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EDITOR’S VIEW

Laser Applications in Implantology: Scientific and Clinical Perspectives
Stuart Coleton, DDS

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LITERATURE REVIEW

Efficacy of Laser Therapy in the Treatment of Peri-Implantitis: A Literature Review
Georgios E. Romanos, DDS, PhD, Prof. Dr. med. dent. and Fawad Javed, BDS, PhD.

How effective are lasers of different wavelengths in treating peri-implantitis? The authors examine the evidence from representative articles appearing in the literature.
CLINICAL CASE

Er:YAG Laser-Assisted Bone and Gingival Augmentation Around Implants
Walid Altraye, DDS, MDS, PhD
How can an Er:YAG laser be used around ailing dental implants? The author demonstrates multiple applications of this laser for soft and hard tissue surgery and implant surface decontamination.

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Antimicrobial Effects of the 810-nm Diode Laser in the Treatment of Peri-Implantitis: An Ex Vivo Pilot Study
Erica Lavere, BS, DDS; Juliana Sagor, BA, DDS; Lynn Mikulski; Sebastiano Andreana, DDS, MS
How effective are different laser parameters in reducing a particular bacterial species, and how does adjunctive use of a photosensitizer affect the outcome? The authors demonstrate results using a novel tissue model.

LETTERS

More on Laser-Assisted Orthodontic Tooth Movement
For facilitating tooth movement, is low-level laser stimulation or inhibition the underlying mechanism? How does emission mode affect the laser dose? A reader and the investigator share their dialogue.

RESEARCH ABSTRACTS

Antimicrobial Effects of Photosensitizers
Experimental findings of the intraoral bactericidal effect of certain chemical substances, whether they are used alone or in photodynamic therapy

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Laser Applications in Implantology: Scientific and Clinical Perspectives

Stuart Coleton, DDS, New York Medical College, Valhalla, New York, and Westchester University Medical Center, Valhalla, New York

For this and future issues of the Journal, we are expanding our regular scientific treatment of dental laser-related topics to include clinical cases. This complementary approach is designed to address the clinical needs of our readers while continuing to explore and relate the scientific foundations behind laser treatment of dental patients.

This issue concentrates on some of the applications of laser use in implantology. The American Academy of Implant Dentistry* estimates that 3 million Americans have dental implants, and that number is growing by one-half million yearly. Further, it is estimated that the U.S. and European market for dental implants is expected to reach $4.2 billion by 2022. From flap surgery to removal of granulation tissues, from bone contouring to decontamination of implant surfaces, and from second-stage recovery to peri-implantitis treatment, lasers provide several means to support the placement and maintenance of dental implants.

Dr. Georgios Romanos and Dr. Fawad Javed furnish a literature review of the efficacy of laser therapy in treating peri-implantitis. For their analysis, they selected clinical and experimental studies cited in indexed databases. Their findings underscore the adjunctive role that laser therapy offers in treating this condition, providing clinicians adhere to safety and clinical application protocols.

Dr. Walid Altayeb presents a detailed case involving the use of an Er:YAG laser to assist in osseous and gingival augmentation surgery around ailing dental implants. The functional and aesthetic outcomes demonstrate how healthy and satisfactory results can be achieved by a cross-disciplinary dental team while considering relevant aspects of patient peri- and intraoral anatomy and respecting patient preferences for treatment.

Dr. Erica Lavere, one of the Academy of Laser Dentistry’s 2015 Dr. Eugene M. Seidner Student Scholarship Program honorees, and her colleagues present their research on “Antimicrobial Effects of the 810-nm Diode Laser in the Treatment of Peri-Implantitis: An Ex Vivo Pilot Study.” Their experimental model and approach examines the effects of 810-nm diode laser irradiation, with and without a photosensitizer, on a particular bacterial species.

Complementing Dr. Lavere’s study, the Research Abstracts explore the role that photosensitizers, whether or not they are used in conjunction with photodynamic therapy, could furnish in reducing the bacterial load of concern in peri-implantitis, periodontal disease, and other dental applications.

Finally, a letter to the editor and author response concerning laser irradiation doses in orthodontics continue the dialogue on “The Effect of Pulsed 810-nm Low-Level Laser Therapy on the Rate of Orthodontic Tooth Movement: A Randomized Clinical Trial,” as initially reported in the previous edition of the Journal.

Future issues will continue to provide scientific and clinical perspectives on the numerous applications of lasers in dentistry.

Yours for the future of lasers in dentistry,

Stuart Coleton, DDS
Editor-in-Chief, Journal of Laser Dentistry

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Efficacy of Laser Therapy in the Treatment of Peri-Implantitis: A Literature Review

Georgios E. Romanos, DDS, PhD, Prof. Dr. med. dent.
Department of Periodontology, School of Dental Medicine, Stony Brook University, Stony Brook, New York, USA

Fawad Javed, BDS, PhD
University of Rochester, Eastman Institute for Oral Health, Department of General Dentistry, Rochester, New York

ABSTRACT

The aim of the present study was to review indexed literature regarding the efficacy of laser therapy in the treatment of peri-implantitis. Indexed databases were searched from using different combinations of the following key words: “peri-implantitis,” “bone loss,” “photodynamic therapy,” “laser,” and “light-activated disinfection.” Titles and abstracts of studies that fulfilled the eligibility criteria were screened by the authors and checked for agreement. Full texts of studies judged by title and abstract to be relevant were read and independently assessed against the eligibility criteria. The pattern of the present review was customized to summarize the relevant data. Laser therapy can effectively be used with conventional mechanical debridement protocols used for treating peri-implantitis. The erbium:yttrium-aluminum-garnet laser effectively strips off the contaminated oxide layer from implant surfaces without damaging implant surface characteristics. Likewise, the carbon dioxide laser application to implant surfaces is a potent strategy for implant surface disinfection and enhancing bone-to-implant contact around previously infected sites. The neodymium:yttrium aluminium garnet laser may melt the surface of failing implants and overheat the implant and surrounding tissues. Diode lasers (810-980 nm) can also be dangerous if they are used without following strict protocols. Photodynamic therapy exhibits high target specificity and destroys pathogens associated with the etiology of peri-implantitis. Laser therapy significantly reduces the clinical markers of peri-implant tissue inflammation (bleeding on probing and clinical attachment loss) without jeopardizing the integrity of the implant and alveolar bone. In conclusion, laser therapy, as an adjunct to conventional mechanical debridement therapy, is a modern therapeutic method that can effectively be used for the treatment of peri-implantitis when safety and clinical application protocols are cautiously followed.

Key words: Dental lasers, Peri-implantitis, Peri-implant bone loss, Photodynamic therapy

INTRODUCTION

Peri-implantitis is a disease of the tissues surrounding dental implants. Roos-Jansåker et al.1 defined peri-implantitis as an inflammatory condition in which implants with varying degrees of bone loss are accompanied by a probing depth of at least 4 mm, bleeding on probing, and purulent discharge on placid probing. It has been reported that peri-implantitis occurs on the order of 10% of implants and in 20% patients within 5 years to 10 years after implant placement. However, the individual reported figures are variable.2-4 Risk factors that have been associated with the etiology of peri-implantitis encompass poor
oral hygiene (poor plaque control), past history of periodontal disease, stagnation of residual cement in or around the gingivae after implant prosthesis cementation, occlusal overloading (Figure 1), systemic diseases (such as poorly controlled diabetes mellitus and osteoporosis), and tobacco smoking.

Figure 1a: Advanced peri-implant bone loss due inflammatory reaction and overloading, The implant was 5 years in function. (Figure 1a is provided courtesy of José Luis Calvo Guirado, DDS, PhD, Eu PhD, MS, Universidad Católica San Antonio de Murcia (UCAM), Murcia, Spain)

Figure 1b: Implant after removal due to increased mobility shows no signs of osseointegration (Figure 1b is provided courtesy of José Luis Calvo Guirado, DDS, PhD, Eu PhD, MS, UCAM, Murcia, Spain)
Ideally, treatment of peri-implantitis focuses on infection control, detoxification of implant surfaces (Figure 2), regeneration of lost tissues, and plaque control regimes via mechanical debridement (with or without raising a surgical flap). However, with advancements in modern clinical dental practice and research, new innovative therapeutic regimes (such as laser-supported- and photodynamic therapy [PDT]) have emerged that have been shown to be potentially useful (Figures 3-4) in the treatment of periodontitis and peri-implantitis. In a case-series, Romanos and Nentwig investigated the efficacy of a carbon dioxide (CO$_2$) laser (10,600 nm) in the decontamination of failing implants before augmentation. The results showed that after a mean follow-up of 27 months, virtually complete bone occurred in the peri-implant defects. Likewise, in a preclinical canine study Nevins et al. assessed the efficacy of erbium:yttrium-aluminum-garnet (Er:YAG) laser in reestablishing bone-to-implant contact around sites with peri-implantitis. After 3 months of treatment, the animals were sacrificed, jaw segments resected and prepared for histologic assessment. The results showed complete depletion of inflammatory cells from the peri-implant tissues and there was new bone in contact with the implant surface for implants treated with the Er:YAG laser as compared to control implants (implants treated without Er:YAG laser therapy).

Figure 2: Detoxification of an implant surface with tetracycline
The aim of the present study was to review the efficacy of laser therapy in the treatment of peri-implantitis.
MATERIALS AND METHODS

PubMed/MEDLINE (National Library of Medicine, Bethesda, Md., USA), Embase, Scopus, ISI-Web of Knowledge, and Google Scholar databases were searched from 1991 up to and including August 2014 using different combinations of the following key words: “peri-implantitis,” “bone loss,” “photodynamic therapy,” “laser,” and “light-activated disinfection.” Clinical and experimental studies were included. Letters to the editor, case reports, historic reviews, and articles published in languages other than English were excluded. Titles and abstracts of studies that fulfilled the eligibility criteria were screened by the authors and checked for agreement. Full texts of studies judged by title and abstract to be relevant were read and independently assessed against the eligibility criteria. Following this, reference lists of original and review studies that were found to be pertinent in the previous step were hand-searched and checked for agreement via discussion between the authors. Since indexed literature has a dearth of studies assessing the role of lasers in the treatment of peri-implantitis, the pattern of this review was customized to primarily summarize the pertinent data.

DISINFECTION OF DENTAL IMPLANT SURFACES

It is known that implant surface characteristics (surface roughness) play a significant role in osseointegration and long-term survival of dental implants. It has been reported that the Er:YAG laser has a high absorbability in water and removes the microbial-infiltrated oxide layer from the surface without jeopardizing the implant surface characteristics and surrounding alveolar bone. In their study on dogs, Nevins et al. investigated the ability of Er:YAG laser to treat peri-implantitis by removing the contaminated titanium oxide layer from implant surfaces. After 3 months of follow-up, the animals were clinically examined to assess the severity of peri-implant soft tissue inflammation following which the animals were sacrificed and jaw segments (containing the implants and surrounding tissues) were assessed histologically. Clinically, minimal gingival inflammation was seen and the histologic results showed bone formation and sufficiently enhanced bone-to-implant contact. Experimental results by Yamamoto and Tanabe also reported that the Er:YAG laser is potentially effective in stripping the contaminated titanium oxide layer from implant surfaces without damaging the implant surface and bone. However, controversial results have also been inked. In a 48-month follow-up clinical study, Schwarz et al. assessed the effect of two surface decontamination methods on the long-term outcomes following combined surgical resective and regenerative treatment of peri-implantitis. In Group 1, implant surfaces were treated with an Er:YAG laser, whereas those in Group 2 were treated with plastic curettes + cotton pellets + sterile saline. The 4-year follow-up results showed significantly greater reduction in bleeding on probing, plaque index, and attachment loss among implants in Group 2 as compared to implants in Group 1. An explanation in this regard is that use of laser equipment is technique-sensitive and operators’ experience with this technology plays a role in the overall effectiveness of laser therapy.

It is pertinent to mention that alternations in implant surface characteristics have been reported with laser energies exceeding 140-180 mJ/pulse; however, Er:YAG laser when used at 100 mJ/pulse and 10 pulses/second for 60 seconds has been shown to be safe for use on implant surfaces. In this context, disinfection of implant surfaces using an Er:YAG laser system seems to be a promising therapeutic protocol for the treatment of peri-implantitis.
SIGNIFICANCE OF CARBON DIOXIDE LASER IN THE TREATMENT OF PERI-IMPLANTITIS

The use of CO₂ (10,600 nm) lasers in implant dentistry is increasing since this CO₂ wavelength reduces the risk of temperature-induced tissue damage as this laser is minimally absorbed in the implant surface and it absorbs in water. Romanos et al. assessed the osteoblast attachment on titanium disks after irradiation with and without CO₂ and Er,Cr:YSGG lasers. The results showed that irradiation of titanium surfaces using CO₂ laser or Er,Cr:YSGG laser does not influence negatively the osteoblast attachment to implant surfaces, thereby augmenting bone formation. This is in agreement with the experimental results by Stübinger et al., where CO₂ laser application (as an adjunct to mechanical debridement) augmented new bone formation in peri-implant defect sites (Figure 5). However, an understanding of the characteristics of the applied laser energy to optimize therapeutic implementation is crucial because heat production as a result of CO₂ laser application may jeopardize osseointegration to an extent. In a clinical study on 32 patients, Deppe et al. assessed the efficacy of soft tissue resection with and without adjunct CO₂ laser therapy in the treatment of peri-implantitis. The 5-year follow-up results showed that treatment of peri-implantitis is significantly accelerated by using a CO₂ laser. However, the authors stated that with respect to long-term results in augmented defects, there seems to be no difference between laser and conventional decontamination.

Figure 5a: Intraoperative condition of peri-implant defects due to peri-implantitis
**Figure 5b:** Decontamination of implant surface with a noncontact CO2 laser (10,600 nm) and 2 Watts power setting (pulsed mode).

**Figure 5c:** Augmentation with particulate bovine bone mineral immediately after implant surface decontamination. The blood clot was stable at the implant surface immediately before grafting.
**Figure 5d:** Coverage with a cell-occlusive collagen membrane and fixation with titanium tags based on the principles of guided bone regeneration.

**Figure 5e:** Preoperative radiograph presenting the peri-implant bony defect before decontamination and bone augmentation. The implant had been 2 years in function under loading conditions.
Figure 5f: Postoperative follow-up 5 months after surgery, presenting the bone fill. No signs of inflammation (such as bleeding on probing, exudation, and pain) were noticed.

**ND:YAG LASER-ASSISTED PERI-IMPLANTITIS PROTOCOL**

There is ongoing research on a relatively new technique termed “laser-assisted peri-implantitis protocol” (LAPIP). LAPIP technique is an implant-specific modification of the laser-assisted new attachment protocol (LANAP). LAPIP and LANAP protocols use a laser ablation step to remove inflamed sulcular tissues (using an Nd:YAG laser) and decontaminate the implant/root surface followed by minimally invasive surgical periodontal therapy. The LAPIP protocol works on the concept of creation of blood clot stabilization that allows the defect area to heal apicocoronally by prevention the down-growth of gingival epithelium. However, to our knowledge from indexed literature, there are no randomized controlled trials, which have assessed the efficacy of LAPIP for the management of peri-implantitis. Therefore, studies are warranted in this regard.

However, previous studies using Nd:YAG laser irradiation in a noncontact mode on implant surfaces demonstrate the dramatic effects this laser wavelength can have on the implant surface due to the high absorption and overheating of the implant body. Therefore, there are concerns of the Nd:YAG laser use *in vivo* due to the possible high risks of complications.
ROLE OF PHOTODYNAMIC THERAPY IN THE TREATMENT OF PERI-IMPLANTITIS

Photodynamic therapy (PDT) is a modern therapeutic strategy, which involves interactions between a light source of a particular wavelength (usually between 630 nm and 700 nm) and a photosensitizer (such as toluidine blue [TBO] or methylene blue [MB]) in the presence of oxygen. This phototoxic and chemical reaction induces the production of reactive oxygen species (ROS) that cause oxidative damage to the target cells including microbial cells and tumor cells. In summary, some advantages of PDT encompass: (a) high target specificity; (b) biocompatibility with healthy human cells; (c) unlikely risk of chemical and/or thermal side-effects; and (d) improbable chances of microbes and fungi to develop resistance against PDT.

In the study by Dörtbutak et al., the pathogens existing in the peri-implant sulci of patients with peri-implantitis were significantly reduced following PDT. Likewise, experimental studies have also reported favorable outcomes of PDT in terms of minimizing bacterial counts. These results suggest that PDT may be considered as a useful treatment strategy in the management of peri-implantitis. Based on the experimental results, it is speculated that PDT when used as an adjunct to mechanical debridement is more effective in the treatment of peri-implantitis as compared to when conventional treatment is performed alone. However, clinical studies have reported contradictory results. For example, Esposito et al. compared the effect of mechanical and surgical debridement techniques with and without PDT in patients with peri-implantitis. In their study, peri-implant inflammatory parameters (probing pocket depth [PPD] and plaque and bleeding scores) were investigated at baseline and 52 weeks after the respective treatments. The results showed a comparable reduction in peri-implant inflammatory parameters when conventional treatments were performed either with or without PDT. Similar results were reported by De Angelis et al. An explanation may be derived from the fact that severity of peri-implantitis most probably varied among all the clinical studies. For example, in the studies by De Angelis et al. and Schär et al., the mean PPD was 4.29 mm and 6.34 mm, respectively. Moreover, in the study by Deppe et al., there was no significant effect of PDT in patients with severe peri-implantitis (PPD 5-8 mm) as compared to those with moderate peri-implantitis (PPD 3-5 mm). Therefore, it is hypothesized that the efficacy of conventional debridement either with or without PDT is governed by the severity of peri-implantitis.

It is, however, pertinent to mention that the frequency and duration of PDT (1-4 times and 10 s – 80 s, respectively) considerably varied among the clinical studies. In addition, a standardized test-group (mechanical debridement with adjunct PDT) and control-group (mechanical debridement alone) was also missing in most of the clinical studies. Therefore, the clinical efficacy of PDT as an adjunct to conventional debridement techniques in the treatment of peri-implantitis remains debatable.

FUTURE PERSPECTIVES

To our knowledge, there are no studies that have assessed the efficacy of peri-implantitis using laser therapy in immunocompromised patients. Since the immunity is compromised in patients with systemic disorders (such as poorly controlled diabetes), it is hypothesized that the therapeutic outcomes of peri-implantitis therapy (either with or without adjunct laser therapy) are compromised in immunosuppressed patients as compared to controls. Further studies are warranted in this regard.
CONCLUSION

Within the limits of the present literature review, it is concluded that laser therapy as an adjunct to conventional mechanical debridement therapy is a modern therapeutic protocol that can effectively be used for the treatment of peri-implantitis, provided that safety and clinical application protocols are cautiously followed. However, there is still a need to reach a consensus regarding the standardization of laser-related parameters that could yield the most favorable outcomes in terms of peri-implant infection therapy.

AUTHOR BIOGRAPHIES

Georgios E. Romanos is a Professor of Periodontology at the Stony Brook University School of Dental Medicine, Department of Periodontology, and a Professor in Oral Surgery and Implantology at the University of Frankfurt, Germany. He is fully trained in Periodontics, Prosthodontics, and Oral Surgery in Germany and has been Board Certified in Oral Surgery and Implant Dentistry in Germany. Dr. Romanos has a Certificate in Periodontology and Advanced Education in General Dentistry (AEGD) from the University of Rochester, New York, and is a Diplomate in the American Board of Periodontology and the International Congress of Oral Implantologists (ICOI). He is a former Professor and Director of Laser Sciences at the New York University College of Dentistry, and a former Professor of Clinical Dentistry at the University of Rochester. He maintains his Dental License in the State of New York and in Europe. Dr. Romanos is a Fellow of the Academy of Osseointegration (AO), International College of Dentists, International Congress of Oral Implantologists, International Team for Implantology Foundation, American Society for Laser Medicine and Surgery, the International Academy for Dental Facial Esthetics, and the Leadership Institute of the American Dental Education Association (ADEA). He is also an Editorial Board Member of the International Journal of Oral and Maxillofacial Implants, Clinical Implant Dentistry and Related Research, Journal of Prosthodontics, Odontology, Photomedicine and Laser Surgery, Quintessence International, Compendium, Journal of Periodontology, International Journal of Dentistry, and others. He has published more than 300 articles, authored 5 books, and conducted over 700 presentations worldwide. Dr. Romanos may be contacted by e-mail at georgios.romanos@stonybrook.edu.

Fawad Javed completed his doctoral education and postdoctoral training from the Department of Dental Medicine, Karolinska Institutet, Stockholm, Sweden. He has published more than 90 articles in ISI-indexed medical and dental journals. Presently, he is a postdoctoral fellow and research associate at the Division of General Dentistry, Eastman Institute for Oral Health, University of Rochester, N.Y., USA. Dr. Javed’s research interests include oral oncology research, connection between oral inflammatory disorders and systemic conditions (particularly diabetes mellitus), impact of smoking and use of smokeless tobacco on oral health, cytokine profile in serum and oral fluids in patients with and without systemic conditions, implant dentistry, bone regeneration research, and saliva research. He is on the editorial board and member of peer-review panels of various indexed medical and dental journals.

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Er:YAG Laser-Assisted Bone and Gingival Augmentation Around Implants

Walid Altayeb, DDS, MDS, PhD
Doha, Qatar

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PRETREATMENT

A. Outline of Case

1. Clinical Description
A 35-year-old female patient presented with minimal attached tissue on the buccal aspect of endosseous implants at the #7 and #8 sites that had been placed more than six months earlier. The patient was undergoing prosthetic treatment and wore a tooth-supported maxillary provisional bridge (Figure 1). The referring implantologist’s report indicated that these implants were inserted at the same time of extraction of teeth #7 and #8 (immediate implantation) (Figure 2). The referring doctor noted inflammation and suppuration at the 3-month follow-up visit. He performed a nonsurgical treatment option for peri-implantitis by using antibiotics and antiseptics. He succeeded in arresting the infection process but was left with a compromised result, caused by the loss of bone and attached gingiva around the neck of the implant.

The prosthodontist was uncertain about the success of the implants and the final esthetic result, so he referred the patient to our clinic to give final recommendations about removing the implants or performing bone augmentation around them.

The options of a treatment plan were discussed with both the prosthodontist and the patient, detailing the nature and potential risks of the proposed procedures. The patient was informed that the final treatment plan would be selected after uncovering the implants and checking their stability, since the implants were still submerged and their stability was uncertain.
2. Medical History
The patient was in excellent medical health with no medical concerns or history. She had no known allergies to any medications and was not taking any medication at the time. She had no history of bleeding or clotting disorders.

3. Dental History
The patient was undergoing comprehensive dental treatment including:
- Extraction of hopeless teeth
- Root canal treatment
- Endosseous implant insertion
- Replacement of old bridges and crowns in the maxilla.

4. Occlusion
The occlusion was not stable since the patient was involved in full-mouth rehabilitation for the maxillary and mandibular teeth.

5. TMJ
Examination of both temporomandibular joints, through palpation, revealed normal movements.

6. Radiographic Examination
The height of the alveolar bone and the outline of the bone crest around the implants were examined radiographically (Figure 3). Most of the mesiodistal peri-implant bone support appeared to be adequate. No periapical pathology was detected on the radiographs.

7. Soft Tissue Examination
Full-mouth periodontal probing was performed; there was no gingivitis or periodontitis. The attached gingiva was very thin on the buccal aspect of implant #7 and #8 (Figure 4). Decreasing inflammation around implants #7 and #8 was completed by the referring dentist.

There were no furcation or mobility involvements. Oral hygiene instructions were reviewed with the patient, emphasizing the importance of effective brushing twice daily and flossing once daily.

8. Hard Tissue Examination
Clinical examination revealed that teeth #2, 5, 6, 7, 8, 10, 14, 18, 19, 29, 30, and 31 were missing. All remaining maxillary teeth were under endodontic treatment with provisional crowns and bridges. Endosseous dental implants were inserted during the last six months at sites #5, 7, 8, 14, 18, 19, 29, 30, and 31.

9. Preoperative Photography
A series of intra- and extraoral photos were taken.

10. Other Tests
No other tests were done.
B. Diagnosis and Treatment Plan

1. Provisional Diagnosis
Surgical complications including bone resorption and insufficient attached tissue in the buccal aspect of endosseous implants #7 and #8.

2. Final Diagnosis
Peri-implantitis around implants #7 and #8 caused by inadequate crestal ridge width at the time of implant placement, resulting in a knife-edge ridge of bone around the implants which was lost during the healing phase of treatment.

3. Treatment Plan
Osseous and gingival regenerative surgery around dental implants #7 and #8 was planned in two-stage surgery:
   a. The first stage included bone regeneration using a bone xenograft and a resorbable membrane. Use of an Er:YAG laser (2,940 nm) was to be applied at different settings to open the flap, remove the granulation tissues, contour the bone, and decontaminate the implant surfaces.
   b. The second stage surgery would be performed four months after the first stage, and included uncovering implants #7 and #8 with an Er:YAG laser, the placement of a connective tissue graft, accompanied by a vestibuloplasty and the deepithelization of the gingival margins of the implants.

4. Treatment Plan Outline
   a. General
   Regenerative osseous and gingival surgery would be employed to promote reossesointegration, increasing the attached gingiva on the buccal aspect of the implants, and a vestibuloplasty would be performed to remove the mobile mucosa and release the frenal attachments extending into the area of the implants.
   b. Specific
   Er:YAG laser-assisted regenerative osseous surgery around implants presents several advantages compared to conventional treatment methods:
      • The laser is capable of effectively removing plaque, biofilm, and granulation tissues on the implant surface without damaging their surfaces.
      • The laser is able to recontour both hard and soft tissues with minimal necrosis of surrounding tissues caused by collateral thermal damage.
      • Vestibuloplasty with the laser achieves a more sustainable result, causes minimal pain, and allows for faster and uneventful wound healing.
      • Deepithelization is easily achieved with the laser without bleeding and postoperative discomfort.

5. Indications and Contraindications
   a. Indications
   Treatment: In cases of bone loss around an implant related to peri-implantitis, biomechanical stresses, and overheating of the bone where the implant is still stable and the bone loss is not too severe, the implant can often be treated and saved.
      • Debridement and regenerative osseous surgery is the treatment of choice, accompanied by attempted mechanical removal of all diseased tissue from around the implant, removal of as many bacteria as possible, administration of antibiotics, and application of bone-grafting material in an attempt to regenerate the peri-implant hard tissues.
• A free connective tissue graft is used to increase the width and thickness of the attached gingiva to improve esthetics, and make the gingival margin bind better around the implants.
• A vestibuloplasty is indicated to ensure the presence of adequate vestibular depth around the implants which is important for oral hygiene and to prevent mechanical tension on the neck of the implant.
• Deepithelization of the gingival margins of the implant with a laser may enhance connective attachment around the implant neck by slowing epithelium growth.

Laser: The pulsed Er:YAG laser can cut and ablate tissues with excellent surgical precision and minimal collateral effects resulting in decreased tissue damage and thus faster healing. Implant surface debridement and decontamination are obtained effectively and safely with the Er:YAG laser compared to plastic curettes. With its wavelength of 2,940 nm and associated maximum water absorption, the Er:YAG laser effectively removes biofilm from the implant surface without damage.

b. Contraindications
Treatment: Absolute contraindication would be present if the patient were suffering from serious illnesses of the hematogenic system. Implant mobility is another contraindication for this treatment.

Laser: Lasers are safe to use if the user adheres to protocols, so there was no known contraindication for the chosen wavelength in this case.

6. Precautions
• The clinician must be careful to avoid possible damage to adjacent root surfaces.
• It is appropriate to use minimal power and proper technique, minimizing the risk of collateral tissue and implant surface damage.
• Laser energy vaporizes biological tissue and amalgam restorations; therefore the clinician must be aware of this potential danger.
• Perpendicular aiming of laser beam is to be avoided to minimize laser reflection from implant surfaces to the operator and adjacent tissues.
• Direct laser irradiation of the implant over an extended period is to be avoided so as not to impair the implant/bone surface through overheating.
• During a vestibuloplasty it is important to make sure that the laser is guided parallel to the bone in order to avoid unwanted side effects such as thermal collateral damage or unintentional bone ablation.

7. Treatment Alternatives
Treatment planning in a complex implant case can be confusing because of the many different surgical and restorative approaches to solve the same problem. The possible alternative treatment for this patient could be:

a. Removal of old implants #7 and #8, with a bone augmentation procedure by the conventional approach of augmenting the ridge first and placing the implant(s) after six months of healing.

b. Removal of old implants #7 and #8, with simultaneous implant placement with a bone augmentation approach in which bone grafting is done at same time as implant placement.

c. Use of plastic therapeutic instruments for implant surface debridement and topical citric acid or tetracycline after debridement as substitute for the laser.
Depending on the situation, the removal of infected implants and a two-stage bone augmentation technique of the alveolar ridge are more advantageous than other approaches in achieving esthetic results with better predictability. This is due to the fact that gingival morphology follows the shape of the underlying bone, and it is difficult to build esthetically acceptable gingiva in areas with vertically deficient supporting bone. For financial reasons and the number of treatments, the patient chose to keep the affected implants after the proposed treatment. The prosthodontist supported the patient preference especially since the patient had a low smile line which hid the vertical tissue loss around the implant and expected asymmetry of gingival contour with adjacent teeth.

8. Informed Consent
Following discussion of the relative risks/benefits and treatment alternatives with the patient and prosthodontist, the Er:YAG laser-assisted regenerative tissue surgery in a two-stage procedure was decided. Written consent was signed by the patient and prosthodontist.

TREATMENT

A. Treatment Objectives
Improve the functional and esthetic longevity of dental implants through:

- Regeneration of subsequent bone in the buccal aspect of dental implants #7 and #8.
- Establishing a healthy gingival contour around implants. This can be done through the widening of attached gingiva to enhance plaque removal around the gingival margin, reduce inflammation, and improve esthetics.

B. Laser Operating Parameters
The instrument of choice was an Er:YAG laser (K.E.Y. Laser, KaVo Dental GmbH, Biberach, Germany) with the following operating features:

- Wavelength: 2940 nm
- Pulse energy: 80 to 600 mJ
- Pulse frequency: 2 to 30 Hz
- Pulse Width: 250 µs
- Average Power: 0.16 to 7.8 W
- Emission mode: Free-running pulse
- Delivery system: Flexible quartz-silica optical fiber with an additional rigid quartz or sapphire tip
- Tip diameter: Disposable 400-micron width, lengths either 6 mm or 18 mm

Specific laser operating parameters for this treatment were:

1. The flap incision: 100 mJ/pulse, 25 Hz, with air, no water. Laser handpiece 2062 with fiber insert size 50/10 (0.47 mm diameter and 10 mm length) in contact mode. Total estimated exposure duration was 2 minutes.

2. Vaporization of granulation tissue and bone recontouring: 120 mJ/pulse, 20 Hz, with maximum water and air. Laser handpiece P2061 with cylindrical fiber (1.1 mm diameter, circular flat exit surface) in near-contact mode, as close as possible to the target without direct contact. Total estimated exposure duration was 2 minutes.
3. Implant debridement and decontamination: 100 mJ/pulse, 10 Hz, with maximum water and air. Laser handpiece 2061 and cylindrical fiber (1.1 mm diameter, circular round exit surface) in noncontact (defocused) mode, at least 5 mm from the target tissue. Total estimated exposure duration was 1 minute.

4. Vestibuloplasty: 300 mJ/pulse, 15 Hz, with air, no water. Laser handpiece 2062 with fiber insert size 50/10 in contact mode. Total estimated exposure duration was 2 minutes.

5. Deepithelization: 140 mJ/pulse, 6 Hz, with air, no water. Laser handpiece 2060 in noncontact mode, at least 5 mm from the target tissue. Total estimated exposure duration was 1 minute.

C. Preliminary to Patient Treatment
Prior to the treatment, the following safety precautions were implemented:

- Infection control guidelines were respected for the environment, patient, and dental staff.
- A safe environment was maintained by restricting operating room access to persons not involved in the treatment, posting warning signs, and minimizing highly reflective surfaces.
- All instruments were pre-dispensed prior to treatment commencing. High-volume evacuation was used.
- The patient and all staff members working in the above-mentioned safety controlled area wore protective glasses specific for the laser.
- The laser was first test-fired outside of the patient’s mouth. The patient was then seated and appropriate safety equipment was utilized.

D. Treatment Delivery Sequence
First Stage Surgery
1. After cleansing the preparation site and disinfection with chlorhexidine, a topical anesthetic (benzocaine) was placed from tooth areas #6 to #9, followed by local anesthesia (articaine HCl 4% and adrenaline 1:200,000, Ubistesin™, 3M™ ESPE™, Seefeld, Germany).

2. The implants were accessed with an appropriate laser incision. The procedure was started with a vertical incision for release with handpiece E 2062 and fiber (50/10). The incision was performed on the mesial aspect of tooth #9, followed by a crestal incision on the alveolar ridge, to the mesial of tooth #4. Down-pressure on the tissue was avoided so as to protect the tissue and prevent the fiber from adhering to it. The energy used for the incision was 100 mJ per pulse at 25 Hz. Average power: 2.5 W, in contact mode without water irrigation. It was noted that at site #8 there was bone loss to the sixth thread on the buccal aspect, and at site #7 the implant body was transparent through thin buccal bone (Figure 5).

Figure 5: View after raising the flap shows bone loss to the sixth thread of implant #8 and very thin buccal bone of implant #7
3. Once the implant and the surrounding bone were exposed, the diseased tissue was vaporized by the laser using the 1.1 mm cylindrical tip, 2.4 W average power with air and water irrigation. The tip of the laser was in contact with the bony crest and maximum water spray cooling was applied to avoid thermal damage. By ablating a thin layer of bone, a new, healthy bone surface was achieved and necrotic bone was removed.

4. After the treated site was cleaned by ablating tissue and blood on the threads, the implant surface was decontaminated using a cylinder tip (1.1 mm) with the tip almost parallel to the implant surface, 1 W average power with air and water irrigation (Figure 6).

5. The bone defects around the implant #7 and #8 were filled with anorganic bovine-derived bone mineral matrix (NuOss™, Collagen Matrix, Inc., Franklin Lakes, N.J., USA) and resorbable collagen membrane (RCM6™, Collagen Matrix, Inc., Franklin Lakes, N.J., USA) which was fixed in place with titanium pins (Figures 7 and 8).

6. Sutures were applied using 4-0 silk, and primary closure was achieved. A provisional fixed bridge was placed during the healing period (Figure 9).
Second Stage Surgery

1. After 4 months of healing time (Figure 10), implants #7 and #8 were uncovered again with the same two first steps mentioned above.

![Figure 10: View after 4 months of bone augmentation surgery and before implant uncovering](image1)

2. The titanium pins were removed. Bone regeneration was noted on both implants and all implant threads were covered (Figure 11).

![Figure 11: View of exposure of implants after 4 months demonstrates complete bone covering of implant threads](image2)

3. The flap was extended palatally to harvest a free connective tissue graft. Healing abutments were inserted for both implants, then the soft tissue graft was sutured around the neck of the implants with a resorbable suture (5-0 PGA coated). Then the wound was closed with silk sutures to allow transgingival healing of the implants (Figures 12 and 13).

![Figure 12: Harvesting of free connective tissue graft](image3)

4. Vestibuloplasty was performed to ensure presence of adequate vestibular depth around the implants. The Er:YAG laser was applied in a contact mode with fiber 50/10 and 4.5 W average power. The vestibular periosteal wound was left to heal by secondary intention (Figure 14).

![Figure 13: Connective tissue graft was secured around the implant neck by resorbable sutures](image4)

![Figure 14: View of vestibuloplasty procedure by 0.5 mm fiber tip](image5)
5. Deepithelization of gingival margins was achieved by a 2060 laser handpiece, 0.84 W average power in defocused mode (10 mm away from gingival tissue) (Figures 15 and 16).

E. Postoperative Instructions
After both surgical phases, verbal and written postoperative instructions were given to the patient. No gingival pack was applied in either stage. The patient was placed on clavulanic acid (Augmentin 1 g) one tab every 12 hours for 7 days; ibuprofen (600 mg) one tab every eight hours for three days, and chlorhexidine rinse twice a day for 10 days. The patient was evaluated postoperatively at 3 and 10 days.

F. Complications
The patient had no complications during either surgical phase.

G. Prognosis
The prognosis was very good during the postoperative period.

H. Treatment Records
All procedural details were entered in the patient’s treatment notes, along with the consent forms, radiographs, and chartings.

FOLLOW-UP CARE

A. Assessment of Treatment
The patient was first assessed at one week, and then at one month after each stage of surgery. This case was followed for 16 months. All through the follow-up care, there was no sign of any complications related to the laser treatments. The recovery was relatively uneventful.

During the first three days following each surgical session, the patient reported moderate pain and moderate swelling. There was no tissue bleeding and the site remained closed, and the flap showed signs of attachment and was healing nicely. At 1 week postoperative, the patient returned for inspection and removal of sutures; the swelling had resolved and the patient no longer had complaints of pain (Figure 17).
One month after second-stage surgery (implant exposure), the soft tissue was completely healed (Figure 18), and there were no reported complications, no recession, no bleeding, and no implant mobility. Six months after the start of treatment, the full-mouth rehabilitation was completed by the prosthodontist with ceramic crowns on the teeth and implants. The radiographs confirmed improved bone levels surrounding the implants with no evidence of bony defects (Figure 19).

![Figure 18: One-month postoperative view demonstrating tissue healing. Note increasing width and thickness of attached gingiva with adequate vestibular depth](image)

![Figure 19: Radiograph taken at 6-month postoperative interval showing improvement in bone level](image)

Healing assessment at the 9- and 16-month appointments showed that the patient had excellent healing and improved tissue color, contour, and consistency. Gingival tissue was well attached and gingival margins were stable (Figures 20 and 21).

![Figure 20: View after 9 months of uneventful healing. Note the healthy gingival contour around implants](image)

![Figure 21: Postoperative photograph at 16 months shows excellent healing around implants #7 and #8. Note the improved vestibule depth and stability of attached gingiva](image)

B. Complications

There were no significant postoperative complications. As expected, severe ridge deficiency resulted in a long implant-supported fixed restoration. The prosthodontist used pink porcelain to mask the increased length of the crowns. As anticipated, the patient’s low lip smile line hid this compromised esthetic result.
C. Long-Term Results
The long-term results were felt to be good at the 16-month recall visit, the soft tissue remained healthy, and no gingival or bone recession was observed. The tissue healing remained relatively the same through the postoperative period. The final reconstruction demonstrated a functional and esthetic outcome. The results were satisfactory to the patient and the clinician.

D. Long-Term Prognosis
The use of an Er:YAG laser in regenerative osseous surgery around implants presents several advantages over conventional treatment, with no complications and with high patient and clinician satisfaction and confidence.

AUTHOR BIOGRAPHY

Walid Altayeb received his dental degree from the Faculty of Dentistry, Damascus University in 1998, and completed his Master of Science in Periodontics in 2004 and Doctorate of Philosophy in Periodontics in 2007. He is a Fellow and Master of the Academy of Laser Dentistry. He has served as lecturer in the Department of Periodontics, Damascus University. He has participated in many conferences in the Middle East, Spain, and USA as speaker in the fields of periodontal medicine and laser dentistry. Dr. Altayeb has achieved an advanced level of knowledge about the application of lasers in dental science and patient treatment (Advanced Proficiency certificates from the Academy of Laser Dentistry in 980-nm diode and 2940-nm Er:YAG lasers). He is currently Chair of the ALD affiliate study club in Qatar and is working in private as a periodontist and implantologist in Madina Dental Center, Doha. Dr. Altayeb may be contacted by e-mail at dreltayeb@hotmail.com.

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Antimicrobial Effects of the 810-nm Diode Laser in the Treatment of Peri-Implantitis: An Ex Vivo Pilot Study

Erica Lavere, BS, DDS
University at Buffalo School of Dental Medicine, Buffalo, New York, USA

Juliana Sagor, BA, DDS
Dental Resident, St. Barnabas Hospital, New York, New York, USA

Lynn Mikulski
Research Support Specialist, University at Buffalo School of Dental Medicine, Buffalo, New York, USA

Sebastiano Andreana, DDS, MS
Associate Professor and Director of Implant Dentistry, University at Buffalo School of Dental Medicine, Department of Restorative Dentistry, Buffalo, New York, USA

ABSTRACT

Background: Peri-implantitis is a condition associated with the presence of bacteria along the surfaces of implants, creating deleterious effects to the peri-implant hard and soft tissues.

Objective: This ex vivo pilot study compared three settings on an 810-nm diode laser to determine optimal laser power levels to suppress bacteria growing in a bony defect adjacent to an implant.

Materials and Methods: The study was conducted in two phases. Fourteen sterile titanium implants were placed into sterilized porcine ribs. A 2 mm x 2 mm wide, 3 mm deep defect adjacent to the implant was created. Three microliters of S. sanguinis ATCC® 10556™ in ½ brain heart infusion were inoculated into the defect and left for 24 hours in 5% CO₂ at 37°C. For Study 1, four defects were not treated with the laser, and 3 were treated with an 810-nm diode laser at 0.6 Watt, 4 at 0.8 W, and 3 at 1.0 W. The laser tip was noninitiated and laser energy was delivered in continuous mode. Defects were rinsed with ½ brain heart infusion transport media and bacteria were plated on tryptic soy agar (TSA) media and left for 48 hours to grow. The colony-forming units (CFUs) were counted. The experiment was repeated three times. Following results from Study 1, a second study was performed. Study 2 involved the use of 3 microliters of indocyanine green as chromophore, delivered into the peri-implant bony defect. The same power settings and techniques were followed as in Study 1.
Results: The amount of post-treatment growth varied within the 0.6 W and 0.8 W groups, with an average count of CFUs of 56 and 30 respectively, whereas the 1.0 W group showed a barely detectable growth, 0.33 CFUs. The untreated defects (control group) showed an average CFU count of 207 ($P < 0.5$), with the differences in the 1.0 W group being statistically significant. Study 2 revealed an additional antimicrobial effect when indocyanine green was added in the 0.8 W and 1.0 W, but not in the 0.6 W groups.

Conclusions: The 810-nm diode laser at 1.0 W was successful in minimizing bacteria growth in ex vivo peri-implantitis defects, whereas even lower laser power levels were able to remarkably diminish the amount of bacteria. One Watt diode laser treatment could be an effective setting to use when treating peri-implantitis cases. The addition of a chromophore increased the antimicrobial activity at 1.0 W, indicating a possible clinical advantage of the photodynamic-enhanced antimicrobial therapy.

Key words: Dental lasers, Peri-implantitis, Indocyanine green, Antimicrobial effect

INTRODUCTION

Peri-implantitis is an emerging and growing problem in the dental field due to the increased placement of implants to restore edentulous spaces. This condition is associated with the presence of bacteria along the surfaces of implants creating deleterious effects to the peri-implant hard and soft tissue. Peri-implantitis is detrimental to the health of the tissue around the implant and to the function of the implant due to the way that peri-implantitis causes a loss of supporting bone. A recent review demonstrated that the prevalence of peri-implantitis is 21.7%. This statistic was generated through a meta-analysis of numerous different case studies. It is difficult to generate a true statistic because of the way in which different studies use different thresholds of disease. But from this percentage it may be concluded that the occurrence is high and it is crucial to be able to predictably eliminate disease around implants and to decrease failure rates. There are many different ways to treat this condition but there is no consensus on which treatment is most effective. Mechanical debridement with chlorhexidine and the use of amino acid glycine powder are among the alternative treatments. Other topical treatments have been reported such as the use of tetracycline slurry, and minocycline in a microsphere slow-release system.

The diode laser has been used in various in vitro studies to decontaminate titanium surfaces. In most investigations of the decontamination of titanium surfaces a flat titanium disc has been used. Titanium discs do not represent the true clinical surface properties of implants, therefore using actual dental implants is important in evaluating the effectiveness of laser treatment in decontaminating peri-implant defects and implant surfaces. The main difference between the titanium disc and the implant is that the implant has a curvy surface. The diode laser is one of the most utilized lasers by dental professionals. A study that surveyed five different lasers commonly used in dentistry demonstrated that 63% of dentists who use a laser use a diode laser. The aim of this study was to determine the lowest laser wattage that could be used with the 810-nm diode laser that would still be effective in eliminating bacteria from a peri-implant defect and implant surface.
MATERIALS AND METHODS

Fresh porcine ribs were obtained from a local butcher. All skin and meat were removed from the ribs until just bone remained. The ribs were then cut into 14 bone blocks. Next, the bone blocks were sterilized in a steam autoclave. Under a clean surgical environment, incorporating sterile drapes, gloves, and instrumentation, a sterile number two round bur was then used to create a pilot hole prior to placing implants. The implant space was then prepared using an implant kit (Nobel Biocare, Zurich, Switzerland). Fourteen sterile titanium implants were placed into the bone blocks (Bränemark MKIII, Nobelpharma, Yorba Linda, Calif., USA). Each implant was treated with glow discharge for 60 seconds before placement to maintain surface properties. Previous studies including the ones from Youngblood and Ong\(^8\) and Baier et al.\(^9\) have clearly demonstrated that glow-discharge surface treatment could be used as alternative treatment to sterilize the implant surface, without negatively affecting the biological properties of the titanium surfaces. A 2 mm x 2 mm wide, 3 mm deep circumferential defect was created adjacent to the implant. Three microliters of \(S.\ sanguinis\) ATCC 10556 in ½ brain heart infusion (BHI) were inoculated into the defects. The bone blocks were then placed into separate sterile containers and left for 24 hours in an atmosphere of 5% \(\text{CO}_2\) at 37° C.

After 24 hours the containers were opened and the bone blocks were exposed for decontamination with the laser. Three laser power levels were used in this study. Three bony defects were treated with the laser (Odyssey® 2.4G, Ivoclar Vivadent, Amherst, N.Y., USA) at 0.6 W, four at 0.8 W, and three at 1.0 W. The laser was not initiated and was used in continuous mode for 30 seconds in each defect. Four defects were not treated with the laser to serve as a control group. Defects were rinsed with ½ brain heart infusion (BHI) transport media; 1:1000 dilutions were made of the transport media and bacteria were plated on tryptic soy agar (TSA) plates and left for 48 hours to grow. The colony-forming units (CFUs) were counted. The experiment was repeated three times. The study was then repeated with one added parameter. Indocyanine green (IG), which is a medical diagnostic dye, was used as a chromophore. Three microliters of this dye was pipetted into each defect before laser treatment. The groups in this trial were the control groups, which consisted of no laser treatment and no IG, and no laser treatment with IG. The 0.6, 0.8, and 1.0 W test groups were divided into laser treatment only and laser with IG. Refer to Figure 1 for the model.

Figure 1: Experimental model showing implant inserted into bone block with laser fiber inserted into the defect adjacent to the implant.
RESULTS

The initial experiments showed that bacteria growth on the agar plates decreased from the control group (no laser treatment) to the group that was treated with 1.0 W. After this experiment was repeated three times for the control group, the average number of colony-forming units was 590.5. The average number for the group that was treated with 0.6 W was 56 CFUs, and the group treated with 0.8 W averaged 30 CFUs. The 1.0 W group showed minimal to no detectable growth with an average of 0.33 CFUs (Figure 2). The control group’s average CFU count of 590.5 was statistically significantly different from the group treated with 1.0 W ($P < 0.5$).

Figure 2: Colony-forming unit averages for specific laser power levels in first phase of the study

Results from the trials using IG as a chromophore were similar to the initial study, however the 0.6 W group did not follow the same trend of the 0.8 W and 1.0 W groups. The control group that received no laser treatment and had no green dye present had an average CFU count of 192 (SD 35.0). The control group that contained indocyanine green but no laser treatment had a count of 129.25 CFUs (SD 5). The 0.6 W laser-treated group without indocyanine green averaged 39.5 CFUs (SD 4.3), and the 0.6 W group with IG averaged 103.5 (SD 10.6). The 0.8 W laser treated group without indocyanine green averaged 58.5 CFUs (SD 7.7) and the group with IG averaged 0 CFUs. The 1.0 W laser treated group without IG averaged 18.5 CFUs (SD 24.1) and the group with IG averaged 0 CFUs (Figure 3).
Figure 3: Colony-forming unit averages for specific laser power levels for defects with and without indocyanine green in second phase of the study

**DISCUSSION**

This study reports the effect of suppressing *S. sanguinis* growth on implant surfaces. The results indicate that the 810-nm diode laser at the parameters used in the present study was successful in suppressing bacterial growth in *ex vivo* peri-implant defects.

Several comments, however, have to be reported. The use of this model is of foremost relevance. In *vitro* laboratory studies have been conducted primarily on titanium discs. This allows for the direct access to the implant surface, not representing the true clinical scenario for the dentist. Our study mimics closely the real clinical setting, with real bone facing the contaminated implant surface. It is worthwhile to mention some of the differences with the real-life situation. In our model, there is a lack of body fluids, such as saliva and blood. In addition, the second study involved the use of a specific chromophore. In real life, the blood due to the peri-implant inflammation serves as a chromophore for the diode laser.

In our study we used indocyanine green as a chromophore. This particular dye, while safe, was also tested by Boehm and Ciancio in combination with the 810-nm diode laser on periodontal pathogens. The results of their *in vitro* study indicated that this combination has a successful bactericidal effect, at very minimal wattages ranging between 0.1 and 0.5 W in continuous mode. However their study aimed at investigating the killing effect in bacterial pellets. Our study adopted similar techniques, but the killing effect was evaluated on implant surfaces inserted into real bone. From the results of the present study, when the chromophore was used with 0.8 W, the level of bacteria suppression was similar to the results obtained when the chromophore was not used in the 1.0 W group. It is important to report, however, that the IG had some antimicrobial effect on its own as shown in the nonlaser treated group. This aspect leads to two points of discussion: (1) IG may have some antimicrobial effect, and (2) IG is safe on mammalian cells. These points possibly strongly suggest its clinical use, being safe on the human body and having a killing effect on bacteria.
Our model used *S. sanguinis* as the tested microorganism. *S. sanguinis* is one of the pioneer bacteria colonizing oral surfaces.\textsuperscript{11} Pioneers are the first colonizers on surfaces. With development of the early biofilm, additional bacterial species may colonize, including the pathogenic species. Future studies involving use of a mixed biofilm may provide additional and more clinically relevant data.

It has been reported that the diode laser may overheat the implant surfaces, therefore transmitting high temperatures to the peri-implant bone. An ex vivo study conducted by and Beneduce et al.\textsuperscript{12} has indicated that the temperature rise from 36.5°C is negligible and still within physiological parameters. The above-mentioned study was performed having the specimens at 36.5°C in a water bath, to mimic as much as possible the clinical conditions.

**CONCLUSIONS**

Overall, this ex vivo study has indicated an antimicrobial effect of the 810-nm laser on dental implants laced in real bone. Furthermore, it may be concluded that indocyanine green in combination with low-wattage 810-nm diode laser irradiation could be used to decontaminate implant surfaces colonized by *S. sanguinis*. Further studies are needed to confirm preliminary reports presented by Kutkut et al. on humans.\textsuperscript{13} In their case report, the investigators used an 810-nm diode laser to further decontaminate infected dental surfaces in conjunction with topical antibiotic therapy, prior to successful bone regeneration treatment. The laser was used at 1 W in continuous mode, without the use of IG.

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**CORRESPONDING AUTHOR BIOGRAPHY**

Dr. Lavere is a pediatric dentistry resident at the University at Buffalo School of Dental Medicine and Women and Children’s Hospital of Buffalo, New York. She may be contacted by e-mail at ericalavere@gmail.com.

*Disclosure:* Dr. Lavere has reported no commercial affiliations or personal conflicts of interest relative to this article.

*Editor’s Note:* This research was performed while Ms. Lavere was a 4th-year dental student at the University of Buffalo School of Dental Medicine, Buffalo, New York, USA.
REFERENCES

Letters to the Editor

The *Journal of Laser Dentistry* welcomes letters from readers on topics of current interest to Academy of Laser Dentistry members. The *Journal* reserves the right to edit all correspondence and requires that all letters be signed. The views expressed are those of the letter writer and do not necessarily represent the opinion or official policy of the Academy or the editors of the *Journal*.

MORE ON LASER-ASSISTED ORTHODONTIC TOOTH MOVEMENT


Dear Editor,

A desire to increase the velocity of tooth movement in orthodontics has produced several suggestions for therapeutic approaches, low-level laser (LLL) being one of them. The literature is still unambiguous.¹

LLL can produce two biological effects: stimulation and inhibition, depending on the applied dose and energy. As for inhibition, several studies have confirmed that the application of LLL can reduce postoperative pain. The mechanism is still not quite clear, but the investigation by Chow² suggests the formation of transient axonal varicosities, lowering the rate of neural flow. Reduction of pain requires fairly high energies. On the other hand, lower energies and doses are required for stimulation. The possible influence on tooth velocity should presumably be based upon stimulation, not upon inhibition. This leads to the assumption that there are two separate dose windows for laser orthodontics – low energies for increase of the velocity of tooth movement and high energies for pain reduction. If there is an overlapping area, the two effects would both still be in a suboptimal part of both windows. But indeed, Youseff³ reports considerable pain reduction and increased tooth velocity using 8 J per tooth.

In the present study, the dose is claimed to be 16 J/cm², based upon 200 mW and 80 seconds of irradiation. This is actually 16 joules (J) and not 16 joules per cm².

16 J over one tooth is, according to the available literature, rather in the pain-relieving window than in the stimulatory window. However, the effect on pain reduction was not studied in the present paper.

The dose (J/cm²) is calculated as the applied energy (J) divided by the irradiated area. Applying 1 J over an area of 1 cm² results in a dose (fluence) of 1 J/cm². If the irradiated area is 0.5 cm², the same applied energy results in a dose of 2 J/cm². In the present study, the size of the irradiated area was 0.4 mm (400 microns) and the dose becomes extremely high in each spot. This further contributes to the conclusion that the laser parameters were outside of the stimulatory window. The misunderstanding of what is energy and dose is too common, and the 8 J applied in the study by Youseff is also called “dose.”
A complicating factor in the evaluation of optimal parameters is that stimulation is best achieved with low output and long time.\textsuperscript{4}

The use of traditional Class IV dental lasers for biostimulation has pros and cons. The advantage of course is that with a reduced handpiece, the dentist can perform biostimulation without investing much money. The downside is that the control over the parameters is difficult, if at all possible. Using the thin fiber in a scanning mode from a slight distance is a possibility, but the control is still uncertain. It is recommended that a biostimulation handpiece should preferably have a “laser eye” of 0.25 or 0.5 cm\textsuperscript{2}. That would make the calculation easy: 1 joule with the 0.25 cm\textsuperscript{2} laser eye produces a dose of 4 J/cm\textsuperscript{2}.

Sincerely,

Jan Tunér, DDS
Private Dental Clinic, Grängesberg, Sweden
Editor of Laser Annals

REFERENCES


Dr. Tunér may be contacted by e-mail at \texttt{Jan.tuner@swipnet.se}.

Disclosure: The author has no commercial bond to any laser manufacturer.

THE AUTHOR RESPONDS

Dear Editor,

The comments by Dr. Tunér are appreciated. However, the statement that the laser parameters used were outside of the stimulatory window based on previous studies cannot be made, as all previous studies employed a continuous laser setting.
If the laser light had been delivered in a continuous mode, the dose used in this study would likely have been too high for stimulation of tooth movement. However, as described in the paper, it is very difficult to quantify the level of energy emitted from lasers operating in pulsed modes. There are two distinct power measurements for a pulsed laser: peak power and average power. Peak power is a measure of the rate at which energy is emitted during the pulse, whereas average power measures the average rate at which energy flows from the laser during an entire cycle. As this was the first study to determine the effect of low-level laser therapy (LLLT) on tooth movement using a pulsed mode, an estimate of dose prescription was necessary.

In another arm of this study (not reported in the paper), the same laser parameters were used to examine the effect of the pulsed laser on pain during canine retraction. It was concluded that pain in orthodontic patients can be significantly reduced with the administration of pulsed LLLT.

It would be most advantageous to the clinician if the same laser could be used for both biostimulation of tooth movement and pain reduction. Controlling the size of the irradiated area by varying the handpiece would undoubtedly increase the adoptability of this technology by orthodontists.

Sincerely,

Monica Gawlik, DDS, MSc, FRCD(C)
Private Orthodontic Practice, Toronto, Ontario, Canada
Editor’s Note: The following 4 abstracts are offered as topics of current interest. Readers are invited to submit to the editor inquiries concerning laser-related scientific topics for possible inclusion in future issues. We’ll scan the literature and present relevant abstracts.

**Antimicrobial Effects of Photosensitizers**

In their report on “Antimicrobial Effects of the 810-nm Diode Laser in the Treatment of Peri-Implantitis: An Ex Vivo Pilot Study” (pages 37-43), Dr. Erica Lavere and colleagues observe that the tested chromophore indocyanine green enhanced the antimicrobial activity of laser irradiation. They also found that the chromophore exhibited some antimicrobial effect of its own, without the application of laser light.

How effective are such chromophores – by themselves – in promoting bacterial reduction? How do they work? And what are some of the factors that influence their effectiveness?

Indocyanine green is most commonly used as an intravenous diagnostic dye to determine blood flow in organs such as the eyes, kidneys and lungs; to measure cardiac output; and in liver function tests. 1

Two other chromophores, methylene blue and the related chemical compound toluidine blue, are customarily used as biologic stains. Methylene blue is also used to treat cyanide poisoning and other conditions; as a surgical marker; and as a potent, reversible monoamine oxidase inhibitor. 1-2 When used pharmaceutically, toluidine blue has been utilized to treat menstrual disorders and to detect oral and gastric carcinomas. 1

All three substances have also been tested experimentally as photosensitizers to determine their relative bactericidal effects in a technique called antimicrobial photodynamic therapy, alternately termed photoactivated disinfection, photodynamic inactivation, and lethal photosensitization, among other names.

A photosensitizer is an agent that increases a material’s sensitivity to electromagnetic radiation. 3 In medicine, the term photodynamic therapy (PDT) generally refers to the process whereby a photosensitizing agent is administered intravenously and selectively concentrates in metabolically active tumor tissue. Following exposure to specific wavelengths of light, cytotoxic free radicals are produced that selectively destroy the photosensitized tissue. 1

The principal is similar in experimental dental applications of antimicrobial photodynamic therapy. In such uses, the generation of highly reactive singlet oxygen during excitation of a photosensitizer is considered the main antimicrobial agent. The singlet oxygen oxidizes cellular targets such as membrane, enzymes, and lipids that lead to destruction of bacteria. 4

Wilson, Gibson, and colleagues 5-6 posited that raising the redox potential of the periodontal pocket should create an environment unsuitable for the growth of anaerobic periodontal pathogens. They applied the redox dye methylene blue to test sites in human patients; no light activation was used. They then collected samples from the periodontal pockets via sterile endodontic paper points for bacteriological analysis. The investigators noted reductions in proportions of black-pigmented anaerobes, spirochetes, and motile bacteria.
Table 1 summarizes the findings of selected studies that have examined the antimicrobial effects of a number of photosensitizers on a variety of microorganisms relevant to dentistry. Featured are indocyanine green, methylene blue, and toluidine blue.

Generally, while not shown in the table, antimicrobial properties of photosensitizers are most pronounced when the agent has been “photoexcited” by subablative levels of irradiation from various laser devices, light-emitting diodes (LEDs), or curing lights. Pertinent to the focus of this survey, the last column of the table highlights the effects of the agent itself, when used without the accompanying irradiation, on specified microbes. Results vary from mild or significant reductions in bacteria to no significant effect.

<table>
<thead>
<tr>
<th>Study Design</th>
<th>Investigators</th>
<th>Microbe(s)</th>
<th>Photosensitizer(s)</th>
<th>Light Source(s) for Photoactivation</th>
<th>Effect of Photosensitizer(s) Alone</th>
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</thead>
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<tr>
<td>In vitro suspensions</td>
<td>Omar et al., 2008</td>
<td>Staphylococcus aureus (S.a.), Streptococcus pyogenes (S.p.)</td>
<td>Indocyanine Green</td>
<td>808 nm GaAlAs laser</td>
<td>Small reduction in S.a. viable count; No significant reduction in viability of S.p.</td>
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<td>In vitro suspension</td>
<td>Nagahara et al., 2013</td>
<td>Porphyromonas gingivalis (P.g.)</td>
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<td>805 nm diode laser</td>
<td>No effect on P.g. viability</td>
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<td>S.a., Pseudomonas aeruginosa (P.a.)</td>
<td>Indocyanine Green</td>
<td>809 nm diode laser</td>
<td>No lethal effect on any strain</td>
</tr>
<tr>
<td>In vitro suspensions</td>
<td>Kranz et al., 2015</td>
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<td>Indocyanine Green in presence of Trolox™, a vitamin E analogue</td>
<td>808 nm GaAs diode laser</td>
<td>No remarkable antibacterial effect</td>
</tr>
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<td>In vitro suspension</td>
<td>Fekrazad et al., 2015</td>
<td>Candida albicans (C.a.)</td>
<td>Methylene Blue (MB), Indocyanine Green (IG)</td>
<td>630 nm InGaAlP laser for MB; 810 nm diode laser for IG</td>
<td>Reduced colony counts</td>
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<td>In vitro suspension</td>
<td>Fekrazad et al., 2013</td>
<td>Streptococcus mutans (S.m.)</td>
<td>Toluidine Blue, Indocyanine Green</td>
<td>620-640 nm (peak 630 nm) LED, 662 nm diode laser, 810 nm diode laser</td>
<td>No significant reduction of colonies</td>
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<td>Study Design</td>
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<td>Light Source(s) for Photoactivation</td>
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<td>In vitro biofilm-coated discs</td>
<td>13 Müller et al., 2007</td>
<td>Biofilm: <em>Actinomyces naeslundii, Veillonella dispar, F.n, Streptococcus sobrinus, Streptococcus oralis, C.a.</em></td>
<td>Methylene Blue</td>
<td>665 nm laser</td>
<td>Reduced microbiota of the biofilm by less than 1 order of magnitude, not significantly different from PDT</td>
</tr>
<tr>
<td>In vitro suspension</td>
<td>14 George et al., 2008</td>
<td><em>Enterococcus faecalis (E.f.)</em></td>
<td>Methylene Blue</td>
<td>664 nm diode laser</td>
<td>Viability of bacteria was not significantly affected by MB in deionized water without irradiation. However, viability of E.f. cells was considerably lowered when cells were subjected to MB dissolved in a mixture of glycerol-ethanol-water without irradiation.</td>
</tr>
<tr>
<td>In vitro suspension</td>
<td>15 Miyabe et al., 2011</td>
<td><em>Staphylococcus spp</em></td>
<td>Methylene Blue</td>
<td>660 nm GaAlAs laser</td>
<td>Small reduction in bacterial counts</td>
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<td>In vitro biofilm in acrylic discs</td>
<td>16 Pereira et al., 2011</td>
<td><em>C.a., S.a., S.m.</em></td>
<td>Methylene Blue</td>
<td>660 nm InGaAlP laser</td>
<td>No cytotoxic effect for the microorganisms tested</td>
</tr>
<tr>
<td>In vitro biofilm on acrylic resin</td>
<td>17 De Freitas-Pontes et al., 2014</td>
<td><em>S.m., S.a., Escherichia coli (E.c.), P.a., C.a.</em></td>
<td>Methylene Blue</td>
<td>630 nm LED</td>
<td>Decreased S.m. scores; No effect on S.a; No effect on E.c.; Decreased P.a. scores; Decreased C.a. CFU count</td>
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<td>In vitro biofilms</td>
<td>18 Vilela et al., 2012</td>
<td><em>S.a., E.c.</em></td>
<td>Methylene Blue, Toluidine Blue, Malachite Green</td>
<td>660 nm InGaAlP diode laser</td>
<td>No toxic effects on S.a. and E.c.</td>
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<tr>
<td>Study Design</td>
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<td>Photosensitizer(s)</td>
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<td><em>In vitro</em> suspension</td>
<td>Rolim et al., 2012</td>
<td><em>S.m.</em></td>
<td>Methylene Blue (MB), Toluidine Blue Ortho (TB), Malachite Green (MG); Eosin (EOS), Erythrosine (ERI), Rose Bengal (RB)</td>
<td>636 nm LED for MB, TB, MG; 570 nm curing light for EOS, ERI, RB</td>
<td>For MB, TB, EOS, and MG, no significant effect on the viability of <em>S.m.</em>&lt;br&gt;For RB and ERI, complete bacterial kill and a decrease in cell viability, respectively, were observed, possibly due to high concentration of these photosensitizers</td>
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<tr>
<td><em>In vitro</em> implant surfaces</td>
<td>Haas et al., 1997</td>
<td><em>Actinobacillus</em> actinomycetemcomitans (A.a.), <em>P.g.</em>, <em>Prevotella intermedia</em> (P.i.)</td>
<td>Toluidine Blue</td>
<td>905 nm diode laser</td>
<td>Bacterial lawns were present in all cultures</td>
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<td>15 humans, implants, <em>in vivo</em></td>
<td>Dörtbudak et al., 2001</td>
<td><em>P.g.</em>, <em>P.i.</em>, <em>A.a.</em></td>
<td>Toluidine Blue</td>
<td>690 nm diode laser</td>
<td>Lowered mean values of bacterial counts</td>
</tr>
<tr>
<td><em>In vitro</em> suspension</td>
<td>Matevski et al., 2003</td>
<td><em>P.g.</em>, <em>A.a.</em>, <em>Bacteroides</em> <em>forsythus</em>, <em>F.n.</em>, <em>P.i.</em></td>
<td>Toluidine Blue</td>
<td>Red-filtered xenon lamp, 635 nm diode laser</td>
<td>No statistically significant decline in bacterial survival</td>
</tr>
<tr>
<td><em>In vitro</em> suspensions</td>
<td>Schlafer et al., 2010</td>
<td><em>E.c.</em>, <em>C.a.</em>, <em>E.f.</em>, <em>F.n.</em></td>
<td>Toluidine Blue</td>
<td>628 nm LED</td>
<td>Mild reductions in the viable counts of all 5 organisms, none of which was statistically significant</td>
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<td><em>In vitro</em> suspensions</td>
<td>Mattiello et al., 2011</td>
<td><em>Ag.a.</em>, <em>Streptococcus</em> <em>sanguinis</em> (S.s.)</td>
<td>Toluidine Blue</td>
<td>660 nm AlGaNnP diode laser</td>
<td>No statistically significant bacteria reduction</td>
</tr>
<tr>
<td><em>In vitro</em> tooth specimens</td>
<td>Poggio et al., 2011</td>
<td><em>E.f.</em>, <em>S.m.</em>, <em>S.s.</em></td>
<td>Toluidine Blue</td>
<td>628 nm LED</td>
<td>Showed the lowest bacterial reduction percentages for all tested strains</td>
</tr>
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<td>Study Design</td>
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<td>33 patients with chronic periodontitis</td>
<td>Theodoro et al., 2012</td>
<td>A.a., P.g., P.i., Tannerella forsythia, Prevotella nigrescens (P.n.)</td>
<td>Toluidine Blue (TB)</td>
<td>660 nm GaAlAs laser</td>
<td>At 180 days there were greater reductions in positivity for P.i. and P.n. in the TB group than in the scaling and root planing (SRP) group. At all other time intervals and in all other species, TB showed fewer reductions than other groups</td>
</tr>
<tr>
<td>Biofilms: In vitro hydroxyapatite discs and in situ enamel slab specimens in 21 humans</td>
<td>Teixeira et al., 2012</td>
<td>S.m.</td>
<td>Toluidine Blue</td>
<td>620-660 nm (predominant 638.8 nm) red LED</td>
<td>No significant effect on the viability of S.m.</td>
</tr>
<tr>
<td>In vitro titanium discs and artificial periodontal pockets</td>
<td>Eick et al., 2013</td>
<td>16 species including P.g., A.g.</td>
<td>Toluidine Blue</td>
<td>625-635 nm LED</td>
<td>Reduced CFU counts</td>
</tr>
<tr>
<td>In vitro suspension</td>
<td>Hakimiha et al., 2014</td>
<td>S.m.</td>
<td>Toluidine Blue (TB), Radachlorin</td>
<td>630 nm LED for TB 662 nm laser for Radachlorin</td>
<td>No significant reductions of S.m. colonies were observed</td>
</tr>
<tr>
<td>In vitro suspension and in vivo on tibial bone defects in rats</td>
<td>Dos Reis Junior et al., 2014</td>
<td>S.a.</td>
<td>Toluidine Blue</td>
<td>660 nm diode laser</td>
<td>Significant reduction of bacterial counts</td>
</tr>
<tr>
<td>In vitro suspension</td>
<td>Paschoal et al., 2014</td>
<td>S.m.</td>
<td>Toluidine Blue, Curcumin</td>
<td>400 to 799 nm noncoherent light source</td>
<td>Did not reduce the log10 CFU when compared with control groups</td>
</tr>
</tbody>
</table>
What might account for the variance in findings? Dörtbudak et al.\textsuperscript{21} suggested that a variable bonding behavior of the photosensitizer to different bacterial membranes may occur. The Rolim group\textsuperscript{19} mentioned differing partition coefficients among photosensitizers that affect their ability to permeate and accumulate in the hydrophobic region of the cellular membrane, thus influencing their bactericidal activities. They also stated that the photodynamic efficacy of each photosensitizer varies according to the characteristics of different target microorganisms.

Theodoro and colleagues\textsuperscript{26} identified a number of conditions that may affect biological response to photodynamic therapy: drug ion concentration (a consideration also discussed by the Rolim cohort\textsuperscript{19}), period of retention of the drug within the tissue, time for biological response, pH of the environment, presence of exudates and gingival fluid, and mode of drug application (irrigation, slow release gel).

George et al.\textsuperscript{4} considered the uptake pathways of photosensitizers into bacteria. They suggested that the uptake of anionic photosensitizers (such as indocyanine green) by bacterial cells may be mediated through a combination of electrostatic charge interaction and by protein transporters, while the uptake of cationic photosensitizers (such as methylene blue) may be mediated by electrostatic interactions and by so-called self-promoted uptake pathways which involve the binding of cationic molecules to lipopolysaccharide that results in the progressive weakening of the cell’s outer membrane.

Of course, these and other factors come into play when the photosensitizer is actually “photoexcited” by a light source. Irradiation wavelength, duration of exposure, fluence, and absorption characteristics of the target area are among the additional considerations that must be optimized to achieve the most beneficial bactericidal effect.

To these factors must be added yet another consideration, applicable to those instances in which certain organisms display elevated resistance to photodynamic treatment. As Kossakowska et al.\textsuperscript{32} note below, the use of multicomponent sensitizing agents (such as the combination of protoporphyrin diarginate, toluidine blue, and 5-aminolevulinic acid) could eradicate virulent strains of the target species.

Continuing research is required to clearly define the mechanisms, means, and protocols required to achieve desired outcomes of photosensitizers and antimicrobial photodynamic therapy.

REFERENCES


Antibacterial Photodynamic Treatment of Periodontopathogenic Bacteria with Indocyanine Green and Near-Infrared Laser Light Enhanced by Trolox™

Stefan Kranz,1 Marie Huebsch,2 Andre Guellmar,1 Andrea Voelpel,1 Silke Tonndorf-Martini,1 Bernd W. Sigusch1

1 Polyclinic for Conservative Dentistry and Periodontology, University Hospital Jena, Jena, Germany

2 Polyclinic for Prosthetic Dentistry and Material Science, University Hospital Jena, Jena, Germany


Background and Objectives: It has been shown that certain vitamins can significantly enhance the effect of photodynamic anti-tumor therapy. Unfortunately, there is no sufficient information available about the impact of those antioxidants on antimicrobial Photodynamic Therapy (aPDT). The present study is aimed at investigating the antimicrobial effect of the dye indocyanine green (ICG) in the presence of Trolox™, a vitamin E analogue, upon irradiation with near-infrared (NIR) laser light (808 nm) on the gram-negative periodontopathogenic bacteria Aggregatibacter actinomycetemcomitans (A.a.), Porphyromonas gingivalis (P.g.) and Fusobacterium nucleatum (F.n.).

Methods: Bacteria solved in PBS were incubated with ICG (50-500 mg/ml) in the presence and absence of Trolox™ (2 mM). Irradiation was performed after 10 minutes of dark-incubation with NIR-laser-light (25-100 J/cm², 810 nm). During treatment, temperature was also recorded inside the bacterial solutions. The treated suspensions were serial diluted and plated onto blood agar plates. After anaerobe cultivation for 5 days the colony-forming units (CFU/ml) were determined.

Results: The antibacterial effect was ICG-concentration and exposure dependent. It was found that high ICG-concentrations and light fluence rates caused bacterial reduction due to hyperthermia. Where low ICG-concentrations (< 250 mg/ml) and fluence rates only induced minor regression, additional Trolox™-administration significantly enhanced the photodynamic effect. While treatment of A.a. (250 mg/ml ICG, 100 J/cm²) without Trolox™ caused no bacterial reduction, additional administration led to total eradication. In the presence of Trolox™ reduction to one-fifth of the original ICG-concentration (50 mg/ml) still induced total suppression of P.g. and F.n. at identical fluence (100 J/cm²). Treatment with ICG, NIR-light or Trolox™ alone showed no remarkable bactericidal effect. Application of high ICG-concentrations (500 mg/ml) and exposure values (100 J/cm²) caused peak temperatures of 64.53°C.

Conclusions: The results clearly show that Trolox™ significantly enhanced the antibacterial effect of ICG upon irradiation with NIR-laser-light. Additional administration of Trolox™ may also increase the efficiency of other aPDT systems.

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**In Vitro Photodynamic Inactivation of *Candida albicans* by Phenothiazine Dye (New Methylene Blue) and Indocyanine Green (EmunDo®)**

Reza Fekrazada,1,2 Vadood Ghasemi Bargha,1,3 Arash Poorsattar Bejeh Mird,4 Masoumeh Shams-Ghahfarokhie 5

1Laser Research Center in Medical Sciences, AJA University of Medical Sciences, Tehran, Iran
2Department of Periodontics, Dental Faculty, AJA University of Medical Sciences, Tehran, Iran
3Department of Periodontics, Ardabil University of Medical Sciences, Ardabil, Iran
4Dental Materials Research Center, Dentistry School, Babol University of Medical Sciences, Babol, Iran
5Department of Mycology, Faculty of Medical Sciences, Tarbiat Modares University, Tehran, Iran


**Background:** The application of a new generation of photosensitizers to increase the efficacy of antifungal photodynamic therapy (aPDT) is an important aspect of PDT. Thus, this *in vitro* study is aimed to evaluate the antifungal efficacy of the photo-elimination of *Candida albicans* with photothermal and antifungal photodynamic therapy.

**Method and Material:** aPDT with new methylene blue and photothermal therapy with EmunDo® were applied to a fungal suspension, which was then subcultured in Sabouraud dextrose agar (SDA). The *C. albicans* colonies were counted and are expressed as colony-forming unit per milliliter (CFU/ml).

**Results:** aPDT with either EmunDo® or new methylene blue (NMB) considerably diminished the viability of inoculated *C. albicans* (*P* < 0.001) by log reduction of 1.9 and 3.37, respectively, compared with the control group. The antifungal potency or dark toxicity of the two photosensitizers alone did not significantly differ (*P* = 0.70). The same trend was observed for the light sources (λ: 810 nm vs. λ: 630 nm), which also did not significantly differ (*P* = 0.78).

**Conclusion:** The photo-elimination of *C. albicans* with either new methylene blue or EmunDo® as a photosensitizer can reduce the viability of fungal cells. Although the result of this study is encouraging, further investigations are warranted to determine clear protocols for the reliable and safe application of this method in clinical practice.

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Streptococcus mutans Photoinactivation by Combination of Short Exposure of a Broad-Spectrum Visible Light and Low Concentrations of Photosensitizers

Marco Aurelio Paschoal, DDS, PhD,¹ Lourdes Santos-Pinto, DDS, PhD,¹
Meng Lin, MSc,² Simone Duarte, DDS, PhD²

¹Department of Pediatric Dentistry, Araraquara Dental School, UNESP-Univ Estadual Paulista, Araraquara, SP, Brazil
²Department of Basic Science and Craniofacial Biology, College of Dentistry, New York University, New York, New York, USA


Objective: Investigate the photodynamic antimicrobial effect by the combination of a novel noncoherent broad spectrum visible light and low concentrations of curcumin and toluidine blue over suspensions of Streptococcus mutans.

Background Data: Long illumination times to activate photosensitizers (PS) and the use of high concentrations of these drugs in photodynamic antimicrobial chemotherapy (PACT) are limitations of its application as an antimicrobial technology in dental practice.

Materials and Methods: Planktonic suspensions of S. mutans were standardized and submitted to PACT treatment at low concentrations of curcumin (C) (0.075; 0.75 and 7.5 µM) and toluidine blue (T) (0.25; 2.5 and 25 µM) exposed to 42 J/cm² (12.2 sec; set power: 3.930 mW) of a white light (WL) (output wavelength range: 400-700 nm; beam diameter: 12 mm) (C + WL + and T + WL +, PACT groups; incubation time, C: 60 sec; T: 5 min); isolated effect of both C (C + WL –) and T concentrations (T + WL –); effect of light source (C – WL + and T – WL +) and suspensions neither submitted to PS nor to light-emitting diode (LED) illumination (control groups, C – WL – and T – WL –). Aliquots of each group were diluted and cultured on blood agar plates and the number of colony-forming units (CFU)/mL was recorded, transformed into log₁₀ and analyzed by ANOVA and Tukey’s test at a cutoff value at 0.05.

Results: The groups submitted to PACT presented a bacterial reduction value of > 5-log₁₀ to both tested PS in comparison with control groups (P < 0.05). PS or light source used alone demonstrated no antimicrobial effect on the number of viable bacterial counts.

Conclusions: The combination of a novel noncoherent light at short illumination exposure time with low concentrations of studied PS achieved a lethal photoinactivation of S. mutans, and can be considered an effective antimicrobial in vitro approach for reducing the number of microorganisms involved with the dental caries process.

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Discovering the Mechanisms of Strain-Dependent Response of *Staphylococcus aureus* to Photoinactivation: Oxidative Stress Toleration, Endogenous Porphyrin Level and Strain’s Virulence

Monika Kossakowska,1 Joanna Nakonieczna,1 Anna Kawiak,2,3 Julianna Kurlenda,4 Krzysztof P. Bielawski,1 Mariusz Grinholc, PhD1

1Laboratory of Molecular Diagnostics, Department of Biotechnology, Intercollegiate Faculty of Biotechnology, University of Gdansk and Medical University of Gdansk, Gdansk, Poland

2Department of Biotechnology, Division of Plant Protection and Biotechnology, Intercollegiate Faculty of Biotechnology, University of Gdansk and Medical University of Gdansk, Gdansk, Poland

3Laboratory of Human Physiology, Medical University of Gdansk, Gdansk, Poland

4Department of Clinical Bacteriology at the Provincial Hospital, Koszalin, Poland

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Background: *Staphylococcus aureus* is generally known to be susceptible to photoinactivation. However, the phenomenon of its strain-dependent response to photodynamic treatment has been reported. Moreover, the factors determining the emerging variation among strains according to photoinactivation remain unclear.

Methods: This work aimed to investigate any relevant correlation between bacterial toleration of oxidative stress, porphyrin level, photosensitizer uptake and strain’s virulence of studied methicillin-susceptible and methicillin-resistant *S. aureus* strains and their response to photodynamic inactivation (using protoporphyrin diarginate, toluidine blue O and 5-aminolevulinic acid).

Results: Obtained data demonstrated that studied factors have limited impact on strain response to PDI. However, we have shown that a multicomponent sensitizing agent, i.e., consisting of PPArg2, ALA, and TBO, would eliminate the *S. aureus* elevated resistance to photoinactivation and that both highly virulent and low virulent *S. aureus* strains could be easily eradicated with the use of PDI. Moreover, we have shown that photodynamic inactivation could decrease the virulence of *S. aureus* extracellular fraction.

Conclusion: The mechanism underlying strain-dependent response to photoinactivation is complex and multifactorial, nevertheless with the use of several sensitizing agents the elevated resistance to photodynamic treatment can be omitted.

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