The effect of therapeutic and Nd:YAG laser as an adjunct treatment modality in periodontal therapy

Talat Qadri
THE EFFECT OF THERAPEUTIC AND ND:YAG LASER AS AN ADJUNCT TREATMENT MODALITY IN PERIODONTAL THERAPY

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Dedication

To my mother.  
She died on the day that I received my DDS degree. She struggled with her disease until she knew that her son had finished his education and became a dentist.
Abstract

Laser irradiation has been proposed as an adjunct to conventional scaling and root planing in the treatment of periodontitis. However, the reported outcomes of studies to date are contradictory and the literature provides limited evidence to support an additional benefit of laser application. The overall aim of the present thesis was to explore the potential of adjunctive application of therapeutic and surgical lasers to improve treatment outcomes, expressed in terms of clinical, radiographic and immunological parameters.

The present thesis is based on a series of four clinical studies of patients with moderately severe periodontitis, treated by scaling and root planing. Two different types of dental laser were investigated. Therapeutic lasers, which are claimed to stimulate cell regeneration and boost the immune system, were investigated in studies I and II: the general effect was investigated in Study I, while Study II compared the difference between gas and diode lasers in the same spectrum, in order to evaluate the importance of the length of coherence in biostimulation. In studies III and IV, the surgical Nd:YAG laser, which is usually applied for sulcular debridement and pocket decontamination, was evaluated in a novel approach. The test procedure comprised one single application of the laser with water coolant after conventional scaling and root planing. In study III, the outcome was evaluated after 3 months and in Study IV the long term outcome was evaluated, at least one year post-treatment.

The split mouth design was used in all four studies. Study I showed a better clinical outcome on the laser treated side and some improvement in immunological parameters. The results of Study II support the hypothesis that a laser with a long length of coherence is superior to one of a shorter length, although both lasers had some positive clinical effect. In Study III a single application of the Nd:YAG laser as an adjunct to scaling and root planing improved the short-term outcome and Study IV confirmed that this improvement was sustained.

In conclusion, the results of these studies confirm the potential role of laser irradiation as a non-invasive adjunctive to scaling and root planing in the treatment of periodontitis.

Key words: Low level laser, Nd:YAG laser, protease activity, coherence length, periodontal inflammation, cytokines, scaling and root planing.
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<th>Abbreviation</th>
<th>Definition</th>
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<tbody>
<tr>
<td>Aa</td>
<td>Aggregatibacter actinomycetemcomitans</td>
</tr>
<tr>
<td>bFGF</td>
<td>Basic Fibroblast Growth Factor</td>
</tr>
<tr>
<td>cfu</td>
<td>Colony forming units</td>
</tr>
<tr>
<td>EMD</td>
<td>Enamel Matrix Protein derivative</td>
</tr>
<tr>
<td>Er:YAG</td>
<td>Erbium Yttrium Aluminium Garnet</td>
</tr>
<tr>
<td>GaAs</td>
<td>Gallium Arsenide</td>
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<tr>
<td>GCF</td>
<td>Gingival Crevicular Fluid</td>
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<tr>
<td>HeNe</td>
<td>Helium Neon</td>
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<td>HSA</td>
<td>Human serum albumin</td>
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<tr>
<td>InGaAlP</td>
<td>Indium Gallium Aluminium Phosphide</td>
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<tr>
<td>LLLT</td>
<td>Low Level Laser Therapy</td>
</tr>
<tr>
<td>LPT</td>
<td>Laser phototherapy</td>
</tr>
<tr>
<td>mJ</td>
<td>Millijoule</td>
</tr>
<tr>
<td>mAbs</td>
<td>Milliabsorbance</td>
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<tr>
<td>MMP</td>
<td>Matrix metalloproteinase</td>
</tr>
<tr>
<td>Nd:YAG</td>
<td>Neodymium Yttrium Aluminium Garnet</td>
</tr>
<tr>
<td>ng</td>
<td>Nanogram</td>
</tr>
<tr>
<td>nm</td>
<td>Nanometer</td>
</tr>
<tr>
<td>ns</td>
<td>Nanoseconds</td>
</tr>
<tr>
<td>OPG</td>
<td>Osteoprotegerin</td>
</tr>
<tr>
<td>PBS</td>
<td>Phosphate buffered saline</td>
</tr>
<tr>
<td>pg</td>
<td>Porphyromonas gingivalis</td>
</tr>
<tr>
<td>PG</td>
<td>Prostaglandin</td>
</tr>
<tr>
<td>pg</td>
<td>Picogram</td>
</tr>
<tr>
<td>PMNL</td>
<td>Polymorphonuclear leukocytes</td>
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<tr>
<td>TGF</td>
<td>Transforming Growth factor</td>
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</table>
INTRODUCTION

LASER LIGHT

The word LASER is an acronym for Light Amplification by Stimulated Emission of Radiation. The first such device, a ruby laser, was introduced by Maiman in 1960 (http://laserstars.org/history/ruby.html). According to the European Standard IEC 601, the definition of a laser is: “Any device which can be made to produce or amplify electromagnetic radiation in the wavelength range from 180 nm to 1 mm primarily by the process of controlled stimulated emission.” Laser light has two unique characteristics: a very narrow band width and a high level of coherence.

Laser light is generally considered to be visible and collimated, *i.e.* travelling in a long, straight line. This is true for many lasers: the most well-known collimated laser is the laser pointer. However, medical lasers are generally neither collimated nor visible to the naked eye. In surgery, as with the carbon dioxide laser (10600 nm), the beam can be either focused for cutting or defocused for tissue ablation. Today lasers are widely used, even in domestic appliances and are basic components of modern technology. In medicine, lasers have been applied for decades in such diverse fields as surgery, ophthalmology and blasting of kidney stones.

In physics, coherence is a property of waves that enables stationary (i.e. temporally and spatially constant) interference. More generally, coherence describes all properties of the correlation between the physical quantities of a wave. Two waves can combine to create a larger wave (constructive interference) or detract from each other to create a smaller wave (destructive interference), depending on their relative phase. Two waves are said to be coherent if they have a constant relative phase (Figs.1,2). (http://en.wikipedia.org/wiki/Coherence_%28physics%29).

The degree of coherence is measured by the interference visibility, a measure of how perfectly the waves can cancel each other out by destructive interference. The beam may or may not be parallel and the intensity can vary from a fraction of a milliwatt to many watts. Coherence is reported to be important in biostimulation. It appears to have
an additional positive effect in laser surgery, but the main advantage of surgical lasers has little to do with the coherence.

**Figure 1. Coherent light**

The length of coherence varies considerably between different types of lasers. The shorter the bandwidth, the longer the length of coherence. The light from a gas-based laser such as the HeNe (632.8 nm), has a coherence length directly from the tube of many metres and a very narrow spectral bandwidth (Fig. 3). However, passage through an optic fibre reduces the length of coherence considerably. Diode lasers, such as the InGaAlP, can have a wavelength similar to the HeNe, but the length of coherence from a laser diode is considerably shorter.

**Figure 2. Incoherent light**

**Figure 3. Spectral bandwidth of different light sources**

From: Laser Therapy, Clinical Practice and Scientific Background. Prima Books AB, 2002
THERAPEUTIC LASERS

The first commercialised biostimulative laser was a HeNe laser of less than 1 mW. With its high degree of coherence the HeNe is an attractive laser for biostimulation but limited by the need for an optic fibre, the size of the machine and the still rather low power option, now typically in the range 5-25 mW. It has generally been replaced by the InGaAlP laser, a diode producing red laser in the range 600 - 700 nm and able to deliver as much as 500 mW. The most frequently used laser in dentistry is the GaAlAs laser. It often operates in the spectrum between 780 and 830 nm. The 808 nm diode dominates the market. Output is typically between 10 and 500 mW. An advantage of the diode lasers is the small size and option for battery operation, making them rather handy and portable. These lasers all work in continuous mode, but can be mechanically or electronically pulsed ("chopped"). The optical penetration of the light varies with several parameters. The short wavelengths in the red spectrum have less penetration than those in the infrared spectrum. The type of tissue also influences the penetration. Mucosa is rather transparent, bone and cartilage fairly transparent whereas penetration into muscles is poor, due to the thickness of the tissue and the high vascularisation. Blood is a major absorber of the light. Penetration also varies with distance from the laser source to the target tissue: contact irradiation forces the light into the tissue, while irradiation from a distance causes more reflection of the light.

The GaAs laser is different, being a superpulsed laser working at 904 nm. Superpulsed lasers produce very powerful, pulsed peaks in the Watt range, but the duration of the peak is typically only 200 nanoseconds. A GaAs laser presenting a Peak Power of 10 W typically has an average output of 10 mW. Pulsing is reported to be of importance in biostimulation, but the evidence to date is based entirely on in vitro studies (Karu, 2007). Little is known of the role of pulsing in clinical application.
Laser phototherapy (LPT) Mechanisms

To achieve an effect, the photon must be absorbed by photoreceptors. There are many photoreceptors in the human body, e.g. the porphyrins. However, the most important receptor has been identified as cytochrome c-oxidase, the terminal enzyme of the Kreb’s cycle. Cytochrome c-oxidase is an ATP producer (Passarella et al. 1984, Pastore et al. 1996, Karu 2007). A cell in a reduced condition can be revitalized by stimulating production of ATP. The laser light in the red spectrum severs the bond between NO and cytochrome c-oxidase, allowing the enzyme to initiate production of ATP (Huang et al. 2010). This production in itself leads to a cascade of events, such as increased permeability of the cell wall and the Ca\textsuperscript{2+} circulation. It has been speculated that infrared laser light bypasses this process and acts directly on the cell membrane permeability and the calcium ion channels. Cells in a normal redox situation are not particularly responsive to LPT: the best effect is seen in cells in a reduced redox situation (Almeida-Lopes et al. 2001). To date, studies of LPT have confirmed the effects as natural processes and no effects outside the box have been reported.
THE ND:YAG LASER

This type of laser produces light in a single crystal of Yttrium-Aluminium-Garnet with the addition of - for example - elemental neodymium (Nd). The full name of this laser is thus Neodymium-Yttrium-Aluminium-Garnet. Normally the laser is pumped by a very strong flash lamp. A new type of Nd:YAG laser is the diode laser pumped YAG:lasers, in which instead of a flash lamp, powerful GaAlAs lasers are used to pump optical energy to the Nd:YAG laser rod. The wavelength is 1064 nm. The light is distributed via optical fibres, typically 300-600 micrometers in diameter.

The pulses are always in the millijoule (mJ) range and both the number of pulses per second and the pulse length can be tailored by the operator to suit the intended target. Most Nd:YAG lasers do not have a water cooling system.

The Nd:YAG lasers are in the watt (W) range. For dental use they are always pulsed, each pulse providing a short energy in the millijoule range. The length of the pulse is measured in nanoseconds (ns). Thus, the actual energy at the tips is determined by several factors, such as basic output power, number of pulses per second and the pulse length. These are often pre-programmed on the laser but can be chosen individually to adapt to the situation or the experience of the operator. These parameters describe the energy applied: the dose (energy density) is also influenced by the size of the optical fibre. A thin fibre produces higher energy density at the tips: hence a 300 micron fibre has an energy density four times greater than that of a 600 micron tip. The use of water cooling will also influence the actual dose locally. Thus many parameters influence the actual energy delivered. In this context, the technique adopted by the operator is also an important determinant.

Modern dental Nd:YAG lasers are free-running and pulsed, in contrast to other continuous wave lasers with gated pulse options. The ablative capacity is set either by increasing the output power or the pulse repetition rate. The procedure is undertaken in tissue contact mode and in constant motion.

For pulsed lasers, peak powers are orders of magnitude higher than average powers. There are pronounced spikes, with peak power 1000 times higher than the average and relatively long rest periods. Pulse width (the duration of each pulse) varies from 90 to
1200 microseconds in different pulsed Nd:YAG lasers and is an important component of this technology. The number of pulses (frequency, pulse repetition rate) per second is one of the crucial variables in pulsed Nd:YAG lasers. With a high repetition rate from 10 to 100 Hz in different devices, smoother cutting can be achieved at a very low power setting, because the peak power in each pulse can be very high (White et al. 1994).

The 1064 nm wavelength is invisible, which complicates objective evaluation of the actual effected area. Observation made by the author, using an infra-red camera has revealed that the light is not concentrated around the fibre tip, but is spread like a small sphere over a rather large area.
The mechanisms underlying the Nd:YAG (surgical) laser

Nd:YAG laser energy is absorbed by tissue and it is this absorbance that allows surgical excision and coagulation of tissue (Goldstein et al. 1995). Absorption by different dental tissues is illustrated in Figure 5: absorption by hydroxyapatite is moderate. At this wavelength, the ablative effect on hard dental tissue is obviously rather low. This wavelength has a particular affinity for melanin or other dark pigments. Therefore dark-pigmented microbes are more sensitive to this laser and can be eliminated at quite low power settings, with no collateral damage to the adjacent tissue. The choice of wavelength is important to reach a bactericidal effect. Harris & Yessik (2004) developed a method for quantifying the efficacy of ablation of Porphyromonas gingivalis (Pg) in vitro for two different lasers. The ablation thresholds for the two lasers were compared in the following manner: Pg were cultured on blood agar plates under standard anaerobic conditions. Haemoglobin is a primary absorber of the wavelengths tested: thus in this context the blood agar simulated gingival tissue. Single pulses of laser energy were delivered to the Pg colonies and the energy density was increased until a small smoke plume was observed coincident with a laser pulse. The energy density at this point was denoted as the ablation threshold. Ablation thresholds to a single pulse were determined for Pg and for blood agar alone.

The investigation showed a major difference in ablation thresholds between the pigmented pathogen and the host matrix for pulsed Nd:YAG, representing a significant therapeutic window. Pg could be ablated without visible effect on the blood agar.

An 810 nm diode laser, on the other hand, destroyed both the pathogen and the gel. Clinically, the pulsed Nd:YAG may selectively destroy pigmented pathogens, leaving the surrounding tissue intact. The 810 nm diode laser may not demonstrate this selectivity due to its longer pulse length and greater absorption by haemoglobin (Harris & Yessik 2004).

It is postulated that the Nd:YAG laser eliminates primarily the dark-pigmented microbes, such as Pg, whereas Aggregatibacter actinomycetemcomitans (Aa) which
has no pigments, would not be similarly reduced. However, in a study by Andrade et al. (2008) Aa was completely eliminated directly after irradiation, but had regained approximately 50% of baseline level after 6 weeks. Such recurrence is reported in several studies and is attributed to cross contamination from non-treated pockets and/or saliva (Teughels et al. 2000).

The Nd:YAG laser has a certain biostimulative effect and this contributes to the enhanced postoperative healing after Nd:YAG laser surgery. The energy densities in the most peripheral zone (LPT) fall within the biostimulative range, as illustrated in figure 4.

**Figure 4.** Schematic illustration of the different light intensity zones (surgical lasers)
Courtesy: Edson Nagib
Negative thermal effects of Nd:YAG laser have been reported from in vitro studies (Liu et al. 1999, Israel et al. 1997). However, in vivo, effects on the root surface and the pulp are not well-documented (Gaspirc 2001; Schwarz et al. 2008). The effect of laser irradiation on the surrounding tissues is influenced by parameters such as power, pulsing, fibre size, fibre angulations and cooling/no cooling. A study by White (1994) suggested that powers between 0.3 to 3.0 W should not cause a damaging rise in intrapulpal temperature. Likewise, Gold and Vilardi (1994) and Spencer (1996) also reported that use of laser at 4 W is safe and does not damage the root surface.

Nd:YAG, which has little absorption in water, may be effectively cooled with simultaneous air and water spray. Lasers with limited transmission through enamel and dentine may also be effectively cooled by an air and water spray immediately after lasing. Several studies have confirmed that application of an air and water spray provides adequate heat protection to the pulp, comparable with cooling of the conventional rotary bur (Miserendino et al. 1994). The absorption in different dental tissues is illustrated graphically in figure 5.

Figure 5. The absorption spectrum for melanin, haemoglobin, enamel and water.
HISTORY OF MEDICAL AND DENTAL APPLICATIONS OF LASERS

The first laser to be used in medicine was a ruby laser (wavelength 694 nm) and it was soon applied in surgical procedures. The ruby is a solid state laser with a ruby rod as the lasering medium. The first gas laser for surgery was the carbon dioxide (CO₂) laser. It had several appealing features in that it was able to remove superficial tissue without harming the underlying tissues, due to the very high absorption of the 10600 nm in water. Although this laser was expensive and large, it was soon accepted as a useful tool in dental surgery, performing tissue ablation with a good degree of coagulation. Conditions such as haemangiomas, leukoplakias and fibromas could easily be ablated and malignancies could be removed surgically by focusing the beam. One of the first Scandinavian papers on this topic was an animal study published by Luomanen (1987).

The Nd:YAG laser was also readily adopted in medicine, especially in the field of ophthalmology. With a wavelength of 1064 nm, this laser could coagulate ocular bleeding in diabetics, among other things. Myers (1991) was the first to apply the Nd:YAG laser in dentistry: in fact, the first laser tested belonged to Myers' brother, an ophthalmologist. This laser proved useful for minor dental surgery, with a good coagulatory effect. An unexpected observation was that little or no analgesia was required. The laser could also be used to numb a tooth before drilling. Application as a substitute for the dental drill attracted much public attention, but was not a great success. To be absorbed into the dental hard tissues, a dark dye had to be applied to the tooth before drilling and the process was very slow. It was not until the advent of the Er:YAG lasers in the late 1990s that application of lasers for removal of hard dental tissue became more widely adopted. These versatile lasers can penetrate dental hard tissue at almost the same rate as a high-speed turbine drill. A major advantage is that little or no analgesia is necessary. Laser-based methods have also been introduced as aids for detection of early carious lesions, such as quantitative light-induced laser fluorescence, using a diode laser with 655 nm (Tranaeus et al. 2005).
The most recent additions to the dental laser family are the diode lasers. These typically emit at wavelengths of 808, 940 or 980 nm, with outputs ranging from 3-7 watts. The light is transmitted through an optical fibre. They are commercialised for soft tissue management but are also used for endodontic decontamination and sulcular debridement (Romanos et al. 2004). The diode lasers are much smaller than Nd:YAG and Er:YAG lasers and less expensive.

Originally, the lasers introduced for medical application were all surgical in that they were able to cut, evaporate and coagulate. However, another application was reported very early by McGuff et al. (1965), studying the potential effect of the ruby laser on tumours in hamsters. Different doses of ruby laser light were applied to various tumours implanted in the animals’ cheek pouches. The results were unexpected: the hamsters receiving laser light lived longer and even recovered completely, while none of the control hamsters survived. The underlying mechanisms were not clarified and the published papers do not appear to have attracted much attention. However, the results were noted by the Hungarian surgeon Endre Mester (1967), who undertook some basic experiments with a ruby laser on mice. The fur was shaved and wounds were created bilaterally (Fig. 6). One side was irradiated with low doses of ruby laser and the other side served as the control. Initially it was intended to increase the dose gradually, but it was soon discovered that the irradiated wounds healed faster than the non-irradiated wounds, while at higher doses the irradiation inhibited the wound healing. Even the shaved fur grew back more quickly on the irradiated side. This was the first documentation of the phenomenon of biostimulation. These lasers have then been applied for a great variety of indications, such as radiation induced mucositis (Bensadoun et al. 1999) and paresthesias of the inferior alveolar nerve (Khullar et al. 1996).
Safety and contraindications

The therapeutic lasers used in dentistry are classified as 3B, considered as low risk devices and according to Swedish authorities (Strålskyddsmyndigheten - SSM) may be used freely by anyone. Although the risk of eye injury is very low, protective goggles are nevertheless recommended for the patient. There is no harmful heating of the tissue when lasers are used in the recommended energy ranges. Since the limit of the ionising radiation is around 320 nm, there is no risk of cancer induction in tissues.

None of several alleged contraindications have been verified during 40 years of use. There are, however, some caveats. Due to the risk of stimulating malignant cells, laser irradiation should not be used over known malignancies. However, the use of the therapeutic laser is well documented for reducing the incidence of mucositis in patients receiving chemo- and radiation therapy. Laser treatment is also contraindicated in patients with coagulation disorders, because the effects of lasers on the mechanisms of coagulation have yet to be determined.
**Dosage**

To reach the dosage (also called fluence or energy density) the power of the laser must be known. The power is expressed in milliwatts (mW). The energy delivered is a function of the time. Thus, mW x seconds = energy. The energy is expressed in joules (J). For instance, a laser of 100 mW used for 10 seconds delivers 1000 mJ = 1 J.

The dose is a function of the size of the irradiated area, expressed in cm². For instance, if 1 J is applied to an area of 1 cm² the calculation is 1 J/1 cm² = 1 J/cm² (dose). However, if the irradiated area is 0.25 cm² the calculation is 1 J/0.25 cm² = 4 J/cm².

Another important factor in biostimulation is the power density, meaning the number of mW over an area. If the laser emits 100 mW over an area of 1 cm², the calculation is 100/1 = 100 mW/cm². If the area is only 0.25 cm² and receives the same number of mW, the calculation is 100/0.25 = 400 mW/cm². In laser phototherapy, it is important that all these variables are controlled, because each evokes different cellular reactions.

In the field of dentistry, the expression power density is quite familiar, because the “power” of the dental curing light is expressed in mW/cm².

LPT follows the Arndt-Schultz law, (Fig. 7) which stipulates that for every substance, small doses stimulate, moderate doses inhibit, and large doses destroy.

![Figure 7. Arndt-Schultz law in phototherapy](image)

From: Laser Therapy, Clinical Practice and Scientific Background. Prima Books AB, 2002
LASER PHOTOTHERAPY IN PERIODONTOLOGY

Inflammation

Local inflammation is the central process in gingivitis and periodontitis. Acute clinical manifestations include gingival swelling, redness and bleeding on probing. Inflammation is basically a functional reaction necessary to protect the body from bacterial invasion. Histologically an influx of leukocytes can be seen, primarily neutrophils and monocytes/macrophages. When the inflammation becomes more chronic the number of plasma cells and lymphocytes increases.

In the studies on which this thesis is based, clinical inflammation has been registered as the Gingival Index (Silness & Löe 1964). This index assesses a combination of swelling, redness and bleeding on probing. Changes in gingival pocket depth were also measured: initially these reflect changes in the inflammatory condition. To complement the clinical registration of inflammation, gingival crevicular fluid (GCF) volume has been measured. GCF is an exudate/transudate that continuously flows out of the gingival pocket. The volume increases with increasing inflammation and may thus be considered a surrogate marker of inflammation, that is more objective than clinical assessment of gingivitis (Golub & Kleinberg 1976).

To further assess the local inflammation a number of inflammatory mediators in GCF have been analysed. Interleukin-1β (IL-1β) is a proinflammatory cytokine that is released by many different cells, among them macrophages. IL-1β can be considered a general marker of the severity of inflammation in the tissues (Dinarello 2005). MMP-8 is a collagenase produced and released by several cells but mainly by neutrophilic granulocytes during their migration from the blood capillaries to the inflamed tissues (Sorsa et al. 2004). MMP-8 can thus be seen as an expression of neutrophil influx and as such as a marker of inflammation. Elastase is a protease typical for polymorphonuclear leukocytes (PMNL). It is mainly released from the neutrophils during phagocytosis and may be regarded as an indicator of neutrophil activation (Janoff 1985). IL-8 is a chemokine and an important inflammatory mediator released from endothelial cells (Gamonal et al. 2000).
In some cases the basically protective inflammatory response becomes tissue destructive, i.e. periodontitis. The reasons for this change from a protective to a tissue degrading inflammation is unclear but a Gram Negative anaerobic microflora together with a susceptible host is probably necessary. The Swedish Council on Health Technology Assessment estimates that signs of periodontitis are present in more than 40% of the Swedish adult population. Hugoson & Norderyd (2008) reported a 13% incidence of severe periodontitis, although this is regional and age-related. Periodontitis is more pronounced in those above the age of 40 years. Some forms of periodontitis are very aggressive and may result in rapid loss of periodontal attachment and destruction of alveolar bone. A major characteristic of the disease is the presence of bacteria in the gingival pocket. Conventional therapy aims at reducing the bacterial load and suppressing inflammatory signs through mechanical or chemical intervention, sometimes including antibiotics. The outcome of mechanical treatment may be compromised by the presence of furcations, invaginations and concavities. In these cases there is a need for an additional treatment approach.

Periodontitis is primarily an inflammatory process which generally causes only minor pain or discomfort. Thus scaling and root planing (SRP) are undertaken in order to remove calculus and granulation tissue adhering to the root surface, and to create conditions which facilitate maintenance of good oral hygiene. While SRP is considered to be fundamental periodontal treatment, it is not always completely successful and adjuvant therapies have been suggested.

In this context, laser therapy has been proposed, the goal being to target the inflammation. However, to date the scientific basis for this treatment modality is not well documented. The optimal parameters for each laser and for each particular intervention have yet to be determined.
**Therapeutic lasers**

Studies using *therapeutic lasers* have reported an effect on inflammation, mainly by shortening the inflammatory process which in itself is essential for healing (Choi *et al.* 2005, Pejcic *et al.* 2010). Sawasaki *et al.* (2009) and Silveira *et al.* (2008) reported significantly increased mast cell degranulation after 670 nm laser irradiation of human mucosa and gingiva, respectively. The degranulation leads to a release of histamine and should theoretically stimulate an increased inflammatory response. It is speculated that the increased mast cell degranulation accelerates the inflammatory process, which eventually leads to wound healing via fibroblast proliferation and collagen synthesis.

Chronic periodontal inflammation leads to the destruction of the periodontal ligament and subsequently to loss of alveolar bone. The latter is mediated primarily by osteoclasts and triggered by the pro-inflammatory molecule Prostaglandin E2 (PGE2) (Choi *et al.* 2005). There is some evidence in the literature that patients receiving LPT in conjunction with conventional periodontal treatment experience improvement in clinical inflammation (Pejcic & Zivkovic 2007).

Although gingivectomy is not a common procedure in modern periodontal therapy, studies by Amorim *et al.* (2006) and Özcelik *et al.* (2008a) report improved healing associated with application of 685 and 588 nm irradiation, respectively.

Garcia *et al.* (2009) compared LPT as an adjuvant to SRP for treatment of induced periodontitis in rats. Treatment was compared to dexamethasone or saline solution. Radiographic and histometric analysis showed less bone loss in animals treated with SRP + LPT. A study by Pires de Oliveira *et al.* (2008) has confirmed the stimulative effect of LPT on osteoblasts. Özcelik (2008) has reported positive effects of LPT in treating intra-bony defects with EMD – enamel matrix protein derivate.

Periodontal wound healing is an important phase when the composition and integrity of periodontal structures have been threatened by gingivitis, periodontitis or trauma. The restoration of fibrous attachment and lost bone requires regeneration of destroyed connective tissue, formation of new cementum and bone and attachment of new

Several in vitro studies have shown that LPT at certain wavelengths may stimulate fibroblast proliferation, provided that certain combinations of exposure parameters and power densities are used (Yu et al. 1994, Almeida-Lopes et al. 2001, Pereira et al. 2002, Azevedo et al. 2006). At higher energy densities, no effect or even decreased proliferation has been reported (Kreisler et al. 2003). Therefore, Karu (1990) suggested a "window-specificity" at certain wavelengths and energy densities, for which a positive effect of laser phototherapy can be expected.

An important aspect of laser-tissue interaction is the coherence of the laser light. Many studies have compared the biological effect of coherent and incoherent light and to date all studies indicate a superior effect by lasers producing a long length of coherence. Generally the comparisons have been made between lasers and Light Emitting Diodes (LED). These light sources have a spectral width of 30-100 nm, while the spectral widths of the lasers are in the range 0.01 – 1 nm. A study by Rosner et al. (1993) investigated the effect of HeNe laser on regeneration of crushed optical nerves. While HeNe laser delayed the degenerative process, non-coherent infrared light was ineffective or affected the injured nerves adversely.

Coherence seems to be an important parameter in light stimulation of biological scattering in bulk tissue. Karu et al. (1982, 1983) studied the importance of different light characteristics in cell stimulation, such as wavelength, coherence, dose and time regimens and concluded that coherence had no effect. However, in this context it is important to note that these studies were conducted in vitro on monolayers of cells: the cells were directly exposed to the laser and there was no scattering in the medium. As the laboratory conditions do not simulate the clinical setting, the results should be extrapolated with caution.
Nd:YAG laser

Nd:YAG lasers have been used in periodontal treatment for many years but consensus has yet to be reached about the general efficacy or the specific efficacy of different power settings and clinical techniques. An important part of the laser device, which is rarely discussed, is the optical fibre. Most bare fibres consist of a glass rod core made of silica quartz with an outer surface cladding of different refractive index, and an outer protective vinyl jacket. The standard options are diameters ranging from 200 to 600 micrometers. As the fibre diameter decreases, the energy densities increase and fibre flexibility increases. Thin fibres are popular because of the high power density but less than ideal for closed curettage, because they are prone to fracture and the energy density is too high. The energy density of a 300 micrometer fibre is four times as high as that of a 600 micrometer fibre. Thus, the use of a thin fibre in a closed area has disadvantages. The high power densities will char areas in the pocket and carbonized tissue will adhere to the tip. In the dark carbonized areas, absorption of the light increases and so does heat. The aim of the laser treatment is not to use the instrument for cautery, but to take advantage of the interaction between the light and the specific tissue irradiated. Further to that, a thicker diameter makes the fibre stronger and difficult-to-reach areas can be accessed more readily.

A major advantage of Nd:YAG laser periodontal therapy is that the procedure is relatively pain free. From the patient’s perspective this is certainly a major advantage. The degree of pain is largely determined by the skill of the operator. However, in some cases an analgesic gel or spray is advisable during the initial phase of the surgery. After a while, it seems that the laser in itself provides an anaesthetic effect. Sulcular debridement around hypersensitive teeth may sometimes be painful. In these cases, the tooth crown can be irradiated from a short distance without water until an anesthetic effect of the pulp is achieved. For the same reason, no water should be used when hypersensitive tooth necks are treated with Nd:YAG laser. In combination with water the area will be cleaned and the tubuli even more open. Without water there is the potential for the laser to seal the tubuli (Lan & Liu 1996).

In general it can be stated that correctly applied, the lasers themselves are not dangerous or damaging. It is the lack of knowledge that creates damage. The undesirable side effects can vary primarily with power and energy density and secondly with the type of laser used.
AIMS

GENERAL AIMS OF THE THESIS

Several potential roles have been proposed for laser application in periodontal treatment but the reported outcomes of studies to date are contradictory. The available data are inadequate for recommendations with respect to optimal laser treatment parameters.

The present thesis is based on a series of clinical studies of patients with moderately severe periodontitis, treated by scaling and root planing. The studies were undertaken with the overall aim of evaluating the potential of adjunctive application of therapeutic and surgical lasers to improve the short and long-term treatment outcomes, expressed in terms of clinical, radiographic and immunological parameters. Such studies are essential in order to provide evidence on which to base recommendations for clinical application.

Four studies were undertaken, the first two on therapeutic lasers and the third and fourth studies on the Nd:YAG (surgical) laser.

SPECIFIC AIMS

The specific aims of the four studies were as follows:

Study I: to examine the effects of irradiation with laser phototherapy on inflamed gingival tissue

Study II: to determine the possible influence of the length of coherence in laser phototherapy

Study III: to compare the outcome of treatment of periodontitis by combined SRP and a single application of water-cooled Nd:YAG laser irradiation with that of SRP alone

Study IV: a follow-up study of Study III, to determine whether the positive advantages of the laser treatment were sustained over a longer time period
MATERIAL AND METHODS

The following is a brief description of the materials and methods used in the four studies. Detailed descriptions of the material and methods are presented in the original papers (I-IV).

**Periodontal Examination**

Periodontal evaluation included PI (Plaque Index, Løe 1967) and GI (Gingival Index, Silness & Løe 1964). PPD (Probing Pocket Depth) was measured with a graded periodontal probe (PerioWise, Premier Dental, Plymouth Meeting, PA, USA) at 4 sites (mesial, distal, buccal and lingual). In studies I and II, the maxillary teeth, from 17 to 13 and 27 to 23 were registered. In studies III and IV, all the mandibular teeth, except for the third molars, were registered.

**Microbiological Examination**

Subgingival plaque was harvested from the same site as GCF samples, by inserting sterile paper points (size 30) for 30 seconds. The paper points from each side were then pooled in sterile transport vials and sent to the laboratory for analysis. The subgingival microbiota was analysed using a checkerboard DNA-DNA hybridization method (Papapanu et al. 1997) and the frequencies of positive sites and of sites with cfu $\geq 10^6$ were recorded. The following 12 micro-organisms were analysed: *Porphyromonas gingivalis, Prevotella intermedia, Prevotella nigrescens, Tannerella forsythensis, Aggregatibacter actinomycetemcomitans, Fusobacterium nucleatum, Treponema denticola, Peptostreptococcus micros, Selenomonas noxia* and *Streptococcus intermedia*.

**Gingival Crevicular Fluid (GCF)**

In all subjects, two GCF samples were taken from each side of the maxilla, after removal of supragingival plaque from the site to be sampled. The sites were isolated with cotton rolls and gently dried with an air syringe before sampling. To collect GCF, prefabricated paper strips (Periopaper, Oraflow Inc., Plainview, NY, USA) were inserted until resistance was felt and removed after 30 seconds. GCF volume was measured with a calibrated Periotron 8000 (Oraflow Inc). Samples were pooled and
diluted in phosphate buffered saline (PBS) up to 1 ml. After elution for 15 minutes, the strips were removed and the samples frozen at -20°C.

**Laboratory analyses**

**Studies I and II**

**IL-1β**

The IL-1β content of the GCF samples was measured with sandwich ELISA, using a monoclonal antibody (MAB 601, R&D Systems, Minneapolis, MN, USA) diluted 125 times in carbonate buffer, coated onto microtitre plates (Nunc Maxisorb Nanc A/S Roskilde, Denmark) overnight at + 4°C. The plates were blocked with 1 % human serum albumin (HAS) for 1 hour in room temperature. The detection antibody (BAF 201, R&D Systems), a biotinylated polyclonal goat antibody diluted 250 times, was incubated for 45 min at 37°C. After washing, horseradish peroxidase conjugated streptavidine, diluted 200 times in PBS +0.1% HSA, was added to the plates and incubated in the same way as for the detection antibody.

The plates were washed again and the undiluted substrate (TMB, Sigma Chemical, St. Louis, MO, USA) added. The reaction was stopped with 1M H₂SO₄ after 15 minutes. Absorbency was read at 450 nm in a spectrophotometer (Millenia Kinetic Analyser, Diagnostic Product Corporation, Los Angeles, CA, USA).

**Elastase Activity**

Total elastase activity was measured with a chromogenic substrate specific for granulocyte elastase (Tanaka et al. 1990), (L-pyroglutamyl-L-propyl-L-valine-p-nitroaniline, mw 445.5 Da, on a 96-well microtitre plate (Nunc Maxisorb, Nanc A/S). After 2 h of incubation at 37°C, absorbency was read for a second time. The total elastase activity is expressed in mAbs (milliabsorbances).

**MMP-8 & IL-8**

MMP-8 & IL-8 were analysed with commercial kits (Quantikine ®, R&D Systems Inc.) in accordance with the manufacturer’s instructions. A monoclonal antibody specific for MMP-8 had been pre-coated on to a microplate. Samples diluted 10 times
were pipetted into the wells and incubated at room temperature for 2 h. The plates were then washed and a monoclonal antibody against MMP-8, conjugated to horseradish peroxidase, was added and incubated again, as described previously. After another washing procedure, the substrate solution was added and the reaction stopped after 15 min. with a stop solution. Within 20 min., the absorbency at 450 nm was read in a spectrophotometer. The MMP-8 was expressed in ng and the amount of IL-8 in pg.

Study III

**IL-1β, 4, 6, 8 and MMP-8**

IL-1β, IL-4, IL-6 and IL-8 were analysed with Multiplex bead kits, using a Luminex 100 (Luminex Corp., Austin, TX, USA) and commercial immunoassays, Lincoplex high-sensitivity human cytokine panel (Lincoplex/Millipore, St. Charles, MO, USA) according to the manufacturer’s instructions. The result was calculated with Bio-Plex Manager software (Bio-Rad Laboratories, Hercules, CA, USA) and the cytokine levels were determined as the total amount per site (pg) in the fluid. The collagenase MMP-8 was similarly analysed, but with a kit from R&D Systems (Abingdon, UK).

**Radiographs**

Digital bite-wing radiographs (Siemens, Bensheim, Germany) were taken with the vertical long axis of the hemi-mandible using a software programme (Schick Technologies Inc., NY, USA).

In Study IV all radiographs were taken by the author. Two observers recorded baseline and post-operative mandibular alveolar bone levels, in millimetres, at all approximal surfaces, from the mesial of the second molar to the distal of the canine. Alveolar bone loss was measured from the cemento-enamel junction (CEJ) to the most apical portion of the alveolar bone. Teeth with suspected or obvious carious lesions at the CEJ were not included.

**Statistical methods**

In studies I & II, statistical analyses were performed using Statistica 7 (Statsoft Inc., 2005, Tulsa, USA).
In Study I, the significance of the differences in treatment effect between placebo and laser was calculated with the Student paired t-test or the Wilcoxon signed rank test. The frequencies of positive subjects and of subjects with $\geq 10^6$ cfu of the analysed bacteria were calculated with Fisher's exact test.

In Study II, the significance of the differences in treatment effect between the two lasers was calculated with the Wilcoxon signed rank test.

In studies III and IV statistical analyses were performed using Statistica v.6.0 (Statsoft Inc., 2005, Tulsa, USA).

In Study III, changes in the clinical parameters from baseline to follow-up, and between the treatment modalities, were assessed for statistical significance using a paired t-test. The laboratory data were analysed using the Wilcoxon signed rank test. Significance was set at p<0.05.

In Study IV, the paired t test was applied to assess the changes in clinical parameters from baseline to follow-up and between the treatment modalities. Normality was tested with the Kolmogorov-Smirnov test.
THE LASERS USED

Study I
A hand held, battery-operated Combi laser (Lasotronic AG, Baar, Switzerland) was used. The device has two wave lengths that can be used together or separately. In this study the wave lengths were utilized separately. Two lasers of identical appearance were used in the study: (Fig. 8) one active and one placebo, the latter having only a weak red LED diode instead of laser power. The active laser had two wavelengths, 635 and 808 nm, respectively. The output at 635 was 10 mW and at 808 nm 70 mW.

Figure 8. Active and placebo lasers

Study II
The lasers used in this study were a 3 mW HeNe laser 632.8 nm from Irradia AB, Stockholm, Sweden and a Pocket Therapy diode laser, nominally 650 nm, from Lasotronic AG, Baar, Switzerland (Fig. 9). Both had equal power of 3 mW.

Figure 9. The HeNe and the diode laser
**Studies III and IV**

The laser used in Study III and IV was a Nd:YAG (Genius 9 SLD) laser, emitting pulsed light 1064 nm, a fixed pulse repetition rate of 50 Hz, output from 1 W to 12 W and coolant water and air levels available from 1 to 15. The fibre diameter was 600 micron (Genius Dental A/S, Tureby, Denmark).

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**Summary of the four studies**

<table>
<thead>
<tr>
<th>I</th>
<th>Clinical study, double blinded</th>
<th>Plaque Index, Gingival Index, Pocket Depth, Gingival Crevicular Fluid, MMP-8, IL-1ß, elastase, 12 bacterial species</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Split mouth</td>
<td>Clinical, immunological and bacteriological outcome</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>II</td>
<td>Clinical study</td>
<td>Plaque Index, Gingival Index, Pocket Depth, Gingival Crevicular Fluid, MMP-8, IL-8, elastase, 12 bacterial species</td>
</tr>
<tr>
<td></td>
<td>Split mouth, double blinded</td>
<td>Clinical, immunological and bacteriological outcome</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>III</td>
<td>Clinical study, single blinded</td>
<td>Plaque Index, Gingival Index, Pocket Depth, Gingival Crevicular Fluid, MMP-8, IL-1ß, IL-4, IL-6, IL-8.</td>
</tr>
<tr>
<td></td>
<td>Split mouth</td>
<td>Clinical and immunological outcome</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>IV</td>
<td>Clinical study, single blinded</td>
<td>Plaque Index, Gingival Index, Pocket Depth, Gingival Crevicular Fluid, marginal bone loss</td>
</tr>
<tr>
<td></td>
<td>Split mouth</td>
<td>Radiological outcome</td>
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<tr>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>
TREATMENT METHODS

Ethical Approval
These studies were approved by the regional ethical review board in Stockholm, Sweden. All subjects gave their written informed consent before inclusion.

Study I
Seventeen patients with moderate periodontitis were included, 10 women and 7 men. After clinical examination, all teeth were scaled and root planed (SRP). Oral hygiene instructions were given and controlled at each session. Baseline measurements were: Pocket Depth, Gingival Index and Plaque Index, all recorded before SRP. One week after SRP, samples of gingival crevicular fluid (GCF) and subgingival plaque were collected.

The laser therapy started one week later and continued once a week for 6 weeks. One side of the upper jaw was treated with the active laser and the other with the placebo unit.

The treated areas were:

1. The buccal papillae, with 635 nm for 90 seconds (0.9 Joule, 4.5 J/cm², 50 mW/cm²)

2. 6 mm further apically, with 830 nm for 25 seconds (1.75 Joules, 8.75 J/cm², 350 mW/cm²)

3. The sites were irradiated from both buccal and lingual aspects.

After the 6th week, the subjects underwent clinical re-examination, and new GCF and plaque samples were collected.

Study II
The study sample comprised twenty patients with moderate periodontitis. After clinical examination, all teeth were scaled and root planed (SRP). The dental hygienist now started the laser therapy, once a week for 6 weeks. One side of the maxilla was treated with HeNe laser and the other with a diode laser: choice of laser was determined by the toss of a coin. Each dental papilla on the teeth 13, 14, 15, 16, 23, 24, 25 and 26 was irradiated from the buccal aspect and 16 and 26 were also irradiated from the lingual
aspect. All irradiated sites received 0.54 J of energy per session, total energy per quadrant 3.25 J.

Studies III & IV
SRP + laser (SRPL) were used on one side of the mandible and the other was treated by SRP alone. Thirty patients (13 males and 17 females) with a mean age of 50 years (range 26 to 70 years) were originally included and randomly assigned to left or right side. The treatment outcome was evaluated after 3 months.

The laser used in this study was a Genius 9 SLD Nd:YAG (Genius Dental A/S, Tureby, Denmark), emitting pulsed light at a wavelength of 1064 nm. To avoid a thermal effect while maintaining optimal therapeutic effect, the instrument was set at level-five, giving the following parameters: average output 4 watt (W), energy per pulse 80 millijoule (mJ), pulse width 350 microseconds (µs), pulse repetition rate 50 Hertz (Hz), pulse peak power 240 W, average power density at fibre end 1430 W/cm² and peak power density 85800 W/cm². Laser energy per treated tooth was 240 – 480 joules (J). The fibre diameter was 600 µm (0.002826 cm²). Water and air cooling were used during irradiation. The time spent on each tooth varied between 60 to 120 seconds, depending on accessibility.

The fibre was held in constant motion, in contact with the pocket epithelial lining almost parallel to the long axis of the root. The power density and peak power density reported above are calculated by a hypothetical 100% emission through the small fibre tip. However, the energy is not emitted solely from the tip of the fibre; there is also considerable lateral emission. Due to the high uncertainty about the total area of tissue irradiated, the energy density (J/cm²) was not calculated.
RESULTS

None of the participants reported any adverse side effects that could be related to the laser irradiation.

Study I

The results were as follows:
All clinical variables (PPD, PI, GI) showed greater reduction on the laser side (p<0.02). The GCF volume decreased more on the laser side, -0.15 µl, compared to the placebo side, -0.05 µl (p<0.02).

Figure 10. Box plot (above) shows the reduction in the clinical variables probing pocket depth (PPD), plaque index (PI) and gingival index (GI) after SRP and an additional treatment with laser or placebo. Filled boxes indicate the laser side.
Table 1. Change in GCF volume (mean SD) and the laboratory variables (median range) elastase activity, total amount of IL-1β and MMP-8 in samples taken before and after treatment with laser or placebo, n=17 patients

<table>
<thead>
<tr>
<th></th>
<th>GCF Volume µl</th>
<th>Elastase activity mAbs</th>
<th>IL-1β pg</th>
<th>MMP-8 pg</th>
</tr>
</thead>
<tbody>
<tr>
<td>Placebo</td>
<td>-0.05</td>
<td>-9 (-576 - 252)</td>
<td>-1.7 (57.9 - 24.7)</td>
<td>90 ((8180 - 5859)</td>
</tr>
<tr>
<td>Laser</td>
<td>-0.15</td>
<td>32 (23 to 160)</td>
<td>-0.8 (24.4 - 82.8)</td>
<td>-70 (510 - 1145)</td>
</tr>
<tr>
<td>P-value</td>
<td>0.015*</td>
<td>0.15**</td>
<td>0.45**</td>
<td>0.052**</td>
</tr>
</tbody>
</table>

* p value calculated with the Student’s paired t-test
** p-value calculated with Wilcoxon’s signed rank test.

The concentration of MMP-8 increased on the placebo side and was somewhat reduced on the laser side. The difference in treatment effect did not quite reach statistical significance (p=0.052). No differences were disclosed between laser and placebo sides with respect to elastase activity, IL-1β concentration or microbiological analyses.
Study II

All clinical variables (PPD, PI, GI) showed greater reduction on the HeNe side (p-value = 0.001).

**Figure 11.** Box plot showing the reduction in the clinical variable probing pocket depth after SRP and an additional treatment with HeNe or diode lasers. Filled boxes indicate post treatment registrations.

**Figure 12.** Box plot showing the reduction in GCF volume after SRP and an additional treatment with HeNe or diode lasers. Filled boxes indicate post treatment registrations.
Figure 13. Box plot showing the clinical variables plaque index (PI), before and after SRP and an additional treatment with HeNe or diode laser. Filled boxes indicate post-treatment registrations.

Figure 14. Box plot showing the clinical variable gingival index (GI), before and after SRP and an additional treatment with HeNe or diode laser. Filled boxes indicate post-treatment registrations.
Study III

Clinical outcomes
One week post-treatment, the PI (p<0.05), PPD (p<0.001) and GCF volumes (p<0.001) on the irradiated side had decreased significantly compared to the control side. The GI also decreased at the test side but the difference did not reach significance (Table 1). The three-month follow-up confirmed that the improvements were sustained. The treatment outcomes for the test side had improved significantly compared to the control-site (PPD [p<0.01], GI [p<0.01], PI [p<0.01] and GCF volume [p<0.05]) (Table 2). During the three-month follow-up, the mean PPD decreased by 0.6 mm on the test side compared to the control side.
Table 2. Clinical parameters (mean ± SD) in 30 patients with periodontitis. Change 1 indicates changes from baseline to one week follow-up and Change 2 from baseline to three months.

<table>
<thead>
<tr>
<th>Variable</th>
<th>Baseline</th>
<th>1 week</th>
<th>Change 1</th>
<th>3 months</th>
<th>Change 2</th>
<th>Baseline</th>
<th>1 week</th>
<th>Change 1</th>
<th>3 months</th>
<th>Change 2</th>
</tr>
</thead>
<tbody>
<tr>
<td>Pocket depth (mm)</td>
<td>4.41 (0.27)</td>
<td>3.98 (0.42)</td>
<td>-0.43 (0.50)</td>
<td>3.57 (0.48)</td>
<td>-0.84 (0.59)</td>
<td>4.59 (0.44)</td>
<td>3.61 (0.48)</td>
<td>0.96 (0.44)</td>
<td>3.12 (0.60)</td>
<td>-1.47 (0.46)</td>
</tr>
<tr>
<td>Scping and root planing (SRP)</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td>Scping and root planing (SRP) + laser irradiation</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Plaque index</td>
<td>1.96 (0.10)</td>
<td>1.26 (0.26)</td>
<td>-0.70 (0.59)</td>
<td>1.46 (0.57)</td>
<td>-0.48 (0.60)</td>
<td>2.02 (0.65)</td>
<td>1.05 (0.73)</td>
<td>0.97 (0.67)</td>
<td>1.11 (0.69)</td>
<td>-0.51 (0.38)</td>
</tr>
<tr>
<td>Gingival index</td>
<td>1.97 (0.53)</td>
<td>1.46 (0.54)</td>
<td>-0.51 (0.58)</td>
<td>1.53 (0.54)</td>
<td>-0.43 (0.55)</td>
<td>2.11 (0.65)</td>
<td>1.40 (0.58)</td>
<td>0.72 (0.59)</td>
<td>1.10 (0.60)</td>
<td>-1.12 (0.76)</td>
</tr>
<tr>
<td>GCF volume (µl)</td>
<td>1.40 (0.31)</td>
<td>1.53 (0.24)</td>
<td>-0.13 (0.30)</td>
<td>1.26 (0.41)</td>
<td>-0.14 (0.45)</td>
<td>1.44 (0.38)</td>
<td>1.12 (0.43)</td>
<td>-0.32 (0.47)</td>
<td>1.04 (0.41)</td>
<td>-0.40 (0.67)</td>
</tr>
</tbody>
</table>

p-values show the significance of the differences between the two groups, calculated with the paired t-test.

Table 3. Levels (median and interquartile range) of cytokines in pooled GCF samples (n=30). Change 1 indicates change from baseline to one week. Change 2 indicates change from baseline to three months.

| Cytokines (pg) | Baseline | 1 week | Change 1 | 3 months | Change 2 | Baseline | 1 week | Change 1 | 3 months | Change 2 |
|----------------|----------|--------|----------|----------|----------|----------|--------|----------|----------|----------|----------|
| IL-1β          | 0.32 (0.89) | 0.42 (0.34) | 0.02 (0.46) | 0.18 (0.33) | -0.20 (0.78) | 0.40 (1.35) | 0.24 (0.71) | -0.26 (1.66) | p<0.05   | 0.12 (0.71) | -0.08 (0.77) |
| IL-4           | 0.66 (0.84) | 0.21 (1.36) | -0.40 (1.07) | 0.23 (2.01) | -0.09 (0.69) | 0.31 (2.81) | 0.54 (2.94) | -0.16 (0.33) | p<0.05   | 0.09 (2.17) | -0.17 (0.31) |
| IL-6           | 0.08 (0.49) | 0.06 (0.31) | 0.00 (0.32) | 0.01 (0.08) | -0.00 (0.40) | 0.10 (0.56) | 0.01 (0.70) | 0.00 (0.43) | 0.02 (0.20) | 0.00 (0.38) |
| IL-8           | 84.6 (88.8) | 89.0 (86.9) | -5.4 (41.6) | 59.0 (85.2) | -14.7 (16.6) | 100.0 (95.8) | 44.6 (74.9) | -33.0 (100.9) | 45.6 (81.4) | -23.5 (75.9) |
| MMP-3          | 7.06 (29.5) | 9.60 (33.2) | 1.56 (9.4) | 5.70 (14.0) | -1.89 (31.4) | 12.93 (7.4) | 6.91 (29.4) | -5.61 (23.9) | p<0.05   | 2.70 (14.8) | -4.88 (14.9) |

p-values indicate significance of difference between the two treatment regimes (SRP compared to SRP plus Nd:YAG Laser)
Study IV

Clinical and radiological results: At the follow up examination, PI (p<0.01), GI (p<0.01) and PPD (p<0.001) were significantly lower on the test side than on the control side. Radiological results showed a significant reduction in marginal bone loss on the test side compared to the control side (p<0.05).

Gingival crevicular fluid volume: GCF volume was significantly lower on the test side (mean change: -0.57 µl, range: -0.4 µl to 1.68 µl) than on the control side (mean change: 0.15 µl, range: -0.12 µl to 1.11 µl) (p<0.01). These results are summarized in Table 4: clinical and laboratory outcomes.

<table>
<thead>
<tr>
<th>Peridontal Variables</th>
<th>Control Site (SRP alone)</th>
<th>Test Site (SRP with Nd:YAG laser)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Baseline (mean ± SD)</td>
<td>20-months follow-up (mean ± SD)</td>
</tr>
<tr>
<td>Probing pocket depth</td>
<td>441 ± 0.31</td>
<td>386 ± 0.76</td>
</tr>
<tr>
<td>Plaque index</td>
<td>1.36 ± 0.66</td>
<td>2 ± 0.71</td>
</tr>
<tr>
<td>Gingival index</td>
<td>1.97 ± 0.34</td>
<td>2.18 ± 0.36</td>
</tr>
<tr>
<td>Marginal bone loss</td>
<td>204 ± 0.49</td>
<td>216 ± 0.53</td>
</tr>
<tr>
<td>GCF volume</td>
<td>1.41 ± 0.34</td>
<td>1.53 ± 0.42</td>
</tr>
</tbody>
</table>

† p<0.001  * p<0.01  # p<0.05
DISCUSSION

Although lasers have been used in dentistry for many years, systematic reviews of the literature report inadequate evidence to support their application in treatment of periodontal disease. In the series of clinical studies on which this thesis is based, the subjects comprised patients with moderately severe periodontitis, who underwent conventional treatment by scaling and root planing. The split-mouth studies then evaluated the potential of adjunctive application of therapeutic or surgical lasers to improve the short and long-term treatment outcomes. Clinical, microbiological and immunological parameters were recorded.

In the four studies undertaken, the first two using multiple applications of therapeutic lasers and the third and fourth using a single application of the Nd:YAG (surgical) laser, the overall results confirmed the beneficial effect of laser irradiation of the tissues after scaling and root planing. Sites which received laser irradiation exhibited improved clinical parameters and positive responses in terms of changes in inflammatory markers in gingival crevicular fluid. Moreover, in Study IV, the long-term outcome of a single application of the Nd:YAG laser also showed some gain in alveolar bone levels.

The initial study in the series confirmed that as a complement to SRP, LPT can reduce gingival inflammation. Adjunctive laser treatment resulted in significantly better clinical variables such as PPD, PI, GI, and GCF than SRP alone. The decrease in plaque index was also greater on the LPT side; this is in agreement with a study by Iwase et al. (1989). Significant decreases in GI and PPD have also been reported by Kiernicka et al. (2004). Ribeiro et al (2008) reported that LPT following SRP reduces gingival inflammation and MMP-8 expression, while histological examination showed a reduction in inflammatory cells. However, there are also some contradictory reports on the effectiveness of LPT. Rydén et al (1994) and Yilmaz et al. (2002) reported that LPT alone did not have an effect on the inflammatory response. Direct comparisons of studies are however, difficult, due to differences in wavelengths, energy output and irradiation mode. Further to that, Rydén et al. treated experimental gingivitis in healthy individuals. Such experiments, using healthy animals or humans have recently been
questioned. The genetically diabetic rat has, for instance, been a better model (al-Watban *et al.* 2007).

Study I showed that MMP-8 decreased on the LPT side and increased on the SRP-only side, but the change did not reach significance (p=0.052). This in accordance with studies by Luza & Hubacek (1996) and Fujimaki *et al.* (2003).

The microbiota were unchanged in both studies I and II. This may be due in part to the timing of the sampling, after SRP, when the microbial load was already lowered. LPT in itself does not have any bactericidal effect, but stimulation of macrophages (el Sayed & Dyson 1996) could lead to phagocytosis and reduced bacterial load.

Ozawa *et al.* (1997) showed that LPT significantly inhibits the increase of plasminogen activator (PA) induced in human periodontal ligament cells in response to mechanical tension force. PA is capable of activating latent collagenase, the enzyme responsible for cleaving collagen fibers. LPT was also efficient in the inhibition of PGE₂ synthesis. In human gingival fibroblast culture, LPT significantly inhibited PGE₂ production stimulated by lipopolysaccharide (LPS) through a reduction of COX2 gene expression in a dose dependent manner. The decrease on PGE₂ levels in cultures of primary human periodontal ligament cells was also verified after cell mechanical stretching. Nomura *et al.* (2001) verified that LPT significantly inhibited LPS-stimulated IL-1β production in human gingival fibroblasts cells, and that this inhibitory effect was dependent on irradiation time.

Safavi *et al.* (2008) evaluated the effect of LPT on gene expression of IL-1β, interferon γ (IFN-γ) and growth factors (PDGF, TGF-β and bFGF) to provide an overview of laser influence on their interactive role in the inflammatory process. The findings suggest an inhibitory effect of LPT on IL-1β and IFN-γ production and a stimulatory effect on PDGF and TGF-β. These changes may be explained the anti-inflammatory effects of laser and irradiation and its positive influence on wound healing.

Arany *et al.* (2007) in a study of the latent growth factor complex Transforming Growth Factor-β (TGF-β), a multifaceted cytokine reported that the latent form can be activated by LPT.
The findings of the above studies, describing different pathways of inflammatory modulation, support the hypothesis explored in Study I that LPT can modulate the periodontal inflammatory process, especially through the reduction of PGE$_2$ release. In summary, LPT influences the expression of COX2 and IL-$1\beta$, as well as MMP8, PDGF, TGF-$\beta$, bFGF and plasminogen. However, the capacity of LPT to modulate inflammation does not seem to be confined to a single mechanism or to specific wavelength, fluence or power: the different parameters tested in various studies gave divergent results.

Study II demonstrated the importance of the coherence length of laser light. The clinical signs of inflammation were significantly decreased on the HeNe laser side (longer coherence length) compared to the diode laser side (short coherence length). Several studies comparing the biological effects of coherent and non-coherent light have reported that coherent light is superior (Hode 2005). In a study of regeneration of crushed optical nerves, the HeNe laser delayed the degenerative process, while non-coherent infrared light was ineffective (Rosner et al. 1993). Similar conclusions have been drawn from other studies (Haina et al. 1973, Rochkind et al. 1989). It is claimed that coherent light is even more effective in deeper structures (Hode 2005). The cited studies compared coherent and non-coherent light, which has in fact a coherence length, albeit very minor. In Study II, two different coherent light sources of different coherence length were compared. The results confirmed the hypothesis that coherence length is an important determinant in laser phototherapy.

With respect to which wavelength best promotes cell proliferation, contradictory results are reported. However, other factors besides wavelength and the energy dose are important determinants of cell growth stimulation. Azevedo et al. (2006) tested two power densities (428.57 and 142.85 mW/cm$^2$) at the same energy density (2 J/cm$^2$) and showed that a lower power density caused higher stimulation. Moreover, the mode of exposure, pulsing or continuous, may also play a role in optimizing stimulation. The number of irradiation sessions and the treatment schedule will also influence the outcome. The power densities used in studies I and II are low and impractical from a clinical perspective. However, the design of the studies took into account
recommendations in the literature (Huang et al. 2010), that the use of low power densities over a longer treatment time would give an optimal outcome.

While pain is not a characteristic feature of chronic periodontitis, it is of major concern after SRP. LPT application can decrease the pain sensation. However, the applied dose must be considered closely. An approximate dose range of 2-6 J/cm² is considered optimal for wound healing and 6-10 J/cm² for hastening the inflammatory process. A shortening of the inflammatory process will in itself reduce the period of pain perception. A larger dose will cause an inhibition of neural transmission and a rapid decrease of pain (Chow et al. 2007). This dose is however, inhibitory for wound healing and will prolong the inflammatory process. In this context, it is important that the clinician understands the rationale underlying the laser application and is familiar with appropriate dose ranges.

Disruption of collagen fibres in the periodontal ligament is attributed mainly to the two collagenases MMP-1 and MMP-8. MMP-8 is released primarily from polymorphonuclear leukocytes (PMNL) and secreted predominantly into the GCF: thus MMP-8 levels in a GCF sample reflect the number of PMNL present and is an expression of the severity of inflammation (Tervahartiala et al. 2000). IL-1β is a pro-inflammatory cytokine released mainly from monocytes/macrophages, and is present in the gingival tissues and GCF of patients with periodontal inflammation. Laser irradiation is associated with significantly greater reductions in MMP-8 and IL-1β (Liu et al. 1999).

Thus laboratory analyses confirm the clinical signs of improved healing at these sites. The Liu study cited above compared the effects of SRP and SRP plus Nd:YAG laser on the laboratory markers of periodontal inflammation. The six to 12 week follow-up results showed a significant reduction in IL-1β levels after treatment with SRP plus Nd:YAG laser compared to treatment by SRP alone. Similar results have been reported by (Choi et al. 2004 and Ge et al. 2008).

The present studies disclosed no differences between SRP and SRP + laser irradiation with respect to the cytokines IL-1β and IL-8, 6, and 4, and the total amount of elastase activity. Shimizu et al. (1995), in an in vitro study, reported that LPT affects the
production of cytokines. The discrepancy between *in vitro* and *in vivo* findings may be attributable to the fact that *in vitro* the actual energy density at the target would be considerably higher than in the clinical setting.

The relative effects of ultrasonic treatment, carbon-dioxide laser and Nd:YAG laser have been investigated in several studies. Nd:YAG laser (without water-cooling) and ultrasonic scaling resulted in significant improvements in clinical parameters (Israel *et al.* 1997; Spencer *et al.* 1996; Miyazaki *et al.* 2006).

In contrast to the results of Study III, Sjöström and Friskopp (2002) using a similar Nd:YAG laser, with water cooling, immediately following SRP, disclosed no additional benefit for laser irradiation at the four-month control. A reduced need for anaesthetics was the only obvious clinical advantage. The reason for the discrepant results is unclear; however, it might be attributable to differences in the study design: in the Sjöström study the laser was set to 7 W, in accordance with the manufacturer’s recommendations; whereas in Study III the setting was lower - 4 W.

A study by Lizarelli *et al.* (2006) showed that, within a limited range of power Nd:YAG laser is a safe tool for irradiation of primary teeth in a broad range of applications.

The laser fibre used in Study III was 600 µm in diameter and operated with a water cooling system. Compared to a 600 µm tip, the power density of the conventional 300 µm tip is four times higher, causing greater carbonization and tissue adherence, resulting in less control over the energy output at the tip. The 600 µm tip reduces the power density and so does the water spray (Gold and Vilardi 1994; Radvar *et al.* 1996). In the present study, in order to overcome the loss of power at the fibre tip, the following settings were selected: 4 W, 80 mJ per pulse, 50 Hz, and a pulse width of 350 µs. A further advantage of the 600 µm tip is the reduced risk of fibre fracture. Results by Israel *et al.* (1997) showed that high energy, such as 9 W, can have negative effects on the root surface. However, no such damage is associated with laser treatment at 4 W and water coolant (Spencer 1996).

It is difficult to offer a comprehensive explanation for the greater improvement of periodontal status at the laser-irradiated sites. An important contributory factor may be
that laser application results in partial removal of the pocket epithelial lining. The reduction in PI and PPD at the test sites might be associated with the improvement in periodontal inflammation: because they experience less discomfort, patients may be able to brush more thoroughly and maintain good oral hygiene at these sites.

The bactericidal effect of Nd:YAG laser has been tested in vitro by Kranendonk et al. (2010). Suspensions of six different periodontal pathogens (Aggregatibacter actinomycetemcomitans, Porphyromonas gingivalis, Prevotella intermedia, Tannerella forsythia, Fusobacterium nucleatum and Parvimonas micra) were prepared in small tubes and exposed to the Nd:YAG laser for five different intervals, using the following laser settings: Power 6 Watt, Pulse Repetition Rate 50 Hz, Pulse duration 250 ms. After exposure to the laser, aliquots of the suspensions were spread on blood agar plates for bacterial counting. After 5 s of laser exposure, there was a decrease in total colony forming units of all six selected micro-organisms. After laser irradiation for 15, 30 and 45 s, no viable bacterial cells remained.

In Study IV, sites irradiated with a single application of Nd:YAG laser as an adjunct to SRP showed a reduction in periodontal inflammation and bone loss compared to the control side. The improvement in clinical inflammation in terms of GI, was corroborated by the reduction of GCF volume on the test compared to the control side. Similar results have been reported previously (Wakao et al. 1989) Laser irradiation has been proposed as an adjunct to conventional scaling and root planning in the treatment of periodontitis. However, the reported outcomes of studies to date are contradictory and the literature provides limited evidence to support an additional benefit of laser application. The overall aim of the present thesis was to explore the potential of adjunctive application of therapeutic and surgical lasers to improve treatment outcomes, expressed in terms of clinical, radiographic and immunological parameters.

The present thesis is based on a series of four clinical studies of patients with moderately severe periodontitis, treated by scaling and root planing. Two different types of dental laser were investigated. Therapeutic lasers, which are claimed to stimulate cell regeneration and boost the immune system, were investigated in studies I and II: the general effect was investigated in Study I, while Study II compared the difference between gas and diode lasers in the same spectrum, in order to evaluate the importance of the length of coherence in biostimulation. In studies III and IV, the
surgical Nd:YAG laser, which is usually applied for sulcular debridement and pocket decontamination, was evaluated in a novel approach. The test procedure comprised one single application of the laser with water coolant after conventional scaling and root planing. In study III, the outcome was evaluated after 3 months and in Study IV the long term outcome was evaluated, at least one year post-treatment.

The split mouth design was used in all four studies. Study I showed a better clinical outcome on the laser treated side and some improvement in immunological parameters. The results of Study II support the hypothesis that a laser with a long length of coherence is superior to one of a shorter length, although both lasers had some positive clinical effect. In Study III a single application of the Nd:YAG laser as an adjunct to scaling and root planing improved the short-term outcome and Study IV confirmed that this improvement was sustained.

Besides reducing periodontal inflammation laser irradiation has been proposed as an adjunct to conventional scaling and root planning in the treatment of periodontitis. However, the reported outcomes of studies to date are contradictory and the literature provides limited evidence to support an additional benefit of laser application. The overall aim of the present thesis was to explore the potential of adjunctive application of therapeutic and surgical lasers to improve treatment outcomes, expressed in terms of clinical, radiographic and immunological parameters.

Nd:YAG laser treatment also supports new connective tissue formation. A significant reduction in PPD with increased clinical attachment levels is associated with Nd:YAG laser therapy in patients with periodontitis (Yukna et al. 2007). This study demonstrated new cementum and connective-tissue formation, also reported subsequently by Romeo et al. (2009). Used at low energy, the Nd:YAG laser does not cause damage to the cementum or the dental pulp. An earlier in vitro study by Radvar et al. (1995) also showed that the Nd:YAG laser did not have a negative influence on cementum, suggesting the formation of new connective tissues around the periodontium.

New bone regeneration is a goal of periodontal therapy, but is seldom achieved. The receptor activator of the nuclear factor-kB (RANK)/RANK ligand (RANKL)/osteoprotegerin (OPG) system is essential in bone turn over. An animal
study by Xu et al. (2009) investigated the effect of 650 nm irradiation on mRNA expression of receptor activator of NF-kappaB ligand (RANKL) and osteoprotegerin (OPG) in rat calvarial cells. The authors concluded that the irradiation may directly promote osteoblast proliferation and differentiation, and indirectly inhibit osteoclast differentiation, by downregulating the RANKL:OPG mRNA ratio in osteoblasts. These observations support an earlier study by Kim et al. (2007).

Study IV showed minor bone loss on the SRP only side while the side treated with laser and SRP showed some bone gain. Similar results have been reported in a recent experimental study in rats (de Almeida et al. 2008). While more bone regeneration is reported in some clinical studies (Kim et al. 2010), in most such studies the selected subjects exhibited more severe periodontitis at baseline, with pockets >4 mm, whereas in the present series of studies the inclusion criteria stipulated that pocket depth should not exceed 4 mm. Another difference in study design concerned the number of laser applications: better bone regeneration was recorded in studies in which the subjects underwent several laser therapy sessions, while the present studies III and IV included only one session of Nd:YAG irradiation. While one such session may therefore not be optimal, it appears to have been effective.

There are obvious weaknesses in Study IV, such as the small number of participants, the relatively long unsupervised period and varying observation times, and the outcome of only minor differences in alveolar bone height between the groups. A difference in bone level of 0.18 mm is not clinically relevant. However, it is statistically significant and shows that one application of Nd:YAG laser can have a long-term beneficial effect on alveolar levels.

In conclusion, the results of these studies confirm the potential role of laser irradiation as a non-invasive adjunctive to scaling and root planing in the treatment of periodontitis.

Key words: Low level laser, Nd:YAG laser, protease activity, coherence length, periodontal inflammation, cytokines, scaling and root planing.
OVERALL CONCLUSIONS

Study I showed that compared to SRP alone, additional treatment with LPT significantly reduced periodontal gingival inflammation.

Study II showed that in laser phototherapy, a gas laser was more effective than a diode laser in reducing gingival inflammation.

Study III showed that compared to SRP alone, an additional single application of a water cooled Nd:YAG laser significantly improved clinical signs associated with periodontal inflammation.

Study IV showed a long-term positive effect of a single application of Nd:YAG laser in combination with SRP.
FUTURE PERSPECTIVES

A review of the literature confirms that the outcome of laser applications in dentistry is heavily dependent on the parameters selected. With sufficient knowledge, lasers can be used for multiple applications and could be a substantial addition to the armamentarium of the periodontist as well as the general dentist. But considering the great variability of the available parameters, more research is necessary to identify therapeutic windows for each indication and for each wavelength. Only then will dental lasers be more readily accepted and sold in greater numbers, at prices that most dentists will consider affordable. Researchers involved in this field have an obligation to be active in education activities to ensure that dental lasers are applied in an evidence-based, professional way. Future studies should preferably be multi-centre studies, where all centres have identical equipment and methods. The present literature is difficult to interpret due to lack of uniformity in selected parameters.

The reduction of the pocket microflora is an interesting topic. It is obvious that Nd:YAG laser can reduce the bacterial burden, but to date there are few published studies in this field.

In contrast to SRP, Nd:YAG laser can remove the pocket epithelial lining. The practical importance of this property needs further verification. A negative outcome is not necessarily attributable to lack of effect of the laser, but may be due to unsuitable power settings, pulse repetition rates, total energy, treatment technique and fibre size. The present series of studies highlights the importance of the fibre size. Further studies are warranted to elucidate the influence of different fibre sizes on the clinical outcome.

The two Nd:YAG studies in this thesis have deliberately used a closed pocket mode, in order to be able to compare the additional effect of the Nd:YAG laser after SRP. However, a more surgical approach is also possible, where the pocket is opened during the removal of the pocket epithelial lining, offering the operator a better view of the pocket, allowing improved inspection of remaining debris. This technique also needs to be investigated in future studies.
As therapeutic lasers and the Nd:YAG laser were investigated in this thesis and both exhibited beneficial effects, a combined study would be of interest. After reducing the bacterial load and the epithelial lining, a number of subsequent applications of LPT could further improve healing by stimulating periodontal cells such as precursors to osteoblasts. The adjuvant effect of LPT in traditional periodontal treatment modalities such as GTR and organic and/or inorganic bone substitutes should also be highlighted. The anti-inflammatory effect of LPT also needs to be better understood.

There are other lasers on the market such as diodes and Er:YAG. The application of these in periodontology also warrants investigation.

Although the use of different lasers in periodontology has not been extensively investigated, the literature suggests many potential advantages. Future research should focus on establishing such an evidence-based treatment modality.
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