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Review Article

Laser Treatment of Periodontal Disease: A Systematic Review of Histological Outcomes

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Abstract

Lasers are increasingly being used in the treatment of periodontal disease. When utilizing lasers to treat periodontal disease, a clinician should clearly understand the treatment outcomes of the lasers being used. The objective of this systematic review was to evaluate the histological outcomes of laser-assisted treatment of periodontitis.

Electronic databases were searched up to November 2016. Only controlled studies reporting histological outcomes of *in vivo* laser-assisted periodontal treatment in humans were included. Selected studies were evaluated for risk of bias.

The 13 studies selected evaluated lasers for root debridement, for periodontal epithelium removal, for bactericidal effects, and for periodontal regeneration. The lasers evaluated included diode lasers, CO2 lasers, Er:YAG lasers, and Nd:YAG lasers.

The following findings were within the limits of this systematic review: 1) lasers may be possible adjuncts to scaling and root planning, 2) the Er:YAG lasers may be used remove calculus, 3) the Diode lasers, CO_2 lasers and Nd:YAG laser may be used to remove gingival tissue, 4) the Nd:YAG laser may be used to selectively ablate diseased epithelium, 5) all the evaluated lasers can be used to eradicate periodontopathic bacteria in the short-term, and 6) the Nd:YAG laser may be the only laser associated with periodontal regeneration. Therefore, when evaluating a laser for oral use, the histological data must be considered in unison with the clinical data.

Keywords: *Lasers; Periodontitis; Review; Regeneration; Histology; Histomorphometric*

Abbreviations

ADA: American Dental Association; CO2: Carbon Dioxide; BOP: Bleeding on Probing; Er:YAG: Erbium:yttrium-aluminum-garnet; ICAM-1: Intercellular Adhesion Molecule 1; Nd:YAG: Neodymium:Yttrium-Aluminum-Garnet; PICOS: Participants: Humans, Interventions: Laser-Assisted Periodontal Treatment, Comparisons: Laser Treated Sites vs Control, Outcomes: Histological Evaluation, and Study Design: Descriptive Studies

Introduction

The goal of periodontal treatment is to remove the etiology, halt the inflammatory process, and restore connective tissue attachment and alveolar bone loss. Regeneration and new attachment gain has been technique-sensitive using guided tissue regeneration procedure [1,2]. During periodontal treatment, scaling and root planing were often used to remove calculus, bacteria and infected root cementum. However, with the advancement of laser periodontal therapy, lasers may become a viable and less invasive option for periodontal treatment of periodontitis [3]. White., *et al.* [4] reported surgical use of lasers have been successfully applied without anesthesia and with minimal bleeding when compared to scalpel surgery. Lasers currently used in periodontal procedures include Erbium:yttrium-aluminum-garnet (Er:YAG), Neodymium:yttrium-aluminum-garnet (Nd:YAG), Carbon dioxide (CO_2) lasers, and Diode lasers [5-8]. The lasers have different properties, and can be used at varying wavelengths and exposures resulting in varying tissue penetration [9]. Thus, further studies are needed to evaluate and calibrate the type of laser, the optimal wavelength, and the best exposure protocol for the desired outcome in periodontal therapy. The Diode laser has shown a possible decrease in incidence of bacteremia when compared to scaling with ultrasonic instruments [10]. The Er:YAG lasers have been shown to produce a bactericidal effect on the root surface [11,12] and are effective in removing subgingival calculus from the root surface [13]. On the contrary, some studies have found that Er:YAG lasers can inflict a negative effect on the periodontium, including thermal damage, attachment inhibition and delayed wound healing [12]. This is especially when the lasers are not properly used on the root surface. To clearly understand the treatment outcomes of the lasers used in periodontal therapy, histological samples evaluated over certain time intervals can show how each treatment modality would affect the periodontium [14]. The objective of this systematic review is to evaluate the histological effects of various lasers on human periodontal tissue when using lasers to treat periodontal disease.

Materials and Methods

Focused Question

What is the histologic response of the periodontium to laser-assisted periodontal treatment in humans? The following were addressed in the focused question: Participants: humans, Interventions: laser-assisted periodontal treatment, Comparisons: laser treated sites vs control, Outcomes: histological evaluation, and Study design: descriptive studies (PICOS).

Literature Search and Study Design

The following electronic databases: PubMed, Web of Science and Cochrane Central Register of Controlled Trials, were searched from inception to November 2016. A reference librarian was consulted on the most effective search strategy with variations of the following keywords: “histology”, “laser”, and “periodontal” (Table 1). In addition, Google Scholar was searched for gray literature. The reference lists of selected articles were then hand searched for any relevant articles not already identified in the search.

Inclusion and Exclusion Criteria

- Clinical trials investigating the histological effects of lasers on human diseased periodontium were included
- Non-English articles were included, but were selected only if there were an English translation
- All *in vitro* studies, cadaver and animal studies were excluded
- Studies with clinical and *in vitro* data, only clinical histologic data was included
- Clinical trials including patients with systemic disease were excluded
- Laser treatment *in vitro* on extracted teeth were excluded.

Database	Keywords
Pubmed	(histomorphometric[tw] OR histomorphology[tw] OR histologic[tw] OR "Histology"[Mesh] OR histology[tiab]) AND (laser[tw] OR lasers[tw] OR "Lasers"[Mesh]) AND (oral[tw] or dental[tw] or periodontal[tw] or periodontium[tw] or "soft tissue"[tw] or "intraoral"[tw] or teeth[tw] or tooth[tiab] or mouth[tiab] OR "Mouth"[Mesh] OR "Tooth"[Mesh] OR "Periodontium"[Mesh] OR "Periodontal Diseases"[Mesh] OR "periodontal diseases"[tiab] OR gum[tw] OR "gingiva"[MESH] OR gingiva[tiab] OR molar[tiab] OR "molar"[MESH] OR "oral cavity"[tw] OR periodontium[tiab] OR "tooth supporting structure"[tw] OR "tooth supporting structures"[tw]) AND ("humans"[MESH])
Web of Science	(histomorpho* OR histomorphomet* OR histol* OR "Histology" OR histology) AND (laser* OR "Lasers") AND (oral* or dental* or periodont* or "soft tissue" or "intraoral" or teeth* or tooth* or mouth* OR "Mouth" OR "Tooth" OR "Periodontium" OR "Periodontal Diseases" OR "periodontal diseases" OR gum* OR "gingiva" OR gingiva OR molar* OR "molar" OR "oral cavity" OR periodontium* OR "tooth supporting structure" OR "tooth supporting structures") AND ("humans") NOT ("Animals" OR animal)
Cochrane Library	Laser and Histology
Google Scholar	With All the words: histology, laser With at least one of the words: periodontal OR periodontitis OR gingival Without words: animal, animals, vitro

Table 1: *Database and keywords.*

Screening and Selection, and Data Extraction

Three reviewers (BH, SD, and MT) independently screened the "Title and Abstract", articles that obviously did not meet the inclusion criteria was excluded. Articles were included for full-text screening if there was any doubt. The full-text was then independently analyzed by the 3 reviewers (BH, SD, and MT). Data extraction of final selected articles was also independently performed by the same three reviewers with a previously pilot tested data extraction sheet. The independently extracted data extraction were cross-referenced between each reviewer for accuracy and completeness. All disagreements pertaining to the literature screening, selection, and data extraction were resolved by discussion with a fourth reviewer (SMB).

Assessment of Risk and Bias

The risk of bias (Table 2) was assessed using the risk of bias tool by the Office of Health Assessment and Translation (OHAT) [15]. Two reviewers (MT and BH) independently scored the risk of bias for the selected studies, and disagreements were resolved through discussion with a third reviewer (SMB).

Results

The search yielded 869 reviews in Pubmed, 634 in Web of Science, 91 in Cochrane Library, and 726 in Google Scholar (Figure 1). After the title and abstract screening, 16 were selected from Pubmed, 8 from Web of Science, 2 from Cochrane Library, 2 from Google Scholar, and none from hand searching the reference list of the selected studies. The duplicates were removed and 25 articles remained for full-text analysis. After full-text analysis, 12 were eliminated [16-27], and 13 articles remained for data extraction (Table 3). The risk of bias of the selected studies scored mostly "definitely or probably high risk of bias" (Table 2). All selected histological studies utilized a control for comparison except the Nevins., *et al*. [6] study which reported only post-laser treatment histology from the pre-treatment baseline notches on the root surfaces.

Study	Biloklytska., *et al.* 2016 [14]	Castro., *et al.* 2006 [28]	Cobb., *et al.* 1992 [33]	Crespi., *et al.* 2005 [30]	Crespi., *et al.* 2006 [31]	Eberhard., *et al.* 2003 [11]	Giannelli., *et al.* 2012 [34]	Nevins., *et al.* 2012 [6]	Radvar., *et al.* 1996 [36]	Schwarz., *et al.* 2001 [32]	Schwarz., *et al.* 2003 [29]	Ting., *et al.* 2014 [35]	Yukna., *et al.* 2007 [3]
Selection bias													
Was exposure level adequately randomized?	--	--	+	+	--	+	--	--	--	--	+	+	++
Was allocation to study groups adequately concealed?	--	--	NR	NR	--	NR	--	--	--	--	NR	NR	--
Performance bias													
Were the research personnel and human subjects blinded to the study group during the study?	--	--	--	--	--	--	--	--	--	--	--	--	--
Detection bias													
Can we be confident in the exposure characterization?	NR	NR	NR	NR	NR	+	NR	NR	NR	NR	NR	NR	NR
Can we be confident in the outcome assessment?	NR	++	NR	NR	NR	NR	NR	NR	NR	++	++	++	++
Attrition bias													
Were outcome data complete without attrition or exclusion from analysis?	+	++	+	++	++	++	++	+	++	++	++	++	++
Reporting bias													
Were all measured outcomes reported?	-	NR	-	-	-	-	-	-	-	NR	NR	NR	+

Table 2: *OHAT risk of bias assessment.*

++: Definitely Low risk of bias; +: Probably Low risk of bias, -/NR: Probably High risk of bias; --: Definitely High risk of bias; NR: Not Reported

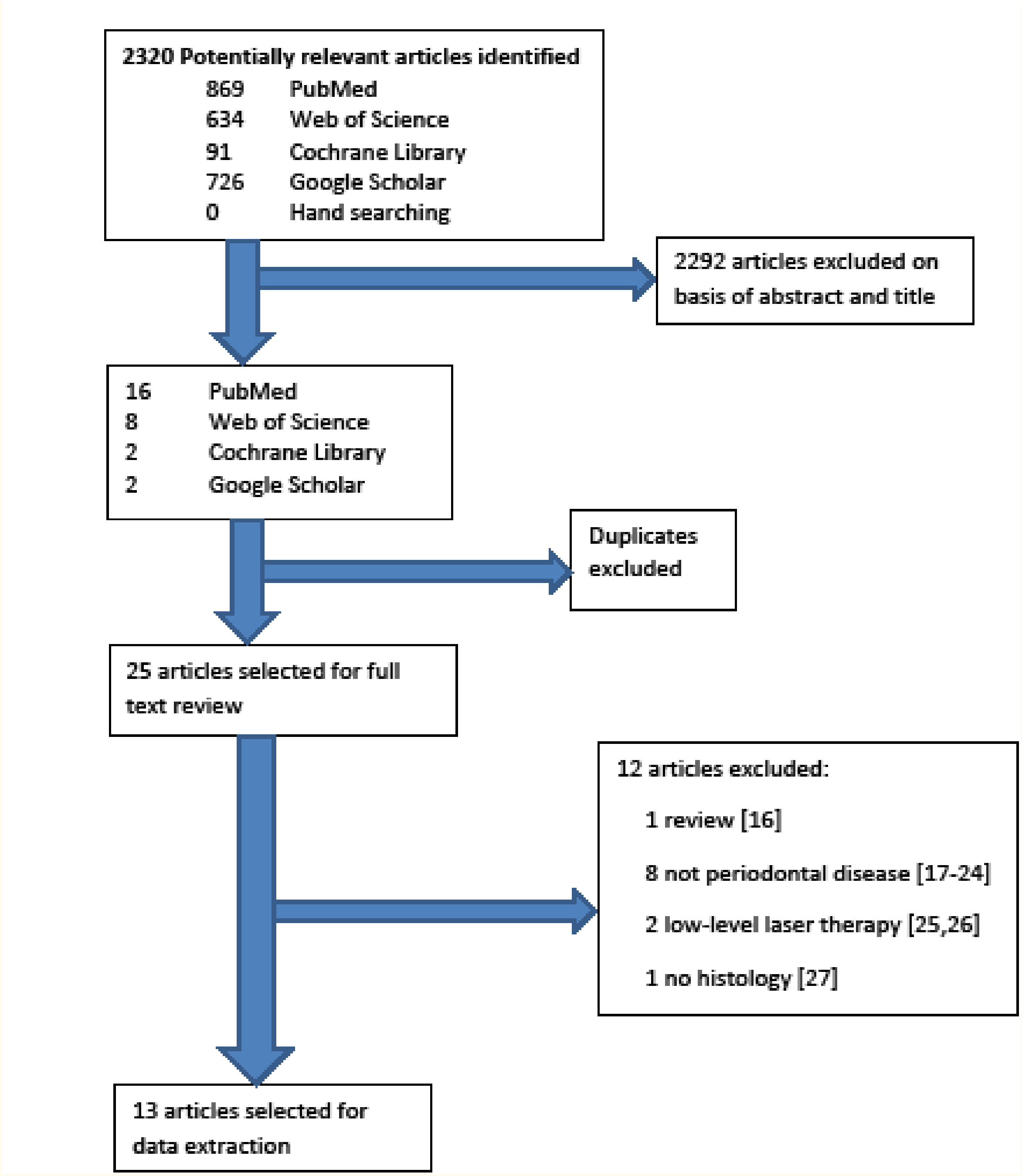

Figure 1: *Search strategy.*

Of the 13 studies (Table 3) selected, 7 evaluated the use of lasers for root debridement [11,28-33], 3 for removal of crevicular epithelium or diseased periodontium [14,34,35], and 7 for bactericidal effects [11,30,31,33-36], and 2 for periodontal regeneration [3,6]. Of these studies, 4 studies evaluated the use of diode lasers [14,28,29,34], 2 studies the use of CO_2 lasers [30,34], 5 studies the use of Er:YAG lasers [11,29,31,32] and 6 studies the use of Nd:YAG lasers [3,6,33-36]. Some of the studies evaluated134 more than one type of laser or mode of application.

Study	Periodontal condition	Treatment groups	No. of patients	No. of teeth/sites evaluated	Laser used and settings	Application of the laser	Clinical findings	Healing period before biopsy	Histologic outcomes
Biloklytska., *et al.* 2016 [14]	Chronic generalized periodontitis	- Conservative therapy, biopsy at 0 days - Conservative therapy, scaling and root planing in conjunction with diode laser curettage, biopsy at 0 days - Conservative therapy, scaling and root planing in conjunction with diode laser curettage, biopsy at 7-60 days - Conservative therapy, scaling and root planing in conjunction with diode laser curettage, and surgical treatment including using enamel matrix protein, biopsy at 7-540 days	20 patients (age range 31-59 yrs, mean age 45.5 yrs)	Samples of soft periodontal tissue harvested from the 20 patients	Diode laser (980 nm, 2 Watt, continuous regimen)	Laser curettage of diseased periodontal tissues	Laser curettage significantly reduced the papillary marginal attachment and bleeding and probing indexes Laser curettage and periodontal surgery significantly reduced bleeding on probing and probing depths	Immediately after treatment 7-60 days post-treatment 7-540 days post-treatment	- Conservative therapy showed an absence of dystrophic, necrotic and ulcerous pathologic changes - Immediately after laser curettage pathological change was minimal - Although injuries to the epithelium was reduced, productive-exudative inflammation was detected in laser treatment groups - However, minimal frequency of cases with productive-exudative inflammation and minimal frequency of hyperplasia was detected in laser followed by periodontal surgery groups that were biopsied after a healing period
Castro., *et al.* 2006 [28]	Severe destructive periodontal disease (All teeth included in the study had probing pocket depths > 8 mm and grade 3 mobility in horizontal and vertical direction	- Conventional scaling and root planing with diode laser irradiation (clinical procedure included scaling and root planing, laser procedure, followed by a second round of scaling and a second laser procedure)	-	5 single-rooted and multiple-rooted teeth with severe periodontal disease were extracted for evaluation	InGaIAsP diode laser device (wavelength of 980 nm, 2 W of power applied with pulse repetition)	Root debridement	-	Immediately after treatment	- Laser irradiation did not cause any degree of alteration on the root surface, there was minimal effect on the cementum tissue - Laser irradiation did not cause any signs of major thermal side effects on the teeth such as charring, melting, carbonization, necrosis, and fusion - Laser irradiation did not cause any signs of grooves and crater-like defects in any cross-sections observed in calculus-free areas

Cobb., *et al.* 1992 [33]	Generalized advanced adult periodontitis (6 mm or greater in depth that exhibited bleeding on probing)	Root planing followed by laser treatment [3.0 Watts (W) at 20 pulses per second (pps)] Root planing followed by laser treatment (2.25 W at 20 pps) Laser treatment (1.75 W at 20 pps) followed by hand instruments Laser treatment (1.75 W at 20 pps) only Untreated controls	8 patients (age range 38 - 66 yrs)	18 teeth tested 5 teeth root planed and then laser (3.0 W) treated 2 teeth root planed and then laser (2.25 W) treated 4 teeth treated by laser (1.75 W) and then hand instruments 4 teeth were treated with laser (1.75 W) only 3 teeth were untreated controls	Nd:YAG laser [wavelength 1064, power of 3.0 Watts (W) at 20 pulses per second (pps), or 150 milli-Joules (mJ) per pulse] Nd:YAG laser (wavelength 1064, power of 2.25 W at 20 pps, or 112.5 mJ per pulse) Nd:YAG laser (wavelength 1064, power of 1.75 W at 20 pps, or 87.5 mJ per pulse) Nd:YAG laser (wavelength 1064, power of 1.75 W at 20 pps)	Root debridement Bactericidal effect	- Pre-treatment microbiology sampling showed detectable levels of *P. gingivalis* or *P. intermedia* - Sites treated by laser (1.75 W) and root planed had negative post-treatment levels for all microbes, or low levels of both *P. gingivalis* and *P. intermedia* - All sites treated by laser (1.75 W) only had persistent low levels of *P. gingivalis* or *P. intermedia* - Observations at 7 days post-laser treatment with no scaling and root planing showed a variety of microbial morphotypes	Immediately after treatment except for 2 teeth which was removed 7 days later	Non-treated controls: - low magnification: root surfaces of the control specimens appeared to be covered with a confluent layer of plaque and calculus - occasional areas of root surface not covered by plaque or calculus were covered by an amorphous crevicular fluid-derived pellicle Laser (3.0 W and 2.25 W) treated specimens: - SEM examination: revealed little difference between the treatment groups - Both sets of specimens exhibited areas of root surface melt-down and resolidification, and root surface ditching or craters - Calculus exposed to the laser were not covered with plaque microbes Laser (1.75 W) treated followed by root planing specimens: - Showed a relatively smooth root surface - Isolated areas that were incompletely root planed showed localized deposits of charred calculus or matted plaque or small craters of resolidified root mineral - Laser treated areas that were thoroughly root planed showed a loosely-textured scale-like smear layer or deep grooves cut in the rough textured root surface

Crespi., *et al.* 2005 [30]	Severe periodontal disease (All teeth included in the study had probing depths >6 mm and clinical attachment levels >8 mm)	- Ultrasonic bacterial curettage with ultrasonic scaler - Hand instrumentation - Hand instrumentation and CO2 laser treatment of roots	-	33 (15 incisors, 9 premolars, and 9 molars) 33 teeth scheduled for extraction due to severe periodontal disease	CO_2 laser (defocused pulsed mode, frequency of 1 Hz, power of 2 W, duty cycle of 6%, and energy density of 2.45J/cm^2)	Root debridement Bactericidal effect	-	Immediately after treatment	Root surfaces treated with the ultrasonic instrument - Showed a scaly and rough topography with some gouges in several areas - Residual bacterial aggregates were observed along some root surfaces and in the interradicular septa of premolars and molars Teeth treated by curettes - Presented smooth root surfaces, especially on convex surfaces - Cementum was completely absent and the dentin layer exhibited open tubules - Defects on the dentin layer were also present along root surface - In some teeth, residual plaque and calculus were present in inter-radicular septa, lacunae, and concavities Teeth treated by currettes and CO_2 laser - Exhibited areas devoid of cementum with completely sealed dentinal tubules - Dentin surface appeared as a melted layer, showing a flat and smooth surface with an apparent fusion of the smear layer - Dentin layer had the appearance of a glazed surface - No residual bacteria were observed on any of the examined roots
Crespi., *et al.* 2006 [31]	Severe periodontal disease probing depths (PD) (>6 mm and clinical attachment levels (CAL) >8mm)	Hand instrumentation Er:YAG laser	15 patients (mean age 57 yrs, age range 42 to 72 yrs)	40 teeth, 26 single rooted and 14 multi-rooted, scheduled for extraction due to severe periodontal disease Control - 20 teeth were treated by hand instruments Test - 20 teeth were treated by Er:YAG laser	Er:YAG laser 160 mJ/10 Hz	Root debridement	-	Immediately after treatment	Control group - Teeth treated by curettes presented smooth root surfaces with elimination of calculi and bacterial flora, and the cementum layer was completely removed, leaving open dentinal tubules - Some samples deep scratches on the dentin layer due to manual strokes of the curette were also observed Test group - The laser-treated root surfaces revealed no major thermal damage; no cracking, carbonization, steep grooves and crater walls were observed along irradiated root surfaces - Bacterial flora was entirely eliminated along all root surfaces observed - Laser scaling was accompanied by an increased removal of tissue; the cementum was ablated through to the dentin in unvarying thickness, leaving a rough and uniform surface - No structural dentin alteration processes immediately beneath the superficial margin

Eberhard., *et al.* 2003 [11]	Advanced chronic periodontitis	hand instrumentation (scaling and root planing) Er:YAG laser irradiation	12 patients (age: 39–59 years)	30 single rooted-teeth scheduled for extraction due to severe periodontal disease Mesial and distal surfaces of these teeth randomly assigned to scaling or laser group 1) ErYAG group: 15 surfaces And Scaling and root planing group: 15 surfaces 2) ErYAG group with twice as much time as scaling and root planing: 15 surfaces And Scaling and root planing group: 15 surfaces	Er:YAG laser (wavelength of 2.94 mm and a pilot wavelength of 635 mm, energy level of 160 mJ and repetition rate of 10 to 15 Hz with water irrigation)	Root debridement	- Scaing and root planing resulted in 93.9% calculus free root surfaces - Laser treatment used for the same amount of time resulted 68.4% calculus free root surfaces - Laser treatment used for twice the time for hand scaling resulted in 83.3% calulus free root surfaces - Initial probing depth was not related to the effectiveness of either treatments - Laser treatment and hand scaling showed similar reduction of subgingival bacteria *Porphyromonas* *gingivalis*, *Prevotella intermedia*, *Bacteroides forsythus* and *Treponema denticola*	Immediately after treatment	Scanning electron microscopic morphology of the root surface after hand instrumentation - Smooth, some traces of the curette - Only limited amounts of remaining calculus were present Scanning electron microscopic investigation of the root surfaces treated with Er:YAG laser - Revealed a rough, acid-etched like surface, interrupted by smooth traces of cementum of about 50 mm width Histological observation of the root surface - Revealed 4.3% residual deposits after manual scaling - Following laser treatment, 30.2% residual deposits were observed in the group treated in the same time as hand scaling group and 22.7% in the group treated in twice the time as hand scaling group - Laser-treated tooth surfaces exhibited no dentin exposition but rather a minimal reduction of cementum for both groups. - 73.2% of the dentin was denuded from the cementum after hand instrumentation, while only a minimal cementum reduction was apparent after laser irradiation.

Giannelli., *et al.* 2012 [34]	moderate-to-severe chronic periodontitis	Diode GaAs (810 nm) Er:YAG Nd:YAG CO2 lasers	16 patients (40–65 age range; mean range 55.6 years)	32 sites, with 16 taken before and 16 (4 per laser type) after laser treatments Small gingival biopsies were evaluated	Nd:YAG laser (wave length 1064 nm, power of 1.4W, pulse duration of 150 µs, pulse frequency of 70 Hz) Er:YAG (wavelength 2940 nm, power of 1W, pulse duration of 400 µs, pulse frequency of 10 Hz) CO_2 laser (wavelength 10600 nm, power of 1W, pulse duration of 250 µs, pulse frequency of 2 Hz) Diode (wavelength of 810 nm, power of 1 W)	Bactericidal effects Removal of the contaminated gingiva	Using real-time PCR analysis - high DNA levels of periodontal pathogens including *P. gingivalis*, *A. actinomycetemcomitans*, *F. nucleatum*, *T. denticola*, *P. intermedia*, and *E. corrodens*, were detected in all the samples Nd:YAG and diode lasers - capable of eradicating pathogenic bacteria within gingival epithelial cells without causing connective tissue damage and microvessel rupture	Upon admission and after laser treatments	Histological and cytodiagnostic analysis of the gingival samples taken outside the periodontal pocket in periodontitis patients performed before the treatment - Showed marked abnormalities - Histological changes consisted of focal epithelial ulceration, diffuse infiltration of inflammatory cells in the lamina propria, and dilated blood vessels - Using confocal microscopy, the endothelial lining of capillaries and post-capillary venules showed a marked immunostaining for ICAM-1, a typical endothelial adhesion molecule regulating leukocyte trafficking during inflammation Histological and cytodiagnostic analysis of the gingival samples from the patients, after photoablative laser irradiation - Showed an almost complete eradication of bacterial contamination, in terms of total bacterial and specific periodontopathogenic DNA Er:YAG laser - Induced marked microvessel rupture and bleeding and failed to completely and selectively ablate the infected gingival epithelium CO2 laser - Yielded a complete removal of the squamous epithelium accompanied by a diffuse heat-induced coagulation of the papillary connective tissue and microvessels Nd:YAG laser - Caused a complete removal of the squamous epithelium, in the absence of appreciable changes of the stromal and microvessel components of the lamina propria - Microvessels appeared collapsed, consistent with reduced bleeding at treatment, and showed a significant reduction of ICAM-1 expression Diode laser (k 810 nm) - Induced a complete ablation of the surface epithelium, prominent microvessel constriction associated with ICAM-1 downregulation, and negligible stromal coagulation, intraoperative bleeding was minimal

Nevins., *et al.* 2012 [6]	Advance periodontitis (probing depth ≥ 7 mm and radiographic suggestion of a 4-mm or deeper intra bony Defect)	Experimental teeth: - Laser first used to remove the diseased pocket epithelium and decontaminate the pocket - Teeth were scaled and root planed with piezo ultrasonic - A second pass with the laser - Occlusal adjustment and extra- or intra-coronal splinting of the teeth - Medications prescribed: 0.12% chlorhexidine mouthrinse bid for 4 weeks, oral antibiotics (amoxicillin 500 mg every 8 hours for 7 days), and anti-inflammatory analgesics (ibuprofen 600 mg, every 6 hrs PRN)	8 patients (age 18-70 yrs)	12 teeth scheduled for extraction due to severe periodontal disease (2 teeth splintered during histologic preparation, thus 10 were analyzed)	Nd:YAG laser (wave length 1064 nm) 1st pass: Power of 4W, pulse duration of 100 µs, pulse frequency of 20 Hz 2nd pass: Power of 4W, 650-µs pulse duration, and 20 Hz	Removal of the crevicular epithelium around the necks of the study teeth, relax the gingival collar, and exposure of the contaminated root surface Clot stabilization for the formation of a solid fibrin clot and a pocket seal Periodontal regeneration	Probing depth reduction: 5.4 mm Clinical attachment gain: 3.8 mm	After 9 months of healing	- No signs of root damage from the laser therapy noted clinically or histologically - Of the 10 teeth evaluated, 5 evidenced a degree of periodontal regeneration with new cementum, periodontal ligament, and alveolar bone, one tooth had new attachment with new cementum and inserting collagen fibers, and 4 teeth healed via a long junctional epithelium - Periodontal regeneration coronal to the calculus notch was confirmed for three teeth presenting with furcation involvement - Although none of the furcations closed or were reduced in grade, they demonstrated significant clinical attachment gain and histologic assessment of regeneration
Radvar., *et al.* 1996 [36]	Chronic adult periodontitis	Laser treatment at 50 mJ, 10 pps for 3 minutes Laser treatment at 80 mJ, 10 pps for 3 minutes Scaling only Untreated control	11 patients	80 sites selected from teeth scheduled for extraction due to severe periodontal involvement 50 mJ laser group: 20 surfaces 80 mJ laser group: 20 surfaces Scaling group: 20 surfaces Untreated control: 20 surfaces	Nd:YAG laser energies of 50 and 80 mJ at 10 pulses per second (pps)	Bactericidal effects	- The microbial samples taken immediately for the scaling group and the laser (80 mJ) group showed a significant reduction in total CFU compared to baseline, but after 6 weeks only the scaling group still showed a significant reduction compared to baseline Nd:YAG laser pulses of 50 mJ and 80 mJ failed to improve the clinical and microbial parameters of periodontal disease	6 weeks	- Scanning electron microscopy examination of laser treated teeth showed no evidence of damage attributable to the laser irradiation

Schwarz., *et al.* 2001[32]	Severe periodontal disease	Each power setting (120, 140, 160, and 180 mJ at 10 Hz Untreated peripheral areas served as control	-	40 single rooted teeth with advanced periodontal destruction scheduled for extraction (only *in-vivo* data for 80 surfaces included in this review) 120 mJ group: 20 surfaces 140 mJ group: 20 surfaces 160 mJ group: 20 surfaces 180 mJ group: 20 surfaces	Er:YAG laser (wavelength of 2940 nm the energy levels of 120, 140, 160, and 180 mJ/pulse at 10 Hz were equivalent to the energy densities of 71, 83, 94 and 106 J/cm^2/ pulse, respectively)	Root debridement	-	Immediately after treatment	- Pulsed Er:YAG laser was able to remove the subgingival calculus from the root surface effectively at all investigated energy levels - At 120 mJ laser energy level, surfaces showed a homogeneous and smooth root surface morphology - Surface alterations were not related to the used energy - Scanning electron microscopy observation of the 80 laser-scaled surfaces revealed no signs of thermal damages, the root surface morphology was homogenous with some slight superficial alterations localized to cementum - The laser produced homogeneous and nearly smooth surfaces - There was no charring during laser application in the patients
Schwarz., *et al.* 2003 [29]	Severe periodontal disease	ERL (Er:YAG laser combined with a calculus detection system with fluorescence induced by 655 nm InGaAsP DL radiation) GaAlAs Diode laser SRP on periodontally diseased root surfaces	-	24 single rooted teeth (48 surfaces) scheduled for extraction due to severe periodontal destruction ERL gp: 8 surfaces Diode laser group: 8 surfaces Scaling with hand instruments group: 8 surfaces	Er:YAG (pulsed infrared radiation at a wavelength of 2.94 mm, parameters were set at 160 mJ/pulse and 10 pulses/ second, and pulse energy 120 mJ/pulse (19.4 J/cm2)) Diode laser (Wavelength of 810 nm and 10,000 Hz to pulse was used, the pulse/ pause relation was set at 1:10 at a power output of 1.8 W, delivered by a 600 mm contact optic fiber (0.63 mJ/mm^2)	Root debridement	-	Immediately after treatment	ERL - Produced homogeneous and nearly smooth root surfaces without visible traces of the fiber tip, loss of cementum was non-existent or minimal - Treated areas could have not been demarcated from untreated areas in respect of the surface morphology, only the presence of calculi has given signs of the treatment borders - Histologic inspection of the root surfaces indicated that subgingival calculus was selectively removed, with no observed thermal damage, such as carbonization, melting, or cracking - Used with a new fluorescent calculus detection system provided a selective subgingival calculus removal on a level equivalent to that provided by hand instruments Root surfaces treated using hand instruments - Generally had a smooth appearance, some scratches and shallow craters caused by the curettes were occasionally observed Diode laser irradiation - Caused severe damages to the root surface, calculus-free areas showed grooves and crater-like defects in all cross-sections - In six of eight samples, cementum was ablated through to the dentin - Histologically, no major thermal damage, such as carbonization, melting, or cracking - craters were observed and were significantly deeper than those following treatment with hand instruments - This power output was unsuitable for calculus removal and altered the root surface in an undesirable manner

Ting., *et al.* 2014 [35]	Moderate chronic periodontitis	Nd:YAG laser for pocket irradiation along the root surface Untreated control	20 patients (age range 47 to 79 years, mean age of 61.5 years)	32 surfaces, specimens were obtained by incisive dissection using a scalpel Test: 14 surfaces Contriol: 18 surfaces	Nd:YAG laser (wave length 1064 nm, 2W, 200 mJ/ pulse, and 10 pps delivered through a 320 µm diameter tip)	Removal of the crevicular epithelium around the necks of the study teeth	All laser-treated test specimens showed a complete absence of microorganisms	Immediately after treatment	Scanning electron microscopy: Untreated control - Pocket wall epithelium of specimens with probing depth of < 3mm and negative bleeding on probing exhibited a smooth layer of cells with no evidence of an inflammatory cell infiltrate - In specimens with probing depth (PD) > 4mm, there was differing morphologic features of pocket epithelium at different probing depths and relative degrees of inflammation - At the greatest depth of untreated periodontal pockets, microbial penetration of the epithelial barrier, especially in specimens with bleeding on probing Laser-treated test specimens - Revealed varying degrees of epithelial cell ablation and/or removal of pocket epithelia - Specimens with a probing of < 3mm and no bleeding on probing, two cellular changes were noted; either no obvious alterations of the epithelial barrier or a localized area of heat-damaged and/or ablated cells - Specimens with PD of > 4mm exhibited a conspicuous ablation and/or removal of epithelia and featured an erythrocytic and lymphocytic infiltrate Histologic evaluation: Untreated control specimens - The epithelium of at the gingival crest of a periodontal pocket was intact and thickened - The thin, nonkeratinized stratified squamous epithelium was observed at a large depth of pocket epithelium - Leukocytic infiltration was usually present near the base of the pocket Laser-treated test specimens - Varying degrees of epithelial cell ablation and/or removal of pocket epithelia - No alterations or only thin layers of evaporated epithelia were observed in specimens with a PD of < 3mm and no bleeding on probing - The specimens with PD > 4mm exhibited a conspicuous ablation and/or complete removal of epithelia in inflamed portion - Epithelium remains in the uninflamed portion - No significant damage on the connective tissues

Yukna., *et al*. 2007 [3]	Moderate to severe periodontal involvement (probing depths and clinical probing attachment loss of 5 to 9 mm with bleeding on probing and subgingival calculus)	Experimental teeth: occlusal adjustment with odontoplasty, and splinting to neighboring teeth, then ultrasonic and hand instruments Control teeth: Scaling and root planing All teeth: No sutures, triple antibiotic ointment, and a light-cured dressing All patients: Nonsteroidal anti-inflammatory medications, doxycycline (100 mg daily for 10 days), and 0.12% chlorhexidine rinses (to be used twice daily)	6 patients (age 26-54 yrs, mean age 45.5 years)	6 pairs of single rooted teeth scheduled for extraction Test: 6 teeth Control: 6 teeth	Nd:YAG laser (wave length 1064 nm) 1st pass: Power of 4W, pulse duration of 100 µs, pulse frequency of 20 Hz 2nd pass: Power of 4W, 650-µs pulse duration, and 20 Hz	Removal of the crevicular epithelium around the necks of the study teeth, relax the gingival collar, and exposure of the contami nated root surface Clot stabilisation for the formation of a solid fibrin clot and a pocket seal Periodontal regeneration	Test: Probing depth reduction: 4.7 mm Clinical attachment gain: 4.2 mm Control: Probing depth reduction: 3.7 mm Clinical attachment gain: 2.4 mm mGI, PI, and BOP were improved on all test and control teeth	3 months	Laser-treated specimens: - All specimens showed new cementum and new connective tissue attachment in and occasionally coronal to the notch - No evidence of any adverse histologic changes around the laser-treated specimens - No evidence of any adverse histologic changes to the root surface or the pulp of any of the teeth Control teeth: - Five of the six control teeth had a long junctional epithelium with no evidence of new attachment or regeneration

Table 3: *Characteristics of selected studies.*

Nd:YAG: Neodymium-Doped Yttrium Aluminum Garnet; Er:YAG: Erbium-doped Yttrium Aluminum Garnet; AlGaAs: Aluminum Gallium Arsenide 810 nm diode; InGaAs: Indium Gallium Arsenide 940-980 nm diode; CO_2 laser: Carbon *Dioxide Laser*

The lasers that can remove calculus as effectively as scaling and root planing include the Er:YAG laser and the Diode laser (wavelength of 980 nm) (Table 4). However, conflicting studies report that the Er:YAG is less effective than scaling and root planing, and may require twice as much treatment time to achieve calculus removal of the same quality as scaling and root planing [11]. For the Diode laser, the wavelength of the laser is crucial, a wavelength of 980nm can avert thermal damage, while a wavelength of 810nm can cause severe damage and is not recommended. Nd:YAG and CO_2 lasers are not effective for calculus removal, and when used as an adjunct to scaling and root planing can further enhance treatment.

All lasers evaluated in the selected studies except the Er:YAG laser, can remove gingival periodontium (Table 4). However, the Diode [14,34] and the CO_2 laser [34] are non-selective in the tissue removal and can cause some damage to the underlying connective tissue. On the other hand, the Nd:YAG lasers can selectively ablate inflamed epithelium, sparing the healthy epithelium and the underlying connective tissue [35].

All lasers evaluated were reported to effectively reduce or eliminate periodontal pathogens immediately after irradiation (Table 4). Compared to scaling and root planing, Er:YAG lasers [11,31] reported similar effectiveness and Nd:YAG lasers reported reduced effectiveness [36]. However, most studies [30,33-35] did not compare laser elimination of periodontal microbes to scaling and root planing. Limited information was available to conclude if the reduction of the pathogenic microbes can be sustained

The Nd:YAG laser [6,3] is the only laser to report periodontal regeneration in the treatment of periodontally involved teeth (Table 4).

Application	Laser used		Study	Key findings of laser treatment	Compared to scaling and root planing (SRP)
Root debridement	Diode laser	980 nm	Castro., et al. 2006 [28]	- No thermal damage, most root surfaces were calculus free	Effective when used as an adjunct to SRP (laser treatment only was not evaluated)
		810 nm	Schwarz., et al. 2003 [29]	- Severe damages to the root surface	Harmful, and not recommended
	CO_2 laser		Crespi., et al. 2005 [30]	- Cementum removed, dentinal tubules completely sealed, flat and smooth root surface with an apparent fusion of the smear layer (only SRP followed by laser treatment evaluated)	Effective when used as an adjunct to SRP (laser treatment only was not evaluated)
	Er:YAG laser		Crespi., et al. 2006 [31]	- Complete elimination of calculi, debris, and cementum - Caused no cracks nor grooves	Less effective than SRP
			Eberhard., et al. 2003 [11]	- No dentin exposure - Cementum spared - Residual deposits present	Similar effectiveness, but take twice the time for the same result
			Schwarz., et al. 2001 [32]	- Removed subgingival calculus from the root surface effectively at all investigated energy levels (120 mJ, 140 mJ, 160 mJ, 180 mJ) - No signs of thermal damage - Produced homogeneous root surface	Effective (Not compared to SRP)
			Schwarz., et al. 2003 [29]	- Produced homogeneous and nearly smooth root surfaces - Subgingival calculus was selectively removed, with no observed thermal damage	Similar effectiveness
	Nd:YAG laser		Cobb., et al. 1992 [33]	- Residual subgingival calculus and plaque in all treatment groups	Not effective used alone or as an adjunct (Not compared to SRP)

Removal of crevicular epithelium or diseased gingiva	Diode laser	810 nm	Giannelli., et al. 2012 [34]	- Complete ablation of the surface epithelium	Effective, non-selective removal of epithelium (Not compared to SRP)
		980 nm	Biloklytska., et al. 2016 [14]	- Minimal pathological change - Reduced injuries to the epithelium	Effective, but some productive-exudative inflammation
	CO_2 laser		Giannelli., et al. 2012 [34]	- Complete removal of squamous epithelium with a diffuse heat-induced coagulation of the papillary connective tissue and microvessels	Effective, but some damage to underlying connective tissue (Not compared to SRP)
	Er:YAG laser		Giannelli., et al. 2012 [34]	- Failed to completely and selectively ablate the infected gingival epithelium	Not effective (Not compared to SRP)
	Nd:YAG laser		Giannelli., et al. 2012 [34]	- Complete removal of the squamous epithelium - No appreciable changes of the stromal and microvessel components of the lamina propria	Effective, no appreciable damage to underlying connective tissue (Not compared to SRP)
			Ting., et al. 2014 [35]	- Specimens with probing depth > 4mm exhibited a conspicuous ablation and/or complete removal of epithelia in inflamed portion - Epithelium remains in the uninflamed portion - No significant damage on the connective tissues	Effective, selective removal of inflamed epithelium (Not compared to SRP)

Bactericidal effects	Diode laser (810 nm)	Giannelli., et al. 2012 [34]	- Almost complete eradication of bacterial contamination	Effective (Not compared to SRP)
	CO_2 laser	Giannelli., et al. 2012 [34]	- Almost complete eradication of bacterial contamination	Effective (Not compared to SRP)
		Crespi., et al. 2005 [30]	- No residual bacteria were observed on any of the examined roots (only SRP followed by laser treatment evaluated)	Effective when used as an adjunct to SRP (Not compared to SRP)
	Er:YAG laser	Giannelli., et al. 2012 [34]	- Almost complete eradication of bacterial contamination	Effective (Not compared to SRP)
		Eberhard., et al. 2003 [11]	- Reduced subgingival bacteria *Porphyromonas* *gingivalis*, *Prevotella intermedia*, *Bacteroides forsythus* and *Treponema denticola*	Similar effectiveness
		Crespi., et al. 2006 [31]	Bacterial flora was entirely eliminated along all root surfaces	Similar effectiveness
	Nd:YAG laser	Radvar., et al. 1996 [36]	- Significant reduction in total colony forming units (CFU) immediately after laser treatment, but not sustained at 6 weeks	Not as effective as scaling and root planing (At 6 weeks, SRP group sustained microbial reduction, but not the laser group)
		Ting., et al. 2014 [35]	- All laser-treated specimens showed a complete absence of micro-organisms	Effective (Not compared to SRP)
		Giannelli., et al. 2012 [34]	- Almost complete eradication of bacterial contamination	Effective (Not compared to SRP)
		Cobb., et al. 1992 [33]	- Substantial suppression was detected as indicated by a lack of DNA probe recovery of *A. actinomycetemcomitans, P. gingivalis,* and *P. intermedia*	Effective (Not compared to SRP)
Periodontal regeneration	Nd:YAG laser	Nevins., et al. 2012 [6]	- Most specimens showed periodontal regeneration with new cementum, periodontal ligament, and alveolar bone	Effective, possible periodontal regeneration (Not compared to SRP)
		Yukna., et al. 2007 [3]	- All specimens showed new cementum and new connective tissue attachment - No adverse histologic changes	More effective, periodontal regeneration reported

Table 4: *Laser application and outcomes.*

Discussion

Lasers has many applications including calculus removal, diseased gingival epithelium removal, elimination of periodontal pathogens, and possibly regeneration of periodontal tissues (Table 4). However, the efficacy of each laser in each application is limited by the hard or soft tissue absorption of its wavelength.

Plaque and calculus removal from the subgingival root surface of the periodontal pocket is the main goal of non-surgical periodontal therapy. Laser studies for calculus removal reported on lasers for direct calculus ablation in the absence of scaling and root planing [11,28,29,31,32], and on lasers as an adjunct to scaling and root planning [30].

Lasers for direct calculus ablation include the diode laser [28] and the Er:YAG laser [32]. The wavelength and power of the laser determined the degree of ablation of calculus and tooth structures [32]. The Diode laser and the Er:YAG lasers have been shown to cause minimal damage to the root surface in laser studies on extracted teeth [37]. Information from *in vitro* studies can suggest optimal power and exposure time required to achieve the desired outcome with minimal damage to the root surface [38]. However, scaling and root planing with lasers in clinical practice differ from experimental *in vitro* studies on extracted teeth. The periodontal sulcus and other clinical differences like periodontal temperature, humidity and blood contamination cannot be duplicated *in vitro*. The presence of a blood film on the root surface may increase the hemoglobin light absorption of the 810 nm diode laser and cause damage to the underlying root surface [29,39].

There are conflicting results as to the effectiveness of laser curettage versus scaling and root planing. The Er:YAG reported twice as much treatment time to remove calculus as effectively as scaling and root planing [11]. In addition, the consensus report by the American Academy of Periodontology stated that laser treatment for periodontal pocket curettage was substantially ineffective [40].

Lasers used as an adjunct to scaling and root planing, are often inefficient for calculus removal [30], but may be useful for hard tissue conditioning and root surface detoxification [41]. After scaling and root planing, the CO_2 laser at a power level of 3.0 W with a time exposure of 0.8 seconds, can remove the smear layer and residual plaque without damage to the dentine [42]. Nd:YAG lasers used as an adjunct to scaling and root planing was reported to significantly reduced the severity of periodontal inflammation [43].

Actinobacillus actinomycetemcomitans, Porphyromonas gingivalis, Fusobacterium nucleatum, Treponema denticola, Porphyromonas intermedia and *Eikenella corrodens* have been shown to invade into the epithelial cells of diseased periodontal pockets [44-46]. The persistence of these periodontal pathogens in the gingival epithelial cells may be linked to refractory periodontitis after conventional periodontal therapy [44]. Hence, there are potential benefits in laser de-epithelialization of the infected gingiva.

However, the clinical relevance of the removal of sulcular epithelium has been questioned, and the American Dental Association (ADA) has since discontinued the use of the curettage insurance code. The above described lasers vary in their ability to effectively ablate diseased gingival tissue. Based on histologic evaluation, the Er:YAG laser was unable to completely remove disease epithelium, and there were persistent intracellular bacteria recovered from Er:YAG treated gingival tissue [34]. The CO2 laser can achieve complete de-epithelization, but can also cause heat-induced damage to surrounding microvessels and papillary connective tissue. Damage to the surrounding gingival tissue forms a nidus for bacterial re-infection and can delay healing [34].

The diode laser [34] and the Nd:YAG lasers [35] can effectively remove gingival epithelium without damage to stromal tissue and microvessels. Both lasers can induce microvessel constriction when removing surface epithelium, this can reduce bleeding and bacteremia during therapy [34]. Furthermore, both lasers can also induce anti-inflammatory effects by reducing endothelium intercellular adhesion molecule 1 (ICAM-1) immunolabelling and inactivating bacterial lipopolysaccharide [47,48]. Areas of chronic inflammation, due to increased hemoglobin in the area, may absorb more of the laser light and show increased thermal ablation compared to non-inflammed connective tissue. This may contribute to the decrease in inflammation and bleeding on probing (BOP) associated with the intrasulcular use of lasers. Other contributory factors may be the biostimulatory and photobiomodulatory effects of lasers that has been demonstrated in the literature [49,50].

The Nd:YAG lasers may have an additional advantage over the diode laser, the Nd:YAG laser is able to target inflamed periodontal tissue and selectively ablate the diseased tissue [35]. Ting., *et al*. reported no appreciable post-laser effects on the stromal and microvessel components of the lamina propria. However, this may need to be interpreted with caution, because the histology was taken at time = 0 and would not report on any likely thermal necrosis that would be expected to occur in the first 7 days. Therefore, to report that this laser will protect the underlying connective tissue, there should be no histological tissue damage at time = 3 days, time = 7 days, and time = 14 days, and this was not evaluated in the Ting., *et al*. [35] study. It is also important to note that this systematic review focused only on the effects of the laser periodontal tissue removal at a cellular level and not on the effects laser tissue removal has on periodontal attachment gains.

Lasers can eliminate or reduce periodontal pathogens. Most of the studies [11,30,33-35] selected in this systematic review did not compare microbial outcomes of laser treatment to scaling and root planing. The few selected studies that compared microbial outcomes of laser treatment to scaling and root planing reported that the effectiveness of lasers compared to scaling and root planing were similar [11,31], and may not be sustained in the long term [36]. However, when laser therapy is applied adjunctively after scaling and root planing, suppression of *Tannerella forsythia, T. denticola* and *P. gingivalis* were more profound than scaling and root planing alone [51].

Proper calibration of the lasers was crucial when ablating periodontal bacterial pathogens without damage to root surface and surrounding tissues. Periodontal pocket decontamination with an improperly calibrated Diode laser can cause damage to the root surface when blood film contamination increases the light absorption on the root surface [39]. The CO_2 laser calibrated at energy densities between 11 - 41 J/cm2 can eliminate bacterial plaque without causing damage to the root surface [52].

In addition to reducing periodontal pathogens [33,51], the Nd:YAG laser is the only laser to report histological outcomes of regeneration and new attachment gain [6,3]. However, the Yukna., et al. [3] controlled study that reported periodontal regeneration is limited, because of the small sample size in their histological assessment of 6 periodontally hopeless teeth. Otherwise, similar findings was also reported in other clinical studies, although these studies were not a controlled studies, there was reported evidence of periodontal regeneration with Nd:YAG laser treatment [6,27]. However, it is important to recognize that the restoration of oral health in the periodontitis patient is not contingent on the type of periodontal attachment, healing by long junctional epithelium or connective tissue attachment are both acceptable outcomes to periodontal therapy. In the treatment of moderate to advance periodontal disease, Nd:YAG laser therapy in combination with selective occlusal adjustments, scaling and root planing, antibiotics and other factors described in the LANAP protocol (US patent #5,642,997) have reported optimistic clinical and histological outcomes [6].

The Nd:YAG lasers when selectively calibrated to surgically remove diseased epithelium [35], facilitate clot stabilization [3] and eliminate putative microbial pathogens, seemed to create a periodontal environment conducive for periodontal regeneration or cementum-mediated new attachment. Even though other surgical techniques have reported regenerative periodontal outcomes [1,53], laser assisted periodontal treatment reported less morbidity [3,4].

All dental lasers available in the United States must have a United States Food and Drug Administration (FDA) 510(k) clearance for marketing. When evaluating dental lasers, it is important to be aware that FDA clearances are specific to the particular laser and specific to the indications were the laser can be applied. Different lasers are cleared by the FDA for different indications. For example, the Er:YAG laser has FDA clearance for hard tissue use in the oral cavity, and the Diode and CO_2 lasers for soft tissue use. The Nd:YAG laser using the LANAP protocol [6] the is the only laser listing periodontal regeneration [54] among its list of oropharyngeal applications that are FDA cleared for marketing in the United States. The specific FDA clearances for each laser device is listed in the operator manual of the laser device, and is specific to each laser manufacturer. It is important to note that further evaluation of the clinical applications and the efficacy of specific lasers are under the purview of the ADA Council on Scientific Affairs.

However, even though the selected studies were the only histological case-controlled *in vivo* human studies found on laser applications in periodontal therapy, the above findings should be interpreted with caution, because of the "definitely or probably high risk of bias" inherent in the selected studies (Table 2). In addition, the reported histologic outcomes may not predictably occur clinically. Furthermore, the histological methods of some of these studies may not comply with the ADA Technical Report No. 110: Standard Procedures for the Assessment of Laser-Induced Effects on Oral Hard and Soft Tissue [55].

It is also important to understand that the scope of this systematic review focused on the periodontal tissue response at a cellular level post-laser treatment. Clinical attachment gains post-laser treatment is not the focused question for this systematic review. Therefore, when evaluating a laser for oral use, the histological data must be considered in unison with the clinical data.

Conclusion

The applications for each type of laser is limited by its wavelength and the tissues that can absorb it. Thus, the clinician needs to be aware of which clinical area each type of laser is most effective histologically; and use the histological knowledge in unison with the clinical knowledge. Proper calibration of the lasers with respect to the exposure time, energy settings, and the amount of irrigation are also crucial to prevent injuries to the surrounding structures.

The following conclusions are within the limits of this systematic review and is focused on histological outcomes:

- Lasers may be possible adjuncts to scaling and root planning.
- Calculus removal may be possible with the Er:YAG lasers. However, the Er:YAG lasers may take more time than scaling and root planing to remove calculus.
- The Diode lasers, CO_2 lasers and Nd:YAG laser may be used to remove gingival tissue. However, in the treatment of periodontal disease, the calibrated Nd:YAG laser may be able to selectively ablate inflamed/diseased epithelium while sparing the healthy adjacent tissues.
- All the evaluated lasers can be used to reduce or eliminate periodontopathic bacteria in the short-term. Although the long-term anti-bacterial effects were not sustained, this short-term antibacterial effect may be a useful adjunct to periodontal therapy.
- Furthermore, if periodontal regeneration is a desired outcome, the Nd:YAG laser may be the only laser to report possible periodontal regeneration.

Conflict of Interests

MT, BH, SD, and SMB declare no competing interests regarding the content of this manuscript. JBS is a certified instructor for Millennium Dental Technologies for 2017 and 2018.

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