

## 1. Introduction

The removal of smear layer and disinfection of canals are important objectives for the success of endodontic treatments. Many methods have been proposed to achieve these objectives (irrigants, disinfecting drugs, ultrasounds, etc.). Several studies suggested the use of laser for smear layer removal and root canals disinfection: Er:YAG laser is shown to be efficient for smear layer removal from root canals [1]. Sahar-Helft et al. [2] demonstrated that smear layer removal was most effective compared to passive ultrasonic irrigation when 17% EDTA solution was activated in root canals using Er:YAG laser at low energy. Interestingly, removal of smear layer along the entire canal was similar when the laser was inserted in the coronal third or 1 mm short of the working length. This effect was not observed with ultrasonic activation or positive pressure techniques [2]. Other laser wavelengths were proposed for the removal of smear layer. Da Costa Lima et al. [3] demonstrated that Nd:YAG laser beam can also be used as an adjunct for smear layer removal with however less efficiency than the passive ultrasonic irrigation.

It is noteworthy that access of irrigants and disinfecting solutions to secondary canals and deep dentinal tubules is difficult. Schoop et al. [4] showed that Nd:YAG, diode, Er:YAG, and Er,Cr:YSGG laser beams are suitable for the disinfection of deeper layers of dentin and may constitute valuable tools in the endodontic disinfection process. Laser light can penetrate areas of canals where irrigating and disinfecting solutions cannot reach, like secondary canals and deep dentinal tubules, and also can eliminate microorganisms [5].

Table 1: Different irradiation conditions are shown in function of predetermined setting of the apparatus. The average of output powers and the average of energy densities are calculated for the fiber diameter of 200 $\mu$m of the Nd:YAP laser.

| Setting parameter: G | Setting parameter: D | Setting parameter: C |
|---|---|---|
| 30 Hz, 150 $\mu$s per pulse | 10 Hz, 150 $\mu$s per pulse | 5 Hz, 150 $\mu$s per pulse |
| G+:<br>(i) Average output power: 10 W (330 mJ per pulse)<br>(ii) Average of energy density per second: 31847 J/cm$^2$ | D+:<br>(i) Average output power: 4 W (400 mJ per pulse)<br>(ii) Average of energy density per second: 12739 J/cm$^2$ | C+:<br>(i) Average output power: 1.8 W (400 mJ per pulse)<br>(ii) Average of energy density per second: 5732 J/cm$^2$ |
| G0:<br>(i) Average output power: 7.5 W (250 mJ per pulse)<br>(ii) Average of energy density per second: 23885 J/cm$^2$ | D0:<br>(i) Average output power: 3 W (300 mJ per pulse)<br>(ii) Average of energy density per second: 9554 J/cm$^2$ | C0:<br>(i) Average output power: 1.4 W (280 mJ per pulse)<br>(ii) Average of energy density per second: 4458 J/cm$^2$ |
| G−:<br>(i) Average output power: 5 W (160 mJ per pulse)<br>(ii) Average of energy density per second: 15923 J/cm$^2$ | D−:<br>(i) Average output power: 2 W (200 mJ per pulse)<br>(ii) Average of energy density per second: 6369 J/cm$^2$ | C−:<br>(i) Average output power: 0.9 W (330 mJ per pulse)<br>(ii) Average of energy density per second: 2866 J/cm$^2$ |

The Nd:YAP laser is a laser using yttrium aluminium perovskite doped with neodymium crystal as active laser medium. It is emitted in the near infrared at 1.34 $\mu$m, which is close to the wavelength of the Nd:YAG laser. The Nd:YAP laser beam shows clinically interesting properties as its good absorption by dark materials and metals. The Nd:YAP is also 20 times more absorbed by water than the Nd:YAG laser [6]. Its flexible fiber optic allows delivering energy in curved root canals where the effect of the ultrasonic instrumentation is limited due to the constraining effect of the curvature of the canal. Some authors also reported that Nd:YAP laser can be successfully used for removal of the smear layer in root canals [7, 8]. However the use of this type of laser in endodontics may generate an increase in temperature and cause periodontal tissue damage. This laser has potentially many clinical applications in dentistry and specifically in endodontics; thus, the safe irradiation conditions should be clearly defined before any future clinical use.

The aim of our study is to determine the safe range of irradiation parameters of Nd:YAP laser that can be used during endodontic treatments without damaging and overheating the periodontal tissues.

## 2. Material and Methods

The study was conducted according to the Ethic Committee Recommendations of Gent University (2014/0579). Twenty-seven caries-free single-rooted adult human teeth extracted for orthodontic reasons were collected and stored before the experiments at 4°C, in a humid atmosphere, on a gauze soaked with Hepes solution (pH 7.2 at 2 mmol/liter containing 0.19 mmol/liter of natrium azide) (Hepes, Merck, Overijse, Belgium). Age of patients ranged between 45 and 60 years. External surfaces of teeth were cleaned using a scaler after which they were decoronated under water cooling at low speed (300 rpm, Isomet, Low Speed Saw, Buehler Ltd., Lake Bluff, IL) as to obtain root segments of 11 mm. Root canals were prepared and enlarged to # 45 K file according to the conventional step-back technique in order to allow for optic fibers (diameter of 200–320 $\mu$m) to be placed inside all the way to the working length of 10 mm.

*2.1. Laser Irradiation Conditions.* A Nd:YAP laser (wavelength: 1340 nm, LOBEL MEDICAL SAS, Les Roches de Condrieu, France) was used. The beam emission is the pulsed mode (5, 10, and 30 Hz) predefined and imposed by the manufacturer's parameters. The pulse duration was 150 $\mu$s for all predetermined irradiation parameters. The laser apparatus was only able to deliver a pulse mode with a high peak output power per pulse. The output powers were predefined by the manufacturer and vary per second of irradiation from 0.9 W (2866 J/cm$^2$ for 200 $\mu$m and 1120 J/cm$^2$ for 320 $\mu$m fiber diameter) to 10 W (31847 J/cm$^2$ for 200 $\mu$m and 12453 J/cm$^2$ for 320 $\mu$m fiber diameter). The emitted power measured by a power meter (UP19K-15S, Gentec-EO, Québec, Canada) represented 90% of the displayed power. The predefined irradiation conditions are summarized in Tables 1 and 2, respectively, for the fiber diameter of 200 $\mu$m and 320 $\mu$m. To facilitate the understanding and the reproducibility of our experiences, only the output powers delivered per second for each irradiation condition (predefined by the manufacturer) will be considered in our study.

*2.2. Experimental Setup for Temperature Rise Measurements during Laser Irradiation.* We followed the setup of protocols used in previous studies for the measurements of temperature increase during laser irradiation [9, 10]. The external root surfaces were covered by thermoconductor paste (Warme Leitpaste WPN 10; Austerlitz Electronic, Nuremberg, Germany) to ensure optimal contact and maximal thermal conduction between the sensor tip of the thermocouple probe and the root surface. The thermal conductivity of the paste was 0.4 cal s$^{-1}$m$^{-1}$K$^{-1}$, which was comparable to the

Table 2: Different irradiation conditions are shown in function of predetermined setting of the apparatus. The average of output powers and the average of energy densities are calculated for the fiber diameter of 320 $\mu$m of the Nd:YAP laser.

| Setting parameter: G | Setting parameter: D | Setting parameter: C |
|---|---|---|
| 30 Hz, 150 $\mu$s per pulse | 10 Hz, 150 $\mu$s per pulse | 5 Hz, 150 $\mu$s per pulse |
| G+:<br>(i) 330 mJ (10 W)<br>(ii) 12453 J/cm$^2$ | D+:<br>(i) 400 mJ (4 W)<br>(ii) 4981 J/cm$^2$·sec | C+:<br>(i) 360 mJ (1.8 W)<br>(ii) 2241 J/cm$^2$ |
| G0:<br>(i) 250 mJ (7.5 W)<br>(ii) 9340 J/cm$^2$ | D0:<br>(i) 300 mJ (3 W)<br>(ii) 3736 J/cm$^2$ | C0:<br>(i) 280 mJ (1.4 W)<br>(ii) 1743 J/cm$^2$ |
| G−:<br>(i) 160 mJ (5 W)<br>(ii) 6226 J/cm$^2$ | D−:<br>(i) 200 mJ (2 W)<br>(ii) 2491 J/cm$^2$ | C−:<br>(i) 180 mJ (0.9 W)<br>(ii) 1120 J/cm$^2$ |

thermal conductivity of soft tissues (0.2–0.5 cal s$^{-1}$m$^{-1}$K$^{-1}$) depending on hydration [11].

Each root was closely rounded by 2 probes of a K-type thermocouple (K-type thermocouples HH806AWE Omega, Manchester, UK) with a precision of 0.01°C. One of the probes was located at 1 mm from the apex and the other at 5 mm from the cervical level. Each root was immerged into a 37°C bath keeping the cervical area above the waterline as to keep water out of the canal. The increases of temperatures caused by the irradiation of the root canal walls were then recorded and analyzed. Canal irradiation was accomplished with a circular and retrograde movement from the apex until the cervical part of the canal during 10 seconds with an axial speed of 1 mm/s. Each irradiation was done in a canal irrigated continuously with 2.25% NaOCl solution. Six records were repeated for each irradiation parameter.

Temperature measurement was performed after seeing the baseline level of temperature of root surface stable during 30 s. Temperature rise was recorded every second for 180 seconds after the end of the irradiation. Temperature increases ($D_t$) were calculated as the difference between the highest recorded temperatures at the root surface ($T_m$) and that recorded as baseline (room temperatures = $T_b$): $D_t = T_m - T_b$. We did a minimum of 5 records for each irradiation condition.

The mean and the standard deviation of recorded temperatures ($D_t$) for each irradiation condition were calculated. Normality tests were performed using the Kolmogorov Smirnov (KS) test.

## 3. Results

Whatever the output power used, the temperature rise after any irradiation condition needed more than 150 seconds to get back to its baseline level.

The KS normality test and Wilcoxon Signed Rank Test ($P$ value, two-tailed) showed that all groups passed normality test and correspond to Gaussian Approximation (alpha = 0.05; KS distance = 0.1788; $P$ value > 0.10). One-way ANOVA and post hoc tests (Newman-Keuls Multiple Comparison Test, $P < 0.05$) showed significant difference between all groups ($P$ value < 0.0001; $F$ 412; $R$ squared 0.9924). In groups using a fiber of 320 $\mu$m as diameter, the statistical difference is not significant between the means of the groups using the energy densities of 1120 J/cm$^2$ and 1743 J/cm$^2$. Also, for the groups using a fiber of 200 $\mu$m as diameter, the statistical difference is not significant between the means of groups using the energy densities, 2866 J/cm$^2$ and 4458 J/cm$^2$, and between means of the energy densities of 9554 J/cm$^2$ and 6369 J/cm$^2$.

After 10 seconds of irradiation (irradiation speed of 1 mm/sec), means and standard deviations of temperature increases at root surfaces were below the threshold level of 10°C, considered as safe for periodontal tissue [12], when the delivered average energy densities per second were equal to or below 4981 J/cm$^2$ (4 W) and 9554 J/cm$^2$ (3 W), respectively, for the irradiations using a fiber diameter of 320 $\mu$m and 200 $\mu$m.

Figures 1 and 2 show the temperature increases caused by 10 seconds of total irradiation time using, respectively, a fiber diameter of 200 $\mu$m and 320 $\mu$m.

For the use of similar irradiation parameters, each diameter of the optical fiber generated different temperature increase. The fiber with smaller diameter (200 $\mu$m) generated less temperature increase than the bigger one (320 $\mu$m) because of the higher distance existing between the edge of the fiber and the canal walls.

## 4. Discussion

Several studies showed some clinical applications using a Nd:YAP laser. It has been used in oral surgeries for lingual frenulectomy and frenectomy [13, 14] and for the initial treatment of periodontitis in adult [15]. In endodontic and restorative dentistry, the Nd:YAP laser was used to enhance canal cleanliness. Moshonov et al. [7] showed significant improved cleanliness into the coronal and the apical part of the root canals treated with Nd:YAP laser beam after manual preparation of the root canal with K-files and 2.5% sodium hypochlorite solution used for irrigation. Unfortunately, authors did not mention any information concerning the time consumed for canal cleaning and the detail about the way they moved the fiber into the canal (circumferential or not) and about the delivered irradiation speed given to the optical fiber (1 mm/s).

Blum and Abadie [16] pointed out that the use of the subsonic device and laser together as adjuncts showed opened tubules and the cleanest preparation was with very little debris and very small particle size. This result suggests that the laser has a potential in ensuring optimal canal cleanliness and opening tubules. The tested irradiation parameters were 260 mJ per pulse, 5 Hz, and 30 sec of irradiation each time with a constant movement of the tip when the laser is used for canal reparation or as adjunct with manual instrumentation. However, the use of Nd:YAP laser for 30 seconds without time off between successive irradiation series can induce a dangerous thermal increase, by cumulative effect, for the bone tissue according to Eriksson et al. [17].

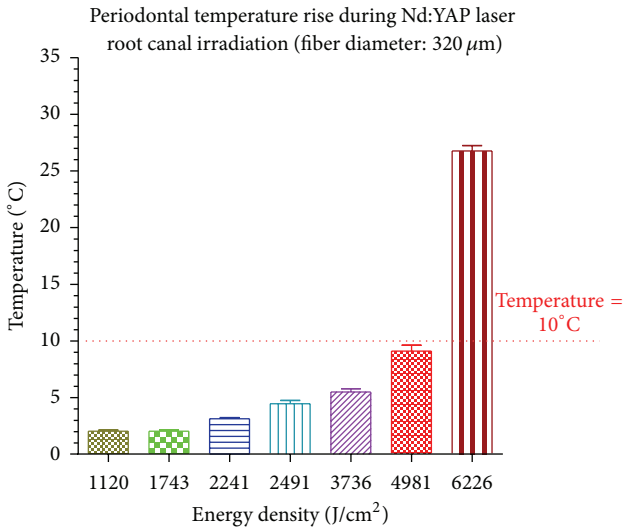



Figure 1: The temperature increases at root surfaces are shown in function of different average energy densities delivered per second after a total irradiation time of 10 seconds by means of fiber diameter of 320 $\mu$m. When the average of delivered energy densities per second was ≤4981 J/cm$^2$ (4 W), the temperature rises were below the safety level of 10°C for periodontal tissue injury.

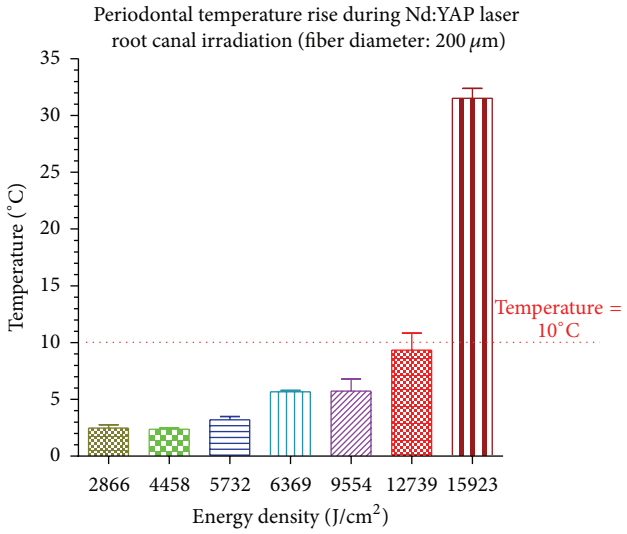



Figure 2: The temperature increases on root surfaces are shown in function of different average energy densities delivered per second after a total irradiation time of 10 seconds. When the average of delivered energy densities per second was ≤9554 J/cm$^2$ (3 W), the temperature rises were below the safety level of 10°C for periodontal tissue injury.

Farge et al. [18] measured the temperature rises on root surfaces with the aim to remove the fillings of root canals by means of Nd:YAP laser. They concluded that the Nd:YAP laser used in combination with hand instrumentation can remove efficiently pulpal debris and smear layer without exceeding the temperature increase of 5.2°C. They concluded that the laser should be used into a dry canal for a total irradiation time of 1 second. The authors recommended a long resting time exceeding one minute between two irradiations in order to allow thermal relaxation.

In our study, we evaluated the harmlessness of different irradiation parameters for endodontic use in empty and unfilled root canals. We used a canal irrigant during the irradiation. Previous tests reported that using the Nd:YAP laser without irrigation can lead to faster temperature increase than with irrigant. Thus, the use of irrigant could increase slightly the irradiation working time and consequently allow longer exposure time of dentinal walls and a reduction of bone injury risk.

Any black coloration into the root canal may induce localized higher overheating. Thus, the use of the Nd:YAP laser beam for endodontic treatment into dark colored teeth should be done with precautions. In this case, it is highly recommended to reduce the total irradiation time. More studies about this subject should be done.

According to the conditions of our study we found that we can use a Nd:YAP laser beam safely with a circumferential movement with a speed of 1 mm/sec moving backward from the apex to the cervical part of the root canal (under constant irrigation flow) during 10 seconds without inducing periodontal temperature overheating exceeding the trigger temperature of 10°C if some irradiation parameters are considered. In our study, we decided to follow the root canal irradiation protocol (circumferential movement, backward movement) proposed by Gutknecht et al. [19]. Authors justified the use of circular movements' protocol to ensure that the applied laser energy is distributed as uniformly as possible on the canal walls because of the variation of the diameter of canals from the apex to the coronal part [19].

Finally further investigations should be done in order to confirm the capacity of Nd:YAP laser coupled with the irrigant to remove the smear layer of the root canal according to the safe irradiation parameters that we found.

## 5. Conclusion

Based on our study and on our in vitro irradiation conditions, the use of Nd:YAP laser for endodontic treatments may be considered as harmless for periodontal tissues under specific irradiation parameters, equal to or lower than 4981 J/cm$^2$ (4 W) and 9554 J/cm$^2$ (3 W), respectively, for the irradiations using a fiber diameter of 320 $\mu$m and 200 $\mu$m.

## Competing Interests

The authors declare that they have no conflict of interests.

## References

[1] F. H. Takeda, T. Harashima, J. N. Eto, Y. Kimura, and K. Matsumoto, "Effect of Er:YAG laser treatment on the root canal walls of human teeth: an SEM study," *Endodontics and Dental Traumatology*, vol. 14, no. 6, pp. 270–273, 1998.

[2] S. Sahar-Helft, A. S. K. Sarp, A. Stabholtz, V. Gutkin, I. Redenski, and D. Steinberg, "Comparison of positive-pressure,

passive ultrasonic, and laser-activated irrigations on smear-layer removal from the root canal surface," *Photomedicine and Laser Surgery*, vol. 33, no. 3, pp. 129–135, 2015.

[3] G. A. Da Costa Lima, C. M. Aguiar, A. C. Câmara, L. C. Alves, F. A. B. Dos Santos, and A. E. Do Nascimento, "Comparison of smear layer removal using the Nd:YAG laser, ultrasound, ProTaper universal system, and CanalBrush methods: an in vitro study," *Journal of Endodontics*, vol. 41, no. 3, pp. 400–404, 2015.

[4] U. Schoop, W. Kluger, A. Moritz, N. Nedjelik, A. Georgopoulos, and W. Sperr, "Bactericidal effect of different laser systems in the deep layers of dentin," *Lasers in Surgery and Medicine*, vol. 35, no. 2, pp. 111–116, 2004.

[5] A. Moritz, U. Schoop, K. Goharkhay et al., "The bactericidal effect of Nd:YAG, Ho:YAG, and Er:YAG laser irradiation in the root canal: an in vitro comparison," *Journal of Clinical Laser Medicine and Surgery*, vol. 17, no. 4, pp. 161–164, 1999.

[6] N. Gutknecht, *Poceedings of the 1st International Workshop of Evidence Based Dentistry on Lasers in Dentistry*, Quintessence, New Maiden, UK, 2007.

[7] J. Moshonov, B. Peretz, T. Brown, and I. Rotstein, "Cleaning of the root canal using Nd:YAP laser and its effect on the mineral content of the dentin," *Journal of Clinical Laser Medicine and Surgery*, vol. 22, no. 2, pp. 87–89, 2004.

[8] J.-Y. Blum and M. J. M. Abadie, "Study of the Nd:YAP laser. Effect on canal cleanliness," *Journal of Endodontics*, vol. 23, no. 11, pp. 669–675, 1997.

[9] A. Namour, S. Nammour, A. Peremans, D. Heysselaer, and R. J. G. De Moor, "Treatment of dentinal hypersensitivity by means of Nd:YAP Laser: a preliminary in vitro study," *The Scientific World Journal*, vol. 2014, Article ID 323604, 7 pages, 2014.

[10] M. Umana, D. Heysselaer, M. Tielemans, P. Compere, T. Zeinoun, and S. Nammour, "Dentinal tubules sealing by means of diode lasers (810 and 980 nm): a preliminary in vitro study," *Photomedicine and Laser Surgery*, vol. 31, no. 7, pp. 307–314, 2013.

[11] F. C. Henriques Jr. and A. R. Moritz, "Studies of thermal injury, I. The conduction of heat to and through skin and the temperatures attained therein. A theoretical and an experimental investigation," *The American Journal of Pathology*, vol. 23, no. 4, pp. 530–549, 1947.

[12] A. R. Eriksson and T. Albrektsson, "Temperature threshold levels for heat-induced bone tissue injury: a vital-microscopic study in the rabbit," *The Journal of Prosthetic Dentistry*, vol. 50, no. 1, pp. 101–107, 1983.

[13] D. De Santis, R. Gerosa, P. F. Graziani et al., "Lingual frenectomy: a comparison between the conventional surgical and laser procedure," *Minerva Stomatologica*, In press.

[14] D. De Santis, D. Bertossi, G. Zanotti et al., "Nd-YAP laser assisted frenulectomy: a case series on 23 patients," *Minerva Stomatologica*, In press.

[15] P. Ambrosini, N. Miller, S. Briançon, S. Gallina, and J. Penaud, "Clinical and microbiological evaluation of the effectiveness of the Nd:Yap laser for the initial treatment of adult periodontitis. A randomized controlled study," *Journal of Clinical Periodontology*, vol. 32, no. 6, pp. 670–676, 2005.

[16] J.-Y. Blum and M. J. M. Abadie, "Study of the Nd:YAP laser. Effect on canal cleanliness," *Journal of Endodontics*, vol. 23, no. 11, pp. 669–675, 1997.

[17] A. Eriksson, T. Albrektsson, B. Grane, and D. McQueen, "Thermal injury to bone. A vital-microscopic description of heat effects," *International Journal of Oral Surgery*, vol. 11, no. 2, pp. 115–121, 1982.

[18] P. Farge, P. Nahas, and P. Bonin, "In vitro study of a Nd:YAP laser in endodontic retreatment," *Journal of Endodontics*, vol. 24, no. 5, pp. 359–363, 1998.

[19] N. Gutknecht, R. Franzen, and F. Lampert, "Finite element study on thermal effects in root canals during treatment with a surface-absorbed laser," *The Journal of Oral Laser Applications*, vol. 5, no. 1, pp. 31–36, 2005.

Hindawi Publishing Corporation
BioMed Research International
Volume 2016, Article ID 5749150, 7 pages
http://dx.doi.org/10.1155/2016/5749150

*Research Article*

# Pilot Study of Laser Doppler Measurement of Flow Variability in the Microcirculation of the Palatal Mucosa

**Pierre Le Bars,[1] Gaston Niagha,[1] Ayepa Alain Kouadio,[1] Julien Demoersman,[2] Elisabeth Roy,[3] Valérie Armengol,[4] and Assem Soueidan[5]**

[1]*Department of Prosthodonthics, UIC Odontologie, CHU de Nantes, 1 Place Alexis Ricordeau, 44042 Nantes, France*
[2]*Department of Periodontology, Dental School of Brest, 22 Avenue Camille Desmoulins, 29238 Brest Cedex 3, France*
[3]*Department of Pedodontics, UIC Odontologie, CHU Nantes, 1 Place Alexis Ricordeau, 44042 Nantes, France*
[4]*Department of Restaurative Dentistry, UIC Odontologie, CHU Nantes, 1 Place Alexis Ricordeau, 44042 Nantes, France*
[5]*Department of Periodontology, UIC Odontologie, LIOAD U-791, 1 Place Alexis Ricordeau, 44042 Nantes, France*

Correspondence should be addressed to Assem Soueidan; assem.soueidan@univ-nantes.fr

Received 19 September 2015; Revised 19 January 2016; Accepted 21 January 2016

Academic Editor: Samir Nammour



*Background.* Histopathological alterations can arise when the denture-supporting mucosa experiences microbial and mechanical stress through the denture base and diagnosis of these diseases usually follows microvascular changes. Microcirculation measurement could allow for detection of such dysfunction and aid in the early diagnosis of palatal mucosa pathologies. *Materials and Methods*. We tested the sensitivity of laser Doppler for measuring the microcirculation of the palatal mucosa, assessing the median raphe (MR), Schroeder area (SA), and retroincisive papilla (RP). A Doppler PeriFlux 5000 System, containing a laser diode, was used. 54 healthy participants were recruited. We compare the measurements of PU (perfusion unit) using ANOVA test. *Results.* The numerical values for palatal mucosa blood flow differed significantly among the anatomical areas ($p = 0.0167$). The mean value of Schroeder area was 92.6 (SD: 38.4) and was significantly higher than the retroincisive papilla (51.9) (SD: 20.2) ($p < 0.05$), which in turn was higher than that of median raphe (31.9) (SD: 24.2) ($p < 0.0001$). *Conclusion.* Schroeder area appeared to have the greatest sensitivity, and vascular flow variability among individuals was also greatest in this region. We suggest that analysis of blood stream modification with laser Doppler of the palatal mucosa can help to detect onset signs of pathological alterations.

## 1. Introduction

The microcirculation of the palatal mucosa around the three anatomical areas tested here has not been previously investigated. The rationale for this study was based on the potential for blood stream measures around these areas to reveal early vascular alterations at the level of the connective tissue. Indeed, the clinical aspects of the mucosa do not always reflect the underlying histological features and can mask infraclinical modifications or alterations. Thus, in the absence of clinical parameters (erythema, pain, or oedema), the investigation of vascular microcirculation is of interest for detecting the onset stages of the pathology.

Denture-supporting mucosa is subjected to microbial and mechanical stress transmitted through the denture base, which can lead to development of certain diseases (e.g., prosthetic stomatitis). Histological and microvascular changes precede the clinical diagnosis of these conditions, and detection of such changes could potentially help in preventing the onset of these pathologies. Measurement of the microcirculation is useful for the early detection of palatal microvessel dysfunction and can help in the diagnosis of numerous palatal mucosa pathologies [1].

The laser Doppler flowmeter (LDF) is a noninvasive measure of capillary blood perfusion (blood flow, volume, and velocity). The laser Doppler measures the flow of blood cells inside a tissue without causing the slightest deterioration of the tissue. Blood cells moving within the volume illuminated by the beam will cause the light frequency [2] and are useful for measuring the microcirculation in healthy tissue in humans and nonhuman animals [3]. This approach was first used in the 1980s [4, 5] and has since been applied for many

tissues, including the skin [6], tongue, and oral mucosa of healthy individuals [7], and the tooth [8], periodontal tissues [9–11], and the masseter muscle [12].

The efficacy of LDF used here has been previously employed in the study of various pathologies including wound healing in cutaneous sclerosis [13] and skin ischemia in rats [14], as well as diseases such as allergic reaction of the human nasal mucosa [15] and psoriasis [16]. Here, we aimed to measure the microcirculation of the healthy palatal mucosa at three specific points, measuring anatomical and histological variation, and to test the reproducibility and sensitivity of the LDF.

## 2. Materials and Methods

Fifty-four healthy students of the dental school at Nantes with no visible palatal mucosal abnormalities were recruited over a 7-month period. Participants were 20 to 26 years old and consisted of 32 men and 22 women, including 12 smokers (10 cigarettes/day). This study was performed in accordance with the ethical standards laid down in the 2002 Declaration of Helsinki and its later amendments.

One operator (GN) made the measurements using Laser Doppler Perfusion Monitor (PeriFlux System 5000; Perimed, Stockholm, Sweden) with a probe, latex particles, and a rotating disc.

The sensor emits monochromatic light at a 780 nm wavelength, which is absorbed by the mucosa. The range of light is 1 $mm^3$ [17]. Students were seen in two sessions on a voluntary basis, and oral informed consent was obtained.

At the first session, a full clinical examination was conducted to determine the general and oral health status. Clinical parameters were also listed, including angle class, palate shape, and the clinical aspect of the palatal mucosa (colour, adhesion, surface, and appearance). Only participants considered healthy based on this examination were included. Exclusion criteria were hypertension, being on medication related to blood circulation, and a pronounced gag reflex.

The first step consisted of the making of an alginate impression that was sent to the laboratory for gutter fabrication. To attach the probe during measurement, thermoformed gutter trays were made by the prosthetic laboratory of the dental school. These gutters, in transparent copolyester (acrylic resin; thickness 1 mm), were fitted with three brackets for attaching the probe during recording (Figure 1).

After completion of the gutters, three perforations were made for the probe supports. The three selected areas represent specific anatomical points: the retroincisive papilla, the median raphe, and the posterolateral Schroeder area. (Figure 1). We positioned our probe precisely halfway along the tangent between the distal surfaces of the first molar to the median raphe.

During the second session, The LDF was calibrated before each data collection session, with a colloidal suspension of latex microparticles (Perimed Mobility Standard), the flow of which corresponds to the value 250 (±15). All recordings were made in the same place at room temperature, but even the temperature of the oral cavity can be somewhat variable between individuals. They were placed on a dental chair in a comfortable position, half-inclined. After the patient rested for 5 min in a prone position, these measures allow us to include patients checking the normality of blood pressure. These measures denote the normality of the pulse and saturation and represent a criterion of selection. Finally, the measurements were performed with the probe that had been previously stabilized by the support. Each recording lasted for 3 min (Figure 2).

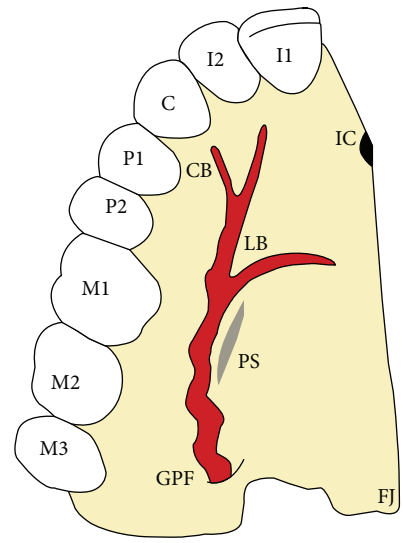



Figure 1: Diagram showing the branches of the artery great palate (GPA) and bony prominences palatine. The GPA emerges through the greater palatine foramen (GPF) from the maxillary artery, runs along the palatal spine (PS), and is divided into lateral branch (LB) and canine branch (CB), and leads to the incisive foramen (IC).

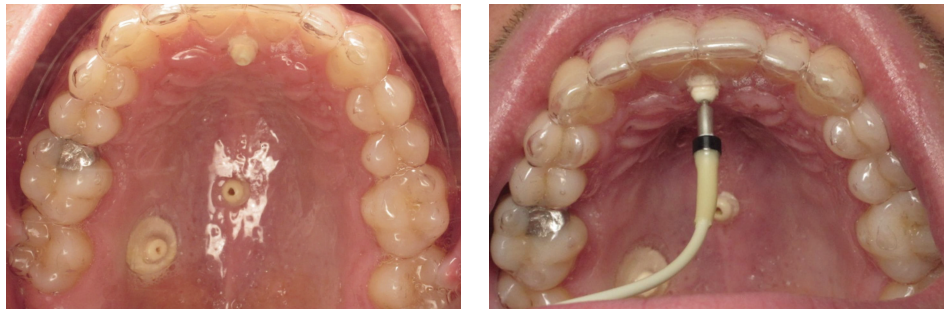
The statistical analysis involved paired-samples $t$-tests. One-way analyses of repeated measures of variance (ANOVA) were applied to compare three areas tested under xlstats®. $p < 0.05$ was taken as indicating statistical significance. According to the central theorem limit, the distribution of the mean of the sample greater than 30 patients would authorize or permit the use of Student's $t$-test to compare each mean of each group of data.

## 3. Results

Differences in the blood flow to the palatal mucosa were expressed as a percentage of the PU value (Figures 2–5).

In the first group ($n = 54$), ANOVA analysis of the repeated measures for the entire group ($n = 54$) (12 smokers + 42 no smokers) identified a statistically significant difference between the tested anatomical areas one to one ($p = 0.0167$). We found that the mean value of Schroeder area was (92.6) (SD: 38.4) and was significantly higher than the retroincisive papilla (51.9) (SD: 20.2) ($p < 0.05$), which in turn was higher than that of median raphe (31.9) (SD: 24.2) ($p < 0.0001$) (Figure 3).

In the second group of only no smokers ($n = 42$), the mean measured values for the three were 81.6 (SD: 27.5)



(a) (b)

Figure 2: Thermoformed trays in the mouth (a) with three brackets for attaching the probe during recording (retroincisive papilla, median raphe, and Schroeder area) (b).

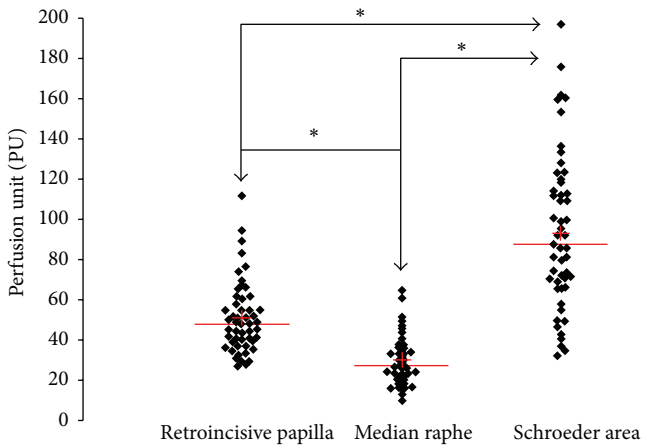



Figure 3: Scatterplot of individual measured values in the three zones. *Statistically significant difference between the different anatomical areas ($p$ value < 0.05). We found that the average value at the Schroeder area (PU = ±92) was significantly higher than that measured at the retroincisive papilla (PU = ±51.92) ($p < 0.05$), which is higher than the median raphe (PU = ±31.97) ($p < 0.0001$). (The red line shows the average blood flow measurements in the three study areas.)

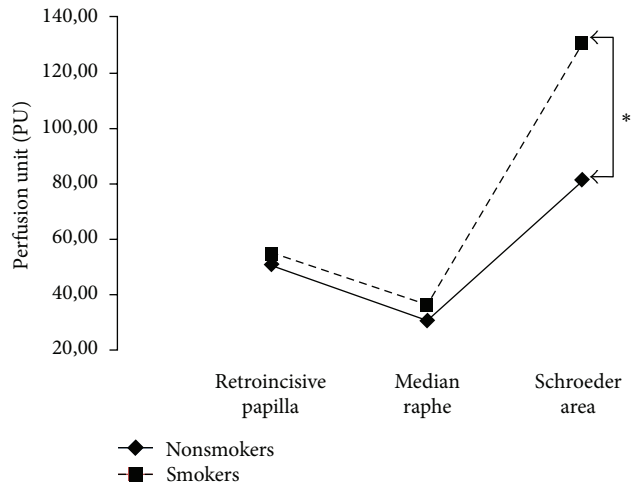



Figure 4: Mean comparisons of PU measure between smokers and nonsmokers patients in Schroeder area. The average values of the three zones were generally higher in smokers than in nonsmokers. However, this difference was only statistically significant for the Schroeder area ($p = 0.005$)*.

(Schroeder area), 51.1 (SD: 18.4) (retroincisive papilla) ($p < 0.0001$), and 30.7 (SD: 26.4) (median raphe) ($p < 0.0001$).

In the third group of only smokers ($n = 12$), we also found that the average values of the three zones were generally higher in smokers than in nonsmokers. However, this difference was statistically significant only for the Schroeder area 130.9 (SD: 47.3) ($p = 0.005$) (Figure 4).

Closer analysis of the dispersion of values for the Schroeder area revealed a dense concentration of the measures in nonsmokers (Figure 5(a)), compared with more dispersed values in smokers (Figure 5(a)).

## 4. Discussion

A removable prosthesis exerts pressure on the oral mucous membranes and in particular on the palatal mucosa during mastication. Under these conditions, the blood supply of the mucous membrane is then modified at histological level before the apparition of clinical signs. The question for us was whether we have the ability to detect and measure these vascular histological changes before the apparition of clinical signs.

To answer this question, we wanted to test the reliability and laser Doppler sensitivity level of vascularization of the palatal mucosa. We conducted, on a sample of 54 healthy patients, measurements at three sites in the palatal mucosa (retroincisive papilla, the median raphe, and Schroeder area).

To validate our measures, several parameters must be apprehended as the depth of the palatal mucosa and the influence of the age of patients.

For the depth, five studies have recently involved the palatal mucosa (in longitudinal and transverse planes) using various techniques. One study examined (transversely) 34 hemimaxillae of cadavers (13 men and 4 women; mean age: 57.2 years). The thicknesses of the palatal mucosa and the *lamina propria* including the epithelium were measured at

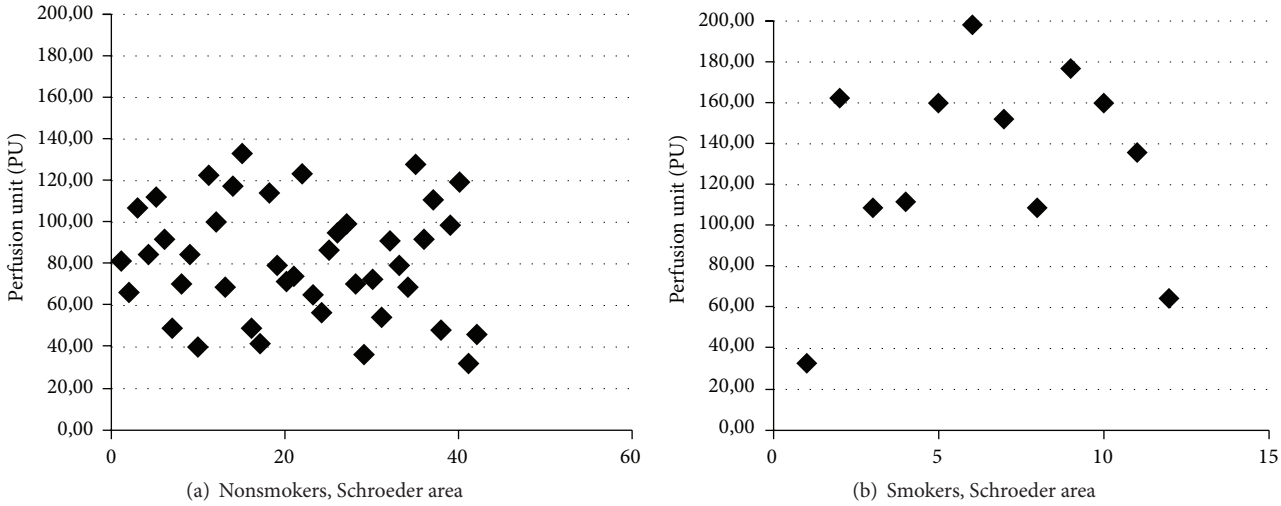



Figure 5: Scatterplot values in Schroeder area in nonsmokers (a) and smokers (b).

three points, starting from the alveolar crest, at intervals of 4 mm and with the aid of Adobe Photoshop®. The thicknesses of the palatal mucosa increase from the alveolar crest toward the midpalatal suture. Conversely, the thicknesses of the *lamina propria* including the epithelium at these same positions decrease toward the midpalatal suture [18].

Kolliyavar et al. [19], Anuradha et al. [20], and Yaman et al. [21] measured (longitudinally) the gingival margin and palatal line area. A bone-sounding method using a periodontal probe was used to assess the thickness of the palatal mucosa at 15 measurement sites, and the difference in mucosal thickness between the groups was determined. The mean thickness of the palatal masticatory mucosa ranged from 2.0 to 3.7 mm.

Cho et al. [22] used light microscopy to investigate the longitudinal depth from the surface of the palatal mucosa. The thickness of the epithelium and *lamina propria* of the palatal mucosa was measured (from the canine distal area to the first molar distal area) at three positions (starting from 3 mm below the alveolar crest and in 3 mm intervals) along the path of the palatine artery. The mean depth from the surface of the palatal mucosa to the greater palatine artery decreased from the canine distal to the first premolar distal but again increased towards the posterior molar. The mean length from the alveolar crest to the greater palatine artery, however, increased toward the posterior molar.

Our measurements are effective to a depth of 1 mm, which corresponds to a *lamina propria* volume of only 1 $mm^3$. Indeed, under this condition, the thickness of the palatal mucosa was not affected in our results.

Secondly, the effects of aging were not consistently reported in these three studies. Although Kolliyavar et al. [19] and Anuradha et al. [20] found that younger participants had thinner mucosa than older participants, Yaman et al. [21] detected no significant difference between age groups. Anuradha et al. [20] also found that, within the same age group, females had thinner mucosa than males whereas Yaman et al. [21] identified no differences according to gender or body mass index. These conflicting findings are likely to result from study differences in age, ethnicity, body mass index, varying measurement methods, and the placement of measurement points.

If we compare these study groups to those of our study, our participants were relatively healthier and younger (mean 23 years) and had a lower weight mean (65 kg).

Another interesting aspect is to compare the measures obtained by laser Doppler between the skin and palatal mucosa; the buccal epithelium is relatively finer than the skin epidermis [23]. Despite this difference, the signal obtained from contact with the oral mucous membrane is less pronounced than that of the skin of the cheek. The density of capillaries varies according to the anatomical zones of the human body, so at the level of the bowel the average density of the capillaries is about 50 capillaries by square millimetre of mucous surface [17]. Another explanation is the fact that the vascular network of oral tissues is less rich than that of the skin [24]. Although it is possible that the presence or absence of epithelial fingering can change the morphology of the epithelium, we propose that our measurements were not influenced by these histological characteristics because the operating range of laser Doppler signal exceeds the thickness of the epithelium.

Concerning the precision of the measures, in our study, for better recording probe support, an in-mouth stabilizing patch was developed. The thickness of the gutters (<2 mm) does not influence measures of blood flow in the mucosa [25]; indeed, amelioration of recording reliability over time could be verified by using this approach.

Another parameter is that the fluctuation in the lower frequencies at 0.1 Hz depends on the sympathetic nervous system at the level of the blood flow to the skin [26]. Under the same conditions for the palatal mucosa, the precision of our measures can be limited. This parameter is much more transient at the level of the oral mucous membrane than at the level of the skin. Histamine is quickly eliminated at the level of the vascular buccal network [24]. Furthermore, at the

level of the skin, the proportion of the nerve network that is influenced by histamine is large and with a prolonged effect [5, 8, 9]. It seems that, in the case of our experiment, this parameter did not influence the result because every measurement was conducted on healthy participants after 30 min of complete rest.

Our anatomical and histological findings enabled us to demonstrate a significant difference in the microcirculation in the three areas. The flow was much more important in the Schroeder area compared to the retroincisive papilla and was significantly higher than the median raphe. The average flux values were higher in the Schroeder area, differences that could have been revealed only by the sensitivity of the laser Doppler device.

*4.1. Smoking and Nonsmoking Patients.* It is well established that smoking modifies the vascular network, with a hyperaemic response in the palatal mucosa of smokers compared to nonsmokers. In the present investigation, the increased PU value of smokers was probably the result of a local vascular vasoconstriction effect, particularly at the most peripheral portion of the mucosa. The elevation of microcirculation in the palatal mucosa serves as a trigger for angiogenesis of the palatal vascular plexus, and our results confirm this other research [27].

In our study, when comparing smokers to nonsmokers, we observed increased microcirculation in smokers, particularly in the connective tissue of the Schroeder area. A local effect of smoking on the palatal mucosa is well established [28, 29] and can potentiate interaction of the flow, particularly in the Schroeder area. There we propose that the Schroeder area would likely be the most informative/interesting zone in which we measure the flow and compare findings between participant groups. Concerning our sample, we took into account interindividual variability including general condition, similar hemodynamic and ambient temperatures [30], age, gender, site, posture, and ethnicity. Other parameters that can influence intraindividual comparisons include menstrual cycle, circadian rhythm, and physical activity whereas mental activity does not influence the results [31].

The relatively homogeneous age of the participants in this study (22 to 26 years old) means that age had little influence on our results [32]. The flexibility of blood vessels is reduced with increased age. In the same way, a decrease in the thickness of the oral mucous membrane occurs with aging. Another aspect is fibrosis of connective tissue in the *lamina propria*, which appears with aging and can influence the measures [33].

*4.2. Influence of a Covered Removable Prosthetic Denture on the Palatal Mucosa.* In an interesting study [34], laser Doppler was used to investigate recovery after removal of a partial denture (bilateral posterior maxilla edentulous) in the palatal mucosa of the Schroeder area [34]. The results of this referenced study did not reveal significant changes in the blood flow of the palatal mucosa in the Schroeder area over a 12-minute period. It seems that wearing a resin partial denture modifies the vascularization of the palatal mucosa in the area of Schroeder, independently of disease. It would also seem that bearing a partial removable prosthesis in resin increases the stream of the vascularization of the palatal mucous membrane of the zone of Schroeder, independently of any clinically detectable sign of local pathologies.

It has been suggested that a denture on tissue surfaces must irritate the mucosa, and the Schroeder area is often implicated in the prevalence of denture-related stomatitis [35]. Changes to blood flow at the level of the palatine mucous membrane are not initially clinically detectable, and for this reason laser Doppler can constitute an appropriate tool for diagnosis or early investigation of certain pathologies (e.g., burning mouth syndrome [9]) that present considerable difficulties with visual detection. Recently, after clinical observations, a new laser Doppler perfusion tool, PeriScan PIM II, has been used to evaluate the distribution maps of blood flow corresponding to specific areas [36].

## 5. Conclusion

Application of LDF to measure blood flow in the palatal mucosa can differentiate three anatomical areas, with measurement of the Schroeder area being the most sensitive. The incidence of the mucosa thickness variability highlights the value of routine measurement of microcirculation by laser Doppler in patients presenting mucosal lesions. This study represents a technical advance and opens new possibilities for investigating diseases of the palatal mucosa, including tissue changes related to dentures, such as denture-related stomatitis. The influence of certain technical impressions or treatments (e.g., radiotherapy or bisphosphonates) can alter the subepithelial microcirculation, and these alterations can be measured at the blood vessel level using LDF. This possibility also applies for more systemic diseases such as diabetes. Therefore, further investigation will be required to better establish the clinical potential of LDF. We currently are extending this study of the palatal mucosa blood microcirculation in the same areas of denture wearers with clinically healthy mucosa and those with different denture-related stomatitis diseases.

## Conflict of Interests

The authors declare that they have no conflict of interests.

## Acknowledgments

The authors are grateful to ParoNantes association for financial support, Jonathan Bodin for statistical advice, and San Francisco Edit for reviewing the paper.

## References

[1] L. Orekhova and A. A. Barmasheva, "Doppler flowmetry as a tool of predictive, preventive and personalised dentistry," *The EPMA Journal*, vol. 4, no. 1, article 21, 2013.

[2] D. Evans, J. Reid, R. Strang, and D. Strirrups, "A comparison of laser Doppler flowmetry with other methods of assessing the vitality of traumatised anterior teeth," *Endodontics & Dental Traumatology*, vol. 15, no. 6, pp. 280–290, 1999.

[3] C. M. Choi and R. G. Bennett, "Laser Dopplers to determine cutaneous blood flow," *Dermatologic Surgery*, vol. 29, no. 3, pp. 272–280, 2003.

[4] G. E. Nilsson, U. Otto, and J. E. Wahlberg, "Assessment of skin irritancy in man by laser Doppler flowmetry," *Contact Dermatitis*, vol. 8, no. 6, pp. 401–406, 1982.

[5] S. Hellem, L. S. Jacobsson, G. E. Nilsson, and D. H. Lewis, "Measurement of microvascular blood flow in cancellous bone using laser Doppler flowmetry and $^{133}$Xe-clearance," *International Journal of Oral Surgery*, vol. 12, no. 3, pp. 165–177, 1983.

[6] H. Svensson, P. Svedman, J. Holmberg, and S. Jacobsson, "Laser Doppler flowmetry and transcutaneously measured carbon dioxide tension for observing changes of skin blood flow in fingers," *Scandinavian Journal of Plastic and Reconstructive Surgery*, vol. 17, no. 3, pp. 183–186, 1983.

[7] D. B. Singh, G. Stansby, and D. K. Harrison, "Assessment of oxygenation and perfusion in the tongue and oral mucosa by visible spectrophotometry and laser doppler flowmetry in healthy subjects," *Advances in Experimental Medicine and Biology*, vol. 614, pp. 227–233, 2008.

[8] E. Chen and P. V. Abbott, "Dental pulp testing: a review," *International Journal of Dentistry*, vol. 2009, Article ID 365785, 12 pages, 2009.

[9] M. L. Kaplan, M. K. Jeffcoat, and P. Goldhaber, "Blood flow in gingiva and alveolar bone in beagles with periodontal disease," *Journal of Periodontal Research*, vol. 17, no. 4, pp. 384–389, 1982.

[10] H. Develioglu, B. Kesim, and A. Tuncel, "Evaluation of the marginal gingival health using laser Doppler flowmetry," *Brazilian Dental Journal*, vol. 17, no. 3, pp. 219–222, 2006.

[11] M. L. Macedo Paizan and J. F. Vilela-Martin, "Is there an association between periodontitis and hypertension?" *Current Cardiology Reviews*, vol. 10, no. 4, pp. 355–361, 2014.

[12] D. A. Curtis, S. A. Gansky, and O. Plesh, "Deep and superficial masseter muscle blood flow in women," *Journal of Prosthodontics*, vol. 21, no. 6, pp. 472–477, 2012.

[13] P. Broz, M. Aschwanden, S. Partovi et al., "Assessment of cutaneous microcirculation in unaffected skin regions by transcutaneous oxygen saturation monitoring and Laser Doppler flowmetry in systemic sclerosis," *Clinical Hemorheology and Microcirculation*, vol. 60, no. 3, pp. 263–271, 2015.

[14] Y.-K. Jan, B. Lee, F. Liao, and R. D. Foreman, "Local cooling reduces skin ischemia under surface pressure in rats: an assessment by wavelet analysis of laser Doppler blood flow oscillations," *Physiological Measurement*, vol. 33, no. 10, pp. 1733–1745, 2012.

[15] S. Juliusson and M. Bende, "Allergic reaction of the human nasal mucosa studied with laser Doppler flowmetry," *Clinical Allergy*, vol. 17, no. 4, pp. 301–305, 1987.

[16] A. G. M. Hendriks, W. Steenbergen, E. Hondebrink, J. C. G. Van Hespen, P. C. M. Van De Kerkhof, and M. M. B. Seyger, "Whole field laser Doppler imaging of the microcirculation in psoriasis and clinically unaffected skin," *The Journal of Dermatological Treatment*, vol. 25, no. 1, pp. 18–21, 2014.

[17] D. A. L. Hoff, H. Gregersen, and J. G. Hatlebakk, "Mucosal blood flow measurements using laser Doppler perfusion monitoring," *World Journal of Gastroenterology*, vol. 15, no. 2, pp. 198–203, 2009.

[18] S.-K. Yu, B.-H. Lee, M.-H. Lee, K.-H. Cho, D. K. Kim, and H.-J. Kim, "Histomorphometric analysis of the palatal mucosa associated with periodontal plastic surgery on cadavers," *Surgical and Radiologic Anatomy*, vol. 35, no. 6, pp. 463–469, 2013.

[19] B. Kolliyavar, S. Setty, and S. L. Thakur, "Determination of thickness of palatal mucosa," *Journal of Indian Society of Periodontology*, vol. 16, no. 1, pp. 80–83, 2012.

[20] B. R. Anuradha, B. S. Shankar, B. John, K. A. R. V. Prasad, A. Gopinadh, and K. N. N. Devi, "Assessment of palatal masticatory mucosa: a cross-sectional study," *The Journal of Contemporary Dental Practice*, vol. 14, no. 3, pp. 536–543, 2013.

[21] D. Yaman, S. Aksu, R. Dişçi, and K. Demirel, "Thickness of palatal masticatory mucosa and its relationship with different parameters in Turkish subjects," *International Journal of Medical Sciences*, vol. 11, no. 10, pp. 1009–1014, 2014.

[22] K. H. Cho, S. K. Yu, M. H. Lee, D. S. Lee, and H. J. Kim, "Histological assessment of the palatal mucosa and greater palatine artery with reference to subepithelial connective tissue grafting," *Anatomy & Cell Biology*, vol. 46, no. 3, pp. 171–176, 2013.

[23] C. A. Squier, M. J. Kremer, and P. W. Wertz, "Effect of ethanol on lipid metabolism and epithelial permeability barrier of skin and oral mucosa in the rat," *Journal of Oral Pathology & Medicine*, vol. 32, no. 10, pp. 595–599, 2003.

[24] B. Bay, M. Hilliges, C. Weidner, and G. Sandborgh-Englund, "Response of human oral mucosa and skin to histamine provocation: laser Doppler perfusion imaging discloses differences in the nociceptive nervous system," *Acta Odontologica Scandinavica*, vol. 67, no. 2, pp. 99–105, 2009.

[25] H. Akazawa and K. Sakurai, "Changes of blood flow in the mucosa underlying a mandibular denture following pressure assumed as a result of light clenching," *Journal of Oral Rehabilitation*, vol. 29, no. 4, pp. 336–340, 2002.

[26] T. Söderström, A. Stefanovska, M. Veber, and H. Svensson, "Involvement of sympathetic nerve activity in skin blood flow oscillations in humans," *The American Journal of Physiology—Heart and Circulatory Physiology*, vol. 284, no. 5, pp. H1638–H1646, 2003.

[27] M. M. Bornstein, K. Klingler, U. P. Saxer, C. Walter, and C. A. Ramseier, "Tobacco-associated lesions of the oral mucosa," *Schweizerische Monatsschrift fur Zahnmedizin*, vol. 116, no. 12, pp. 1261–1274, 2006.

[28] D. A. Baab and P. A. Oberg, "The effect of cigarette smoking on gingival blood flow in humans," *Journal of Clinical Periodontology*, vol. 14, no. 7, pp. 418–424, 1987.

[29] T. N. Meekin, R. F. Wilson, D. A. Scott, M. Ide, and R. M. Palmer, "Laser Doppler flowmeter measurement of relative gingival and forehead skin blood flow in light and heavy smokers during and after smoking," *Journal of Clinical Periodontology*, vol. 27, no. 4, pp. 236–242, 2000.

[30] J. Allen and K. Howell, "Microvascular imaging: techniques and opportunities for clinical physiological measurements," *Physiological Measurement*, vol. 35, no. 7, pp. R91–R141, 2014.

[31] K. Zeghal, P. Geslin, A. Maurel, G. Lagrue, and F. Lhoste, "Laser-Doppler velocimetry: a new technic for evaluating of microcirculation. A reproducibility study," *La Presse Médicale*, vol. 15, no. 40, pp. 1997–2000, 1986.

[32] G. Shklar, "The effects of aging upon oral mucosa," *The Journal of Investigative Dermatology*, vol. 47, no. 2, pp. 115–120, 1966.

[33] M. W. Hill, "Influence of age on the morphology and transit time of murine stratified squamous epithelia," *Archives of Oral Biology*, vol. 33, no. 4, pp. 221–229, 1988.

[34] C. Okada, T. Ueda, and K. Sakurai, "Blood flow in denture-supporting maxillary mucosa in response to simulated mastication by loading," *Journal of Prosthodontic Research*, vol. 54, no. 4, pp. 159–163, 2010.

[35] A. V. Newton, "Denture sore mouth. A possible aetology," *British Dental Journal*, vol. 112, pp. 357–360, 1962.

[36] H. Hirai, "Application of a laser Doppler perfusion imaging Periscan PIM II for measuring the blood flow of the oral mucosa," *Nihon Hotetsu Shika Gakkai zasshi*, vol. 49, no. 1, pp. 26–35, 2005.