Effect of photodynamic therapy by 810 and 940 nm diode laser on Herpes Simplex Virus 1: An in vitro study

Mahsa Alavi Namvar[a], Mohammad Vahedi[b], Hamid-reza Abdolsamadi[c], Alireza Mirzaei[d], Younes Mohammadi[e], Farid Azizi Jalilian[f],*

[a] Postgraduate Student, Department of Oral and maxillofacial Medicine, Faculty of Dentistry, Hamedan University of Medical Sciences, Hamedan, Iran
[b] Associate Professor, Dental research center, Department of Oral and maxillofacial Medicine, Faculty of Dentistry, Hamadan University of Medical Sciences, Hamadan, Iran
[c] Professor, Dental research center, Department of Oral and maxillofacial Medicine, Faculty of Dentistry, Hamadan University of Medical Sciences, Hamadan, Iran
[d] DDS, MS, DMD, member of dental laser research of Hamadan university of medical science, Hamadan, Iran
[e] Ph.D. in Epidemiology, Modeling of Noncommunicable Diseases Research Center, Department of Epidemiology, School of Public Health, Hamadan University of Medical Sciences, Hamadan, Iran
[f] Associate Professor of Medical Virology, Department of Medical Microbiology, Hamadan University of Medical Sciences, Hamadan, Iran

ARTICLE INFO

ABSTRACT

Introduction: Herpes simplex virus (HSV) is among the most common viruses in humans. HSV1 is often responsible for oral and perioral herpetic lesions. Photodynamic therapy (PDT) is a novel antimicrobial modality that involves the use of laser and a photosensitizer with a specific wavelength. This study aimed to assess and compare the effect of PDT with 810 and 940 nm diode laser and indocyanine green (ICG) photosensitizer on HSV1.
Methods: In this in vitro study, HSV1 isolated from herpes labialis and there were 6 experimental groups. The irradiation parameters were the same for all groups. Number of remaining viruses per milliliter in each group was determined using real-time polymerase chain reaction (PCR) and statistically analyzed by ANOVA.
Results: The virus count in all groups significantly decreased compared to the control group (P < 0.05) except in group ICG- without irradiation (P > 0.05). Comparison of groups 810- and 940- (use of each laser alone) with groups 810+ and 940+ (use of each laser plus ICG) revealed that reduction in virus count in groups 810+ and 940+ was significantly greater than that in groups 810- and 940-.
Conclusion: 810 nm diode laser irradiation and ICG causes the greatest reduction in number of HSV1 compared to all the other groups. ICG without laser irradiation has not significant efficacy on reduction of virus count.

1. Introduction

Herpes simplex virus (HSV) belongs to the herpesviridae family and has double-stranded DNA genome. HSV is common worldwide; infection with HSV1 has an acute phase in which the virus multiplies rapidly at the site of infection and then migrates to neural ganglia to initiate the latent phase [1].

The binding and penetration of HSV into the host cells are mediated by superficial glycoproteins. Viral DNA replication and protein expression then occur by cellular proteins. In addition, the virus impairs the immune system [2].

The majority of middle-aged individuals have herpes antibody in their serum and about one-third of the world's population experience the recurrence of herpes infection.

HSV1 is responsible for herpes labialis while HSV2 causes herpes genitalis; however, both types can cause infection in other areas of the human body as well and manifest similar clinical signs and symptoms. Primary HSV1 infection is characterized by mucosal and skin lesions. Buccal mucosal and gingival lesions are common in primary HSV1 infection. Herpetic simplex keratitis is the most common cause of blindness caused by corneal inflammation in the United States and is usually hard to treat [1–3]. Herpetic infections are treated with nucleotide analogs such as acyclovir and penciclovir. Resistance to acyclovir is now a major problem caused by mutations in the viral thymidine kinase gene and is seen in 95% of the patients [4].

Considering the emergence of drug resistance, search is ongoing for other methods to eliminate HSV. Photodynamic therapy (PDT) is one suggested technique to eliminate viruses using light at a specific

---

wavelength and a light-sensitive molecule known as photosensitizer, which is activated by light. This method has long been used to disable viruses [5–7]. A wavelength of light with maximum absorption by the photosensitizer activates the photosensitizer and releases singlet oxygen. The efficacy of PDT depends on the characteristics of the tissue and damaged cells as well as oxygen generation. Singlet oxygen remains within the cells for a short time and has limited range of action. If the photosensitizer binds to the target cells, it can selectively destroy the damaged cells without injuring the adjacent cells [8].

Indocyanine green (ICG) is a commonly used photosensitizer through intravenous infusion to test liver function and for endoscopy. ICG has a wide range of light absorption from 600 to 800 nm wavelength. Its maximum absorption occurs at around 800 to 805 nm wavelength [9,10].

A photosensitizer should be able to produce singlet oxygen in a wide range of wavelengths and should have a strong affinity for binding to damaged cell and not the healthy cells [11–13].

ICG binds to plasma proteins. It has no adverse effect on the human body and is quickly excreted via bile. Thus, it can be considered as an ideal photosensitizer [9–14].

Considering the disadvantages of using methylene blue photosensitizer in PDT (such as tooth discoloration) [15] we decided to assess the efficacy of PDT with ICG for elimination of HSV1 using 810 and 940 nm diode laser.

2. Materials and methods

2.1. Preparation of light sources

In this in vitro study, infra-red diode lasers with 810 (Wuhan Gigaa Optronics Technology Co., Wuhan, China) and 940 nm (Biolase, USA) wavelength, with 500 mW power in the area of 0.384 cm^2, and 400 μm fiber tip were used for 60 s as the light source. Energy density was approximately 78 J$_{/cm2}$.

2.2. Preparation of photosensitizer

ICG (Periogreen, Elixxion, AG Radolfzell, Germany) was prepared in 0.1 mg/mL concentration by dissolving a 0.2 mg ICG tablet in 2 mL of sterile water in a dark room and was used as photosensitizer after 5 min (according to the manufacturer's instructions).

2.3. Preparation of cell culture

For cell culture, Vero cells (ATCC CLL-81) were purchased from the Pasteur Institute of Iran and passaged after quality control. RPMI 6040 culture medium supplemented with 10% fetal bovine serum, penicillin-streptomycin and an anti-mycotic with the recommended ratios in 50 μg/mL concentration was used for cell culture. The cells were incubated in 5% CO_2 and > 90% humidity and checked every day under an inverted microscope to ensure their ideal morphology. After reaching 90% confluence in cell culture flasks, the stock solution was prepared. The cell density at the time of addition of viruses was 70%.

2.4. Preparation of HSV1

Using a sampling scalpel and a swab, samples were taken from the fluid within the newly formed vesicles of herpes labialis in a 35-year-old female. The samples were transferred to VTM medium. The mixture was then filtered using 0.45 μm filter and added to cell culture with 70% confluence. It was then incubated for one hour in order for the cells to absorb the viruses. The overlaying medium was then discarded and cells, now infected with viruses, were added to 25 cm flasks containing 5 mL of 2% RPMI. The control flask had the same condition [16].

The cells were evaluated daily for cytopathic effect, which is often characterized by formation of giant cells and ballooning in cells. After observing the cytopathic effect in > 50% of the cells compared to the control medium, the viruses were collected and stored at −70 °C. Real-time polymerase chain reaction (PCR) was used to determine the type of virus and ensure that it was HSV1. PCR is the new gold standard for HSV detection in clinical samples [17]. Next, standard concentration of cells (3×10^5/mL) was seeded as mono-layer with 70% density in specific wells of a 96-well microplate. HSV1 was seeded in 20 wells for each experimental group (every other wells of one row).

We had 6 experimental groups in this study as follows:

1. Control: Only had Vero cells and HSV1 without any laser irradiation and ICG (for the purpose of comparison)
2. 810-: Cells with HSV1 without ICG irradiated with 810 nm diode laser
3. 810 +: Cells with HSV1 and ICG irradiated with 810 nm diode laser
4. 940-: Cells with HSV1 without ICG irradiated with 940 nm diode laser
5. 940 +: Cells with HSV1 and ICG irradiated with 940 nm diode laser
6. ICG-: Cells with HSV1 and ICG without any irradiation (to assess the effect of ICG alone on HSV1)

2.5. Evaluation of the effect of lasers without ICG on HSV1

The wells that only underwent laser irradiation (groups 810- and 940-) contained cells plus virus and were subjected to laser irradiation with a 400 μm fiber tip from 1 cm distance [18] that was same for all groups (to assess the effect of laser without ICG on HSV1).

2.6. Evaluation of the effect of lasers plus ICG on HSV1

The wells in groups 810 + and 940 + contained ICG and cells plus virus and subjected to laser irradiation with a 400 μm fiber tip from 1 cm distance, 5 min after addition of ICG (according to the manufacturer's instructions).

2.7. Evaluation of the effect of ICG without any irradiation on HSV1

In group ICG-, ICG and cells plus virus were added to each well. No irradiation was performed (to assess the effect of ICG alone on HSV1).

The contents of the wells were subjected to quantitative PCR. DNA was extracted using DNA/RNA extraction kit (Roche, Switzerland) and qRT-PCR kit (NovinGene, Iran). The quality and concentration of extracted genome were then determined using a NanoDrop(2000C; Thermo, USA). Quantitative test was performed using FTD kit (Lithuania) and real-time PCR (Roche, USA). Since we did differentiation of HSV1 and HSV2 using PCR kit (AryaTous, Iran), and also quantification of HSV using qRT-PCR kit(NovinGene, Iran), we didn't use primer in this study.

2.8. Statistical analysis

The results were reported as copy numbers/milliliter. Data was analyzed with analysis of variance test (ANOVA) using SPSS version 16 (SPSS Inc., IL, USA) and ANOVA. The results were expressed as mean value ± standard deviation and differences were significant at the level of p < 0.05 (Tukey post hoc test).

3. Results

In all experimental groups, a reduction in the count of HSV1 was seen compared to control group (untreated virus). This reduction in virus count was maximum in group 810 + and minimum in group ICG- (Table 1).

Tukey post hoc test showed that reduction in HSV1 count in groups 810-, 810 +, 940- and 940 + was highly significant (P < 0.001) but

Table 1
Count of HSV1/ml in the six experimental groups. Reduction of Herpes simplex viruses in group ICG- was the least and in group 810 + was the most in comparison of control.

Group	Minimum	Maximum	Mean	Std. deviation	P value
Control	8.2×10^5	9.8×10^5	9.000×10^5	66708.32032	< 0.001 [*]
810-	4.347×10^5	5.02×10^5	4.7038×10^5	24623.09891	
810 +	1.492×10^5	1.62×10^5	1.548×10^5	4859.52673	
940-	4.599×10^5	5.106×10^5	4.871×10^5	20231.90550	
940 +	2.35×10^5	2.606×10^5	2.484×10^5	9176.87311	
ICG-	8.4×10^5	9.11×10^5	8.7348×10^5	25702.85976	

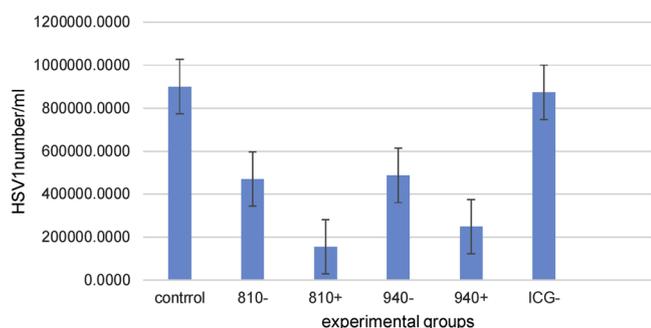[*] statistically significant at level < 0.001.

Fig. 1. mean count of HSV1/ml in the experimental groups at the end of the assay. Reduction in group ICG- was not significant in comparison with the control group.

this reduction in group ICG- was not significant compared to the control group (P = 0.782).

3.1. Effect of laser irradiation without ICG

When we compared 2 groups (810- and 940-) that ICG had not been used (only lasers were used without ICG) both showed a significant reduction in the number of HSV1 compared to the control group but the difference between them was not significant (P = 0.961).

3.2. Effect of PDT by ICG

When we used laser irradiation and ICG in groups 810 + and 940 + (PDT by ICG) both of them showed significant reduction in the number of HSV1 compared to the control group (P < 0.001) and the reduction in group 810 + was the significantly greater than that in group 940 + (P < 0.001).

3.3. Effect of ICG

In comparison between groups 810- and 810 +, both of them experienced a significant reduction in number of HSV1 compared to the control group (P < 0.001) and the reduction in group 810 + was significantly greater than that in group 810- (P < 0.001).

The result was exactly the same with groups 940- and 940 + (P < 0.001).

Reduction in HSV1 count in group ICG- was the least and not significant in comparison of control group and reduction in all other groups (810-, 810 +, 940-, 940 +) was significantly more than group ICG-.

4. Discussion

PDT is currently accepted as an adjunct to other methods for treatment of drug-resistant pathogens and cancers such as skin melanoma and stem cell cancer [18–21]. In PDT, photosensitizer absorbs certain wavelengths of light and transform it into useful energy and also

it can produce cytotoxic agents like reactive oxygen species (ROS) [22]. In PDT, reactive oxygen is produced by a photosensitizer and low intensity light to kill the infectious cells [21]. Several studies have confirmed the positive efficacy of PDT in oral (like endodontics, implant therapy, periodontics and etc.) dermatological and gastric infections [21,23–25]. Several in-vitro studies has been done about effects of PDT of ICG and methylene blue on oral bacterial infections [18,22,26,27]. ICG is a new fluorescent dye from tricarbocyanine dye family with 775 g/mol molecular weight. It is soluble in water because of its sulfonate side chains [28,29]. Considering the fact that no previous study has evaluated the effect of PDT by ICG and 810 and 940 nm diode laser on HSV1, this study was undertaken to evaluate this topic.

As noted in the papers, the peak absorption of ICG is in 600–900 nm, and the results of our study also confirm this [30,31]. It means that ICG could also have absorption in the other wavelengths but it may be less than in 600-900. Based on this spectrum, we decided to investigate the effect of 940 nm laser which is not far from it. Also similar articles, have used 980 and 970 nm lasers for photodynamic therapy by ICG and had favorable results. Since 940 nm is a common wavelength in clinical practice, we decided to investigate the effect of photodynamic therapy with 940 and 810 nm laser on HSV1. In the current study, we used both 810 and 940 nm diode lasers to assess their efficacy for activation of ICG and elimination of HSV1. The results showed that both 810 and 940 nm lasers without ICG (groups 940- and 810-) were equally effective for elimination of HSV1 (Fig. 1). HSV1 treatment with lasers is maybe based on the photochemical and photobiological effects of laser light [32]. Interestingly, in both groups with ICG (940 + and 810 +) significant reduction in HSV1 in comparison with 940- and 810- was seen, but reduction in 810 + was significantly more than that in 940 +, maybe because of the peak absorption wavelength of ICG. Results of our study showed that PDT with ICG and 810 nm laser has the best effect in reducing HSV1 in comparison of other groups (810-, 940-, 940 +, ICG-).

A previous study used 810 and 980 nm laser and ICG for disinfection of implant surfaces and concluded that both laser types without ICG could disinfect the implant surfaces. Valente et al. concluded that 810 nm laser, 980 nm laser can also activate ICG but the reduction of microorganisms in the use of photosensitizer with the two laser types was not significantly different from the use of 810 and 980 nm lasers alone [30]. This difference with our study can be due to different microorganisms and settings.

In this study, we concluded that use of ICG without laser irradiation decreased the virus count but not significantly. In other words, the effect of ICG on viruses is only significant when activated by laser and ICG alone is not capable of eliminating viruses (Fig. 1). In the article "Diode laser activated indocyanine green selectively kills bacteria "Boehm et al. used 810 nm laser and ICG to kill P.gingivalis and they concluded that ICG treatment alone does not significantly kill any bacteria and we reached this result about HSV1 and ICG [33]. Azizi et al. evaluated the effect of PDT with 660 (methylene blue photosensitizer) and 810 nm (ICG photosensitizer) lasers on Streptococcus mutans (S.mutans) and concluded that number of S. mutans decreased to zero in laser plus photosensitizer groups. They also concluded that in groups where photosensitizer was used without laser irradiation the

bacterial count decreased, but did not reach zero; this finding about using ICG without laser treatment was in agreement with our findings [26].

It is not clear how ICG kills microorganisms. There is two possibilities considering killing bacteria: 1- actual phototoxicity and 2- indirect thermal effects. On the other hand, effect of ICG absorption depends on the dye concentration, the solvent medium and the temperature [29]. Laser irradiation (810 and 940 nm) with ICG in groups 810+ and 940+ activated this photosensitizer and caused a significant reduction in virus count, but in none of them, HSV1 number didn't reach to zero. We used only one setting for all groups. Maybe studying about other settings and such more concentrations of ICG can help to know the reason that is the aim of our ongoing study. Beytollahi et al. evaluated S. mutans biofilm reduction by PDT and photothermal therapy and obtained similar results as ours. They stated that PDT and photothermal therapy are suitable methods for reduction of S. mutans biofilm [27]. Fekrazad et al. assessed the effect of PDT with 810 nm laser and ICG on Porphyromonas gingivalis (P. gingivalis) compared to metronidazole and chlorhexidine and concluded that the efficacy of PDT for elimination of P. gingivalis was greater than that of chlorhexidine and metronidazole [18]. Latief et al. assessed the effect of PDT with 660 nm laser and TONS504 on viruses sensitive and resistant to acyclovir and concluded that both types of viruses (sensitive and resistant to acyclovir) were sensitive to PDT with TONS504; TONS504 alone had no antiviral effect [34]. Nagahara et al. evaluated the effect of PDT with 805 nm laser and ICG on P. gingivalis and concluded that PDT eliminates P. gingivalis only if chitosan is used as an intermediate material to mediate the adhesion of ICG to bacteria [35]. The results of the present study showed that 810 nm laser with ICG was the most efficient way to reduce the number of HSV1 viruses and ICG alone didn't have significant reduction in virus numbers. In the present study, we concluded that both 810 and 940 nm diode lasers can cause a reduction in HSV1 count. Irradiation of these lasers with ICG as a photosensitizer results in significant reduction of HSV1 count. We also found that this reduction in viral count was not due to the effect of ICG itself and it should be activated by laser to exert its antiviral effect. Many studies have used 660 nm laser with methylene blue photosensitizer and one study used 670 nm laser without photosensitizer for treatment of recurrent herpes labialis [32,36–40].

It's a very promising result of a Pilot test, given the approximately 8-fold reduction in viruses in this study. As mentioned, with changing the dosage, temperature, duration of radiation, etc., the level of virus reduction may be changed, which is being done in other ongoing studies.

All these results show that PDT by ICG may be an effective treatment for clinical herpetic lesions in the future, based on several basic studies and animal model studies which evaluate several concentrations and powers.

Acknowledgements

References

[1] R.J. Whitley, D.W. Kimberlin, B. Roizman, Herpes simplex viruses, Clin. Infect. Dis. 26 (3) (1998) 541–553 quiz 54-5.

[2] R.J. Whitley, B. Roizman, Herpes simplex virus infections, Lancet 357 (9267) (2001) 1513–1518.

[3] S. Guess, D.U. Stone, J. Chodosh, Evidence-based treatment of herpes simplex virus keratitis: a systematic review, Ocul. Surf. 5 (3) (2007) 240–250.

[4] T.H. Bacon, M.J. Levin, J.J. Leary, R.T. Sarisky, D. Sutton, Herpes simplex virus resistance to acyclovir and penciclovir after two decades of antiviral therapy, Clin. Microbiol. Rev. 16 (1) (2003) 114–128.

[5] R. Ackroyd, C. Kelty, N. Brown, M. Reed, The history of photodetection and photodynamic therapy, Photochem. Photobiol. 74 (5) (2001) 656–669.

[6] I.J. Macdonald, T.J. Dougherty, Basic principles of photodynamic therapy, J. Porphyr. Phthalocyanines 05 (02) (2001) 105–129.

[7] C. Wallis, J.L. Melnick, Irreversible photosensitization of viruses, Virology 23 (1964) 520–527.

[8] M. Ochsner, Photophysical and photobiological processes in the photodynamic therapy of tumours, J. Photochem. Photobiol. B, Biol. 39 (1) (1997) 1–18.

[9] M. Sawa, K. Awazu, T. Takahashi, H. Sakaguchi, H. Horiike, M. Ohji, et al., Application of femtosecond ultrashort pulse laser to photodynamic therapy mediated by indocyanine green, Br. J. Ophthalmol. 88 (6) (2004) 826–831.

[10] I.J. Fox, E.H. Wood, Indocyanine green: physical and physiologic properties, Proc. Staff Meet. Mayo Clin. 35 (1960) 732–744.

[11] W. Baumler, C. Abels, S. Karrer, T. Weiss, H. Messmann, M. Landthaler, et al., Photo-oxidative killing of human colonic cancer cells using indocyanine green and infrared light, Br. J. Cancer 80 (3-4) (1999) 360–363.

[12] K.J. Baker, Binding of sulfobromophthalein (BSP) sodium and indocyanine green (ICG) by plasma α1 lipoproteins, Proc. Soc. Exp. Biol. Med. 122 (4) (1966) 957–963.

[13] G.R. Cherrick, S.W. Stein, C.M. Leevy, C.S. Davidson, Indocyanine green: observations on its physical properties, plasma decay, and hepatic extraction, J. Clin. Invest. 39 (1960) 592–600.

[14] J. Caesar, S. Shaldon, L. Chiandussi, L. Guevara, S. Sherlock, The use of indocyanine green in the measurement of hepatic blood flow and as a test of hepatic function, Clin. Sci. 21 (1961) 43–57.

[15] S. Carvalho Edos, I. Mello, S.J. Albergaria, S.M. Habitante, J.L. Lage-Marques, D.P. Raldi, Effect of chemical substances in removing methylene blue after photodynamic therapy in root canal treatment, Photomed. Laser Surg. 29 (8) (2011) 559–563.

[16] S.M. Kramer, K.M. Ford, M.K. York, Comparison of HSV-1 and HSV-2 isolation in Vero, primary rabbit kidney, and MRC-5 cells, Lab. Med. 19 (1) (1988) 30–31.

[17] H.E. Kaufman, A.M. Azcuy, E.D. Varnell, G.D. Sloop, H.W. Thompson, J.M. Hill, HSV-1 DNA in tears and saliva of normal adults, Invest. Ophthalmol. Vis. Sci. 46 (1) (2005) 241–247.

[18] R. Fekrazad, K. Karamifar, A. Bahador, Comparison of antibacterial effect of photodynamic therapy using indocyanine green (Emundo) with 2% metronidazole and 2% chlorhexidine gel on Porphyromonas gingivalis (an in-vitro study), Photodiagnosis Photodyn. Ther. 15 (2016) 28–33.

[19] Q. Zhang, L. Li, Photodynamic combinational therapy in cancer treatment, J. BUON 23 (3) (2018) 561–567.

[20] S.R. Lucena, N. Salazar, T. Gracia-Cazana, A. Zamarron, S. Gonzalez, A. Juarranz, et al., Combined treatments with photodynamic therapy for non-melanoma skin Cancer, Int. J. Mol. Sci. 16 (10) (2015) 25912–25933.

[21] G.B. Kharkwal, S.K. Sharma, Y.-Y. Huang, T. Dai, M.R. Hamblin, Photodynamic therapy for infections: clinical applications, Lasers Surg. Med. 43 (7) (2011) 755–767.

[22] M. Pourhajibagher, N. Chiniforush, R. Ghorbanzadeh, A. Bahador, Photo-activated disinfection based on indocyanine green against cell viability and biofilm formation of Porphyromonas gingivalis, Photodiagnosis Photodyn. Ther. 17 (2017) 61–64.

[23] N. Chiniforush, M. Pourhajibagher, S. Shahabi, A. Bahador, Clinical approach of high technology techniques for control and elimination of endodontic microbiota, J. Lasers Med. Sci. 6 (4) (2015) 139–150.

[24] M. Leretter, A. Cândea, F. Topala, Photodynamic Therapy in Peri-implantitis, (2013).

[25] L. Lin, L. Xiong, Y. Wen, S. Lei, X. Deng, Z. Liu, et al., Active targeting of nanophotosensitizer delivery systems for photodynamic therapy of Cancer stem cells, J. Biomed. Nanotechnol. 11 (4) (2015) 531–554.

[26] A. Azizi, S. Shademan, M. Rezai, A. Rahimi, S. Lawaf, Effect of photodynamic therapy with two photosensitizers on Streptococcus mutans: in vitro study, Photodiagnosis Photodyn. Ther. 16 (2016) 66–71.

[27] L. Beytollahi, M. Pourhajibagher, N. Chiniforush, R. Ghorbanzadeh, R. Raoofian, B. Pourakbari, et al., The efficacy of photodynamic and photothermal therapy on biofilm formation of Streptococcus mutans: an in vitro study, Photodiagnosis Photodyn. Ther. 17 (2017) 56–60.

[28] C. Shi, J.B. Wu, D. Pan, Review on near-infrared heptamethine cyanine dyes as theranostic agents for tumor imaging, targeting, and photodynamic therapy, J. Biomed. Opt. 21 (5) (2016) 50901.

[29] J. Meister, M. Hopp, J. Schäfers, J. Verbeek, D. Kraus, M. Frentzen (Eds.), Indocyanine Green (ICG) as a New Adjuvant for the Antimicrobial Photo-Dynamic Therapy (aPDT) in Dentistry, SPIE, 2014SPIE BiOS.

[30] N.A. Valente, T. Mang, M. Hatton, L. Mikulski, S. Andreana, Effects of two diode lasers with and without photosensitization on contaminated implant surfaces: an ex vivo study, Photomed. Laser Surg. 35 (7) (2017) 347–356.

[31] A. Mathew, M. Lajevardi, H. Abdullah Al Juboori, N.A. Abu Mukhaimer, A. Al-Radaideh, S. Thomas, An in vivo study on comparison of disinfection of root canal with chemical disinfectants and disinfectant-diode laser-photodynamic treatment combined system, J. Dent. Lasers 9 (1) (2015) 2–10.

[32] C. de Paula Eduardo, A.C. Aranha, A. Simoes, M.S. Bello-Silva, K.M. Ramalho, M. Esteves-Oliveira, et al., Laser treatment of recurrent herpes labialis: a literature review, Lasers Med. Sci. 29 (4) (2014) 1517–1529.

[33] T.K. Boehm, S.G. Ciancio, Diode laser activated indocyanine green selectively kills bacteria, J. Int. Acad. Periodontol. 13 (2) (2011) 58–63.

[34] M.A. Latief, T. Chikama, J.A. Ko, Y. Kiuchi, T. Sakaguchi, A. Obana, Inactivation of acyclovir-sensitive and -resistant strains of herpes simplex virus type 1 in vitro by photodynamic antimicrobial chemotherapy, Mol. Vis. 21 (2015) 532–537.

[35] A. Nagahara, A. Mitani, M. Fukuda, H. Yamamoto, K. Tahara, I. Morita, et al., Antimicrobial photodynamic therapy using a diode laser with a potential new photosensitizer, indocyanine green-loaded nanospheres, may be effective for the

clearance of Porphyromonas gingivalis, J. Periodont. Res. 48 (5) (2013) 591–599.

[36] J. Marotti, A.C. Aranha, P. Eduardo Cde, M.S. Ribeiro, Photodynamic therapy can be effective as a treatment for herpes simplex labialis, Photomed. Laser Surg. 27 (2) (2009) 357–363.

[37] P.J. Munoz Sanchez, J.L. Capote Femenias, A. Diaz Tejeda, J. Tuner, The effect of 670-nm low laser therapy on herpes simplex type 1, Photomed. Laser Surg. 30 (1) (2012) 37–40.

[38] K.M. Ramalho, R.G. Rocha, A.C. Correa-Aranha, S.R. Cunha, A. Simoes, L. Campos, et al., Treatment of herpes simplex labialis in macule and vesicle phases with photodynamic therapy. Report of two cases, Photodiagnosis Photodyn. Ther. 12 (2) (2015) 321–323.

[39] F. Sperandio, J. Marotti, A. Aranha, C. Eduardo, Photodynamic Therapy for the Treatment of Recurrent Herpes Labialis: Preliminary Results, (2009), pp. 415–419.

[40] V.V. Zverev, O.V. Makarov, A.Z. Khashukoeva, O.A. Svitich, Y.E. Dobrokhotova, E.A. Markova, et al., In vitro studies of the antiherpetic effect of photodynamic therapy, Lasers Med. Sci. 31 (5) (2016) 849–855.