EFFECT OF AN ER, CR:YSGG LASER ON P. GINGIVALIS-CONTAMINATED TITANIUM ALLOY DENTAL IMPLANT SURFACES IN VITRO

by

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INTRODUCTION

Statement of the Problem

Implant dentistry has become a widely accepted modality to replace missing teeth. However, dental implants are susceptible to biofilm-mediated inflammatory lesions (peri-implant mucositis / peri-implantitis), similar to that seen around natural teeth (gingivitis / periodontitis). These lesions, in turn, threaten the longevity of implants as anchors for dental prostheses. Because of the similarity in etiology and presentation, comparable treatment modalities are applied to resolve peri-implant and periodontal inflammatory lesions. Such a shared treatment includes mechanical debridement, with or without surgical repositioning of the soft tissue complex. However, most contemporary dental implants feature threads to engage the alveolar bone and a micro/nano-textured surface to stimulate bone-implant contact (osseointegration). Therefore, when the implant threads become exposed and contaminated by biofilm, subsequent surface debridement / decontamination becomes considerably more complex than with that of a natural tooth, which is usually debrided using a metal curette or ultrasonic device. The micro/nano-textured surface of a dental implant is easily damaged by instrumentation using a metal curette. If an efficient method of dental implant surface decontamination could be established, then clinical protocols may be developed that effectively clean the implant surface to achieve peri-implant tissue health. To this end, lasers have been introduced; however, directly applied laser energy may also affect implant surface characteristics, including micro/nano-structure and composition, essential to osseointegration. Therefore, lasers may have disadvantageous clinical effects, in turn compromising peri-implant tissue consolidation.
and health: the very aspects its use is attempting to provide. Commercially available 
Er,Cr:YSGG lasers have been used to remove such implant-attached deposits, however 
the efficacy in removal of bacteria and the safety to the implant surface integrity have 
yet to be demonstrated quantitatively.

**Significance**

If an Er,Cr:YSGG laser is shown to decontaminate an oral implant surface 
without altering its chemical or physical properties, then such treatment could be added 
to a presently short list of potentially effective technologies suggested for the 
management of peri-implant inflammatory lesions: i.e., peri-implant mucositis and peri-
implantitis. Laser treatment may be less time consuming, more effective, less costly, 
and result in decreased tissue trauma and patient morbidity than do conventional, often 
more invasive treatments.

**REVIEW OF THE LITERATURE**

**Introduction**

Implant dentistry has been successfully used for nearly a half century as a 
modality for replacement of missing teeth \(^1\). Research and technological advancements 
have made osseointegration and restoration of dental implants predictable and 
successful. However successful, there are also a number of potential mechanical and 
biological problems associated with dental implants (including infection, crestal bone 
loss, implant mobility, prosthetic screw loosening, cement retention, and prosthesis 
fracture) that may threaten the longevity of an implant-anchored prosthesis \(^2\). Peri-
implant mucositis is characterized by a reversible biofilm-induced inflammation of the peri-implant mucosal tissues. Peri-implantitis features the added characteristic of peri-implant crestal bone loss (Figure 1)\(^3\). For either situation, the long-term retention of the implant may be jeopardized; thus, development of effective methods to treat these conditions is desirable.

![Figure 1. Schematic representation of peri-implantitis (right side) and peri-implant health (left side)](Shawn McLeod, Periimplantitis, 2016, SHMarts, all rights reserved)
**Peri-implant diseases**

Peri-implant diseases include peri-implant mucositis and peri-implantitis. Peri-implant mucositis is defined as an inflammatory lesion of the peri-implant supporting soft tissues, without associated bone loss. Conversely, peri-implantitis is an inflammatory lesion of the peri-implant tissues including crestal bone loss. Studies using vastly different criteria for peri-implant inflammation and bone loss estimate the prevalence of peri-implant bone loss to range from 7% to 47% of implants. A systematic review indicates that prevalence of peri-implantitis encompasses approximately 10% of implants and 20% of patients, when evaluated 5-10 years after implant placement. A more recent review found that within 9 years following implant placement, 45% of patients presented with peri-implantitis, and 14.5% of patients presented with moderate-severe peri-implantitis.

As shown in Figure 2, the implant-soft tissue interface is similar to, but significantly different from, the tooth-soft tissue interface. The sulcular epithelium and junctional epithelial attachments are similar, but the connective tissue attachment is oriented in a different direction, and there is no periodontal ligament around an implant.
The etiology of peri-implant diseases is multifactorial. However, the prevailing theories indicate that the main contributor to loss of implants by peri-implantitis is bacterial in nature. Accumulation of plaque and calculus on implant components leads to inflammation and sometimes to eventual breakdown of marginal bone support: a process similar to that of bone loss around teeth in periodontal disease.

Much like periodontal disease, the early stages of peri-implant disease begins with inflammation of the marginal soft tissue structures, appearing as erythema and bleeding on probing. At this stage of the lesion, inflammation is reversible with treatment and has not yet resulted in any permanent loss of supporting tissue structures around the implant. However, given time, the inflammation, combined with a robust host immune response, can result in bone loss around the coronal aspect of the implant.
implant. At this point, the lesion is termed peri-implantitis and there is now evidence of permanent loss of supporting bone. Like periodontal disease, the loss of supporting structures around implants is progressive in nature\textsuperscript{11}.

A study utilizing subgingival ligatures in dogs to accumulate plaque and stimulate loss of marginal bone around implants demonstrated a rapid, progressive, continual loss of bone\textsuperscript{12}. There is evidence that bone loss continues after the ligatures have been removed from the implant surface\textsuperscript{13}.

**Microbiology of peri-implant diseases**

The microbiota of the oral cavity is exceedingly complex. Many major bacterial species are associated with pathologic events, in particular caries and periodontal disease, and lately, in peri-implant mucositis and peri-implantitis\textsuperscript{14}. It is generally accepted that red complex bacteria, i.e., *P. gingivalis*, *T. denticola*, and *B. forsythus*, associated with advancing periodontal disease also are correlated with the advancement of peri-implant disease\textsuperscript{15}. A recent systematic review indicates that the majority of studies agree that the bacterial population associated with peri-implantitis is mixed, variable among lesions, and consists mostly of gram-negative anaerobes. In addition, the bacteria found in peri-implant lesions are, for the most part, similar to the bacteria found in periodontitis lesions\textsuperscript{16}.

**Treatment of peri-implant diseases**

Treatment of peri-implant diseases generally consists of attempts at debridement and detoxification of the implant surface, and regeneration of the supporting tissue.
structures. An initial, non-surgical phase of therapy, with an emphasis on mechanical debridement and/or (local/systemic) antimicrobial treatment, is sometimes attempted, and is followed by a re-evaluation stage, and determination of the need for surgical intervention. This initial phase is intended to reduce inflammation, hopefully eliminating the need for surgical intervention, or at least to facilitate any future surgery, by decreasing local inflammation.

Debridement

Mechanical debridement of substances on tooth roots, or exposed surfaces of implants, is the principal component of the initial phase of therapy. Gross removal of supragingival and subgingival plaque and calculus are effective in resolving clinical signs of inflammation around implants and implant-supported restorations, such as plaque scores and bleeding on probing. However, probing depths around implants generally remain unchanged after non-surgical debridement.

Open flap debridement consists of providing surgical access to the subgingival implant components, followed by mechanical removal of plaque, calculus, and granulation tissue in the peri-implant defect. After debridement, the tissue flaps are closed, without any removal of bone around the peri-implant defect. This method is effective at reducing probing depths and levels of red complex bacteria.

Antibiotic therapy is often used, in conjunction with non-surgical and surgical approaches, to treat peri-implantitis. Adjunctive systemic antibiotic administration is used with surgical intervention, showing significant reductions in probing depths and signs of inflammation. Locally applied antibiotics are sometimes applied directly to
peri-implant pockets, and show some degree of success, but the significance of their role in treatment remains debatable\textsuperscript{21}.

\textit{Detoxification}

Detoxification of implant surfaces, to eliminate noxious substances, such as LPS and bacterial cells, which might prevent development of a healthy condition, is generally utilized in conjunction with surgical therapy. Multiple protocols for this treatment are available, and many agents are used to accomplish chemical detoxification of the implant surface. Chlorhexidine gluconate is considered a “gold standard” in periodontal post-surgical plaque control, and is also used to treat peri-implant lesions\textsuperscript{22}. Many other agents are used as adjuncts to surgical therapy, including citric acid, sodium hypochlorite, hydrogen peroxide, and essential oils. All of these formulations demonstrate bacterial elimination when applied to titanium-adherent biofilms\textsuperscript{23}.

A recent systematic review determined that non-surgical therapy for peri-implantitis has limited efficacy for reducing probing depths, but the greatest probing depth reduction results from a combined approach of mechanical debridement with locally-applied antibiotics\textsuperscript{24}. Another review found sufficient evidence for recommendation of supplementing mechanical debridement with some form of surface decontamination protocol (i.e. detoxification)\textsuperscript{25}.

\textit{Resection/Regeneration}

A recent meta-analysis showed that each of the most commonly utilized surgical procedures (open debridement, resective therapy, bone grafting, and guided bone regeneration) can be highly successful at resolving inflammation and probing depths
around implants by about 2-3 mm \(^{26}\). Any of these four procedures may be accompanied by implantoplasty, or selective removal of implant surface structure in an effort to smoothen the exposed surface. Open debridement has been discussed previously in this review.

Resective therapy involves tissue flap reflection and mechanical debridement, as utilized in the open flap debridement procedure. However, ostectomy and osteoplasty are used to remove the peri-implant crater defect that is the hallmark of marginal bone loss around implants. No attempt is made at regenerating the lost bone support around the implants. This technique is documented to be effective at reducing probing depth \(^{27}\), but leaves the implant with less bone support.

Attempts to restore the lost bone in peri-implant defects show a substantial degree of success. These treatments involve bone grafting, with or without the use of a barrier membrane. A variety of biomaterials have been used, and bone fill has been observed around the affected implants. However, the question remains whether re-osseointegration can be truly achieved, or if the restored bone around the implant is actually in contact with the implant surface. Regardless, radiographic hard tissue fill and reduction in probing depths are successfully achieved in peri-implant defects using bone grafting \(^{28}\).

Implantoplasty has been advocated as a potential adjunctive procedure to surgical treatment of peri-implantitis lesions \(^{29}\). This procedure involves the mechanical recomtoung, generally with rotary instruments, of the implant surface, with the end goal of removal of exposed thread surfaces and formation of a smooth, non-plaque retentive surface that is exposed to the oral cavity. However, these methods will severely
compromise the carefully engineered structural integrity and surface coatings of the implant, and should only be implemented after careful deliberation.

Some authors, utilizing several of the above-mentioned techniques in conjunction, recommend a combined treatment approach. Flap debridement, implant surface decontamination, application of biologic materials, and bone grafting with a membrane have been used together with variable results. One study reports a 5 mm reduction in probing depth, using this combined method 30.

Predicted efficacy of treatment/re-osseointegration

There is biologic plausibility with all of these mentioned treatment protocols. However, a recent review, comparing the success of peri-implantitis treatment, revealed a large range of successes and failures. The success ranged from 0 to 100%, with a successful outcome reported in a majority of patients in only seven of eleven articles 31. For this reason, a more predictable method of treating peri-implantitis lesions is required in order to be able to effectively treat this common, and serious, condition.

The potential for re-osseointegration of dental implants following bone loss by peri-implantitis has been investigated. The outcome of a recent literature review indicates many different methods have been attempted, including open flap debridement, surface decontamination, and bone grafting with or without membrane use. The results are mixed, with conflicting findings reported by different authors 32. Regardless, bone fill around implants is achievable through surgical methods.

Because implant surface characteristics have a significant impact on osseointegration, it is conceivable that these characteristics will also have an impact on
any chances of re-osseointegration. For this reason, it would be ideal for implants to retain their original surface properties, composition, and textures after peri-implantitis therapy.

**Osseointegration of implants**

The surface of dental implants is a heavily studied topic. The first root form dental implants had a turned, machined surface that was relatively smooth. However, these implants had problems with osseointegration, particularly in the posterior mandible and maxilla. For this reason, new surfaces were developed with the goal of better and faster osseointegration. Surface modifications include both subtractive and additive processes that roughen the metal and create different microstructures and compositions.  

Several studies indicate that implants with a moderately rough surface exhibit better and faster osseointegration. On the other hand, these rough surfaces also contribute to faster and more robust biofilm accumulation. One specific study indicates that there is an optimal roughness for implants, below which and above which the possibility of osseointegration is compromised. Nevertheless, most commercially available dental implants have a roughened surface, which may be accomplished by a variety of methods. Sand blasting, acid etching, surface coatings, anodization, and surface treatments have all been utilized to create specific surface topographies, as opposed to the surfaces of traditional machined-surfaced implants.

A recent review discussed the most popular, commercially available implant surfaces, and their relative micro-roughness values. It was reported that these surfaces mostly demonstrated moderately rough (average roughness of an area (Sa) 1-2 µm).
textures, but that different implant batches from the same manufacturer could vary slightly in this parameter $^{39}$.

The wettability of dental implants has been recently studied with respect to the potential for osseointegration. Surfaces with a higher wettability, as evidenced by a lower contact angle, demonstrate a faster and more robust osseointegration $^{40}$. This finding can be explained by the thought that cells and nutrients may be more quickly and readily supplied to the implant-bone interface if the surface of the implant demonstrates hydrophilicity (a lower contact angle).

It is important to note that roughness and contact angle are not strictly correlated. Rather, increasing roughness will amplify the wettability of a surface. This means that hydrophilic surfaces will become more hydrophilic when roughened, and hydrophobic surfaces will become more hydrophobic when roughened.

**Lasers**

*Theory*

The term “laser” is an acronym for light amplification by stimulated emission of radiation. Laser devices consist of a lasing medium (solid or gas), a flash rod, and two mirrors (Figure 3). The flash rod is used to stimulate electrons in the lasing medium. The electrons are unstable in their excited state and, when returning to their ground state, release energy in the form of photons. The emitted photons excite other electrons in the medium, leading to a propagation of photon emission from the medium. Because the lasing medium is of uniform composition, the electrons are all emitting photons of
the same wavelength and energy, producing a beam of monochromatic light, i.e.; light of a single wavelength.

Figure 3: Schematic of a solid state laser and associated components (Shawn McLeod, Ruby Laser, 2016, SHMarts, all rights reserved)

Lasers are used for many purposes, based on their wavelength and energy of emission. Low-energy lasers emit light in the visible spectrum, and are used for pen-laser pointers and as measurement tools. High-energy lasers emit light outside the visible spectrum, and are used to cut materials. Lasers that emit infrared light are specifically utilized to heat substances, such as in the cutting of metal and other solid parts.\textsuperscript{41}
Types of lasers

The CO$_2$ (carbon dioxide) laser using a gas lasing medium, as opposed to a solid, was one of the first lasers developed. This laser operates as a continuous wave device (non-pulsed), and is one of the most powerful lasers currently available. The CO$_2$ laser emits infrared light, having peaks around 9.4 and 10.6 µm, and is therefore used to heat and incinerate materials. In dental and surgical applications, the CO$_2$ laser is used as a coagulative tool and to incise and excise soft tissues $^{42}$.

The diode laser is a solid state device, typically found in a wide variety of devices, because of its small packaging size and low cost to manufacture. A 940 nm (0.94 µm) diode laser has been used in a wide variety of medical and dental applications including hair removal, soft tissue surgeries, and biopsies. A main advantage of the diode laser is that it can cut through soft tissue while at the same time provide hemostasis, increasing visibility during surgical procedures $^{43}$.

The Nd:YAG (neodymium-doped yttrium aluminum garnet) laser is another solid-state laser. Nd:YAG lasers emit light at a wavelength of 1064 nm (1.064 µm) and are therefore similar to the 940 nm diode laser, effective during surgeries to obtain hemostasis. These lasers are used in a wide variety of soft tissue surgeries, including cancerous lesion removal and incisional procedures $^{44}$. Nd:YAG lasers are also widely used to engrave and etch plastic and metal.

Erbium lasers, such as the Er:YAG (erbium: yttrium-aluminum-garnet) and the Er,Cr:YSGG (erbium, chromium:yttrium-scandium-gallium-garnet) have been adapted for hard tissue removal. The Er,Cr:YSGG laser emits radiation at a wavelength of 2,780 nm (2.78 µm), and is well absorbed by hard tissues including bone, enamel, and dentin $^{45}$.
Dental use of lasers

Soft-tissue lasers are used for oral procedures as an alternative to conventional surgery. Incisional/excisional biopsies, gingivectomy, periodontal pocket removal, and crown exposures are performed using lasers. Laser therapy has the added benefit over scalpels of hemostasis, providing increased visibility for the surgeon as well as decreased morbidity and complications for the patient following treatment. Figure 4 illustrates the wavelengths of lasers commonly used in dentistry.

![Figure 4: Wavelengths of commonly used dental lasers (from https://upload.wikimedia.org/wikipedia/commons/thumb/4/48/Commercial_laser_lines.svg/775px-Commercial_laser_lines.svg.png)](https://upload.wikimedia.org/wikipedia/commons/thumb/4/48/Commercial_laser_lines.svg/775px-Commercial_laser_lines.svg.png)

The use of hard-tissue lasers, especially the Er,Cr:YSGG laser, is being expanded in oral settings. For example, alveoplasty and crown exposure may be performed using a laser instead of conventional rotating burs. Laser therapy may
provide enhanced control by the operator, particularly when in close proximity to vital anatomic structures, as well as decreased discomfort for the patient.

Conventional endodontic procedures attempt to eradicate bacteria within the root canal(s), using combined mechanical and chemical protocols. The persistence of bacteria within the root canal system is a major contributor to endodontic failure, and need for retreatment. In perspective, the Er,Cr:YSGG laser, applied in endodontic settings, effectively eradicates bacteria within the root canal system. The Er,Cr:YSGG laser also has potential use in restorative dentistry and is effective in removing carious tooth tissue, while being somewhat protective to natural structures, albeit somewhat slower than conventional high-speed rotary bur preparation.

In periodontal settings, the Er,Cr:YSGG laser is effective in removing calculus from root surfaces at a power output setting of 1.0W. A clinical case series evaluated use of the Er,Cr:YSGG laser in non-surgical treatment of peri-implantitis. This study utilized the laser as a stand-alone therapy. Out of 28 treated implants, the mean probing depth decreased from 6.6 mm to 3.0 mm at 6 months post-treatment.

A clinical trial evaluated use of an Er:YAG (erbium: yttrium, aluminum, garnet) laser for surgical debridement of peri-implantitis lesions. While this laser is different in terms of wavelength output than the Er,Cr:YSGG laser, the functionality and usage of the two devices are somewhat similar. The clinical trial indicated that there was no added benefit of the Er:YAG laser over application of a sterile saline cotton swab in a surgical setting of peri-implantitis treatment. However, it should be noted that a significant potential benefit of laser treatment is that it can be used in a non-surgical setting, in an attempt to debride and detoxify the implant surface.
A systematic review investigated the efficacy of the Er:YAG laser in non-surgical management of peri-implantitis. The review suggested an added benefit of the Er:YAG laser being related to the initial healing after treatment, compared with conventional mechanical debridement. However, no significant differences between treatments were found at later times. The review concluded that more research on the use, functionality, and effects of the Er:YAG laser was needed before conclusive statements could be made ⁵².

**Antimicrobial activity**

Laser light is known to be bactericidal, when appropriately applied. The antibacterial effects may be attributed to photochemical (due to free radical production), photothermal (due to heat production), photoablative (breaking of chemical structures), or photomechanical (due to intense intracellular plasma vibrations) effects. It is not currently known which of these mechanisms is responsible for the antibacterial activity of the Er,Cr:YSGG laser ⁵³.

**Laser tips**

A wide variety of attachment tips for lasers is available for different applications. Commercial laser companies provide tips that deliver energy to different surface areas and at different working lengths, as well as delivering energy laterally. The latter attachments, known as radial firing tips, apply energy laterally in all directions, extending outward and downward from the tip attachment (Figure 5). This pattern of energy delivery allows radiation to be delivered towards a tooth or an implant when the
attachment is inserted into the periodontal or peri-implant pocket, and to also treat the tissue surface opposing the implant surface.

![Figure 5: Schematic of laser energy from a radial firing tip (from www.biolase.com)](image)

When considering this type of radial energy dispersion, it is important to keep in mind that the applied power setting is also dispersed over this area, and therefore neither the implant nor the tissues are actually exposed to the full power level being generated. Based on personal communication with the manufacturer, 83% of the power output is delivered axially through the distal end of the tip, while 30-45% of the power output is delivered radially.

**Effect of Er,Cr:YSGG laser on titanium alloy surfaces**

The Er,Cr:YSGG laser has been investigated for its usefulness in the management of peri-implantitis. An experiment examining the effect of the laser on root surfaces indicated that a power setting of 1.0 W with a straight-firing tip was effective at removing calculus from root surfaces while not permanently physically affecting the root surface *ex vivo* \(^{49}\). Several studies show that the Er,Cr:YSGG laser can affect the surface of titanium alloy dental implants. One study, using a tricalcium phosphate/hydroxyapatite-
blasted titanium surface, showed minimal alterations to the implant surface with a straight-firing tip at low power settings (1.0 W). However, flattening and melting of the surface were observed at higher power settings 54.

*Temperature Effects*

Lasers are known to increase the temperature of a substrate to which they are applied 55. This temperature increase has also been shown to impact the surface of titanium alloys 56. However, the Er,Cr:YSGG laser, when utilized with appropriate air and water irrigation settings, has been shown to reduce the temperature at the apex of dental implants *in vivo* in pigs 57. Nonetheless, there could be changes in temperature, caused by the laser, at the microenvironment level, which could be affecting the surface characteristics of the titanium alloy.

**SUMMARY**

*Current knowledge*

Implant therapy is a commonly used approach for tooth replacement. However, bacterial-induced crestal bone loss (peri-implantitis) contributes to implant failure and eventual loss. There are many suggested approaches to the resolution of peri-implantitis. One emerging technology is the application of laser decontamination of the implant surface. Efficient calculus removal around teeth and implants has been achieved, using the Er,Cr:YSGG laser. However, this laser is also capable of
irreversibly damaging the implant titanium surface, potentially affecting the possibility of re-osseointegration or bone fill post-treatment.

**Deficiencies in current knowledge**

It is currently unknown whether the Er,Cr:YSGG laser may affect dental implant surface characteristics that, in turn, may impact bone formation and re-osseointegration following application, in the treatment of peri-implantitis. Also, there is limited information regarding the effect of the Er,Cr:YSGG laser on the biofilm harbored on a treated dental implant surface.

**Significance of what remains unknown**

If laser therapy significantly alters the implant surface, in terms of roughness, wettability, or composition, then re-osseointegration or bone fill after therapy may also be affected. In addition, effective microbial elimination from the implant surface using the Er,Cr:YSGG laser could drastically improve the efficacy of peri-implantitis treatment. A study of laser-treated, commercially available implant surfaces would provide guidelines on laser power settings, which could effectively kill bacteria without drastically altering important implant surface characteristics.

**PURPOSE**

The purposes of this study were to 1) investigate surface changes of a commercially available implant surface following irradiation by the Er,Cr:YSGG laser, and 2) examine the effect of the Er,Cr:YSGG laser on *P. gingivalis* biofilm *in vitro*. Specifically, the effect
of laser power output on implant surface properties including composition, surface energy, micro-roughness, and temperature rise, as well as bacterial cell removal and or killing were evaluated.

HYPOTHESES

Specific aim #1

The first specific aim tested the hypothesis that implant surface micro-roughness, surface energy (wettability), composition, and temperature rise of a commercially available titanium alloy will be significantly altered at power levels over 1.0W, but will not be altered at power levels of less than 1.0W.

Specific aim #2

The second specific aim tested the hypothesis that viability of a common periodontal pathogenic bacteria (*P. gingivalis*), grown on titanium disks, will be significantly reduced after irradiation from the Er,Cr:YSGG laser, in a dose-dependent manner, demonstrating greater bacterial elimination with increasing power levels from the laser.
MATERIALS AND METHODS

Pilot Study Overview

Prior to a definitive study, a pilot test was undertaken for the purposes of observing global effects on specific implant properties while using different applied power levels and exposure times. The main goal of pilot testing was to narrow the selection of Er,Cr:YSGG laser exposure times and power setting to those that spanned the range of no detectable difference in implant integrity (SLA-treated titanium alloy disks) was noted to that where overt bacteria elimination (P. gingivalis) was observed. Thus, in the full study, a wider array of test parameters could be applied to this narrower range of exposure times and power settings. Autoclaving was used as a positive control for bacteria killing, and no treatment (no laser, no irrigation) was used as a negative control. The resulting determination of combinations of exposure time and laser power were then utilized to examine the effect of the laser on bacterial viability as well as on several surface characteristics of the disk, as the major emphasis for the thesis work. The specific methods and the results of the pilot study can be seen in Appendix A.
Main Experiment Overview

The results of the pilot study demonstrated a dose-dependent increase in dead bacteria with increasing laser power, as well as qualitative surface changes as visualized from SEM images. The region of interest for laser power and exposure duration was determined to be in the range of 1.0-2.0 W, with no impact of duration. For these reasons, it was decided to retest the control as well as power settings of 1.0, 1.5, and 2.0W, all for 120 s exposure.

The following figure presents the experimental flow chart for the main aspect of research.
Figure 6: Flowchart of main study

Titanium Disk Selection

Details of the titanium disks are available in Appendix A.
Biofilm growth

Details of the biofilm growth parameters are available in Appendix A. In brief, a *P. gingivalis* monoculture was applied to the titanium disks and incubated anaerobically for 48 hours prior to laser application.

Laser Exposure of Disks

Details of the laser exposure are available in Appendix A. In brief, a custom-built moving stage was constructed to hold both the laser handpiece and the disk specimen. This stage allowed for precise, repeatable distancing (0.5mm) of the laser tip to the specimen, and repeatable, uniform application of the laser to the entire surface of the disk.

Contact angle measurement

Prior to surface analysis, the disks were cleaned by sonication (Ultrasonic Cleaner, Fisher Scientific, Waltham, MA, USA) in 70% ethanol for 2 minutes, in order to remove any surface debris, oils, or contaminants that might artificially interfere with surface energy measurement. Disks were tested using a dynamic contact angle instrument (EasyDrop DSA20, Kruss GmbH, Hamburg, Germany). Five microliters of deionized, distilled water was deposited onto the disk surface using an automated syringe, while the instrument digitally recorded a video of the silhouette of the deposited drop on the disk over time. Right and left contact angle measurement values were obtained using software (DSA v1.90.0.14, Kruss GmbH, Hamburg, Germany) at a rate of 3 measurements/second. The data were copied into a spreadsheet program (EXCEL 2010, Microsoft Corporation, Redmond, WA, USA), where the right and left angles were
averaged and reported as a single data point at each measured time interval. A plot of the measured contact angle with time, Figure 7, was made to identify the duration of time after droplet deposition at which a steady state in results was obtained. Thereafter, the contact angle for each specimen was reported at that time interval. This time interval was determined, after several trials, to be optimized at 20 seconds.

![Contact Angle Graph]

Figure 7. Example of change in contact angle with time of deposition of the water droplet. After 20 seconds, the data were deemed to be “steady state”, and only the value of contact angle at that time were reported.

An example of the image of the water droplet after the recommended deposition time is seen in the following figure, 8.
Figure 8  
Example of the silhouette of water droplet recorded from which the contact angle was measured.

A two-tailed Student’s t-test was used to analyze the contact angles from before to after treatment. A paired test was utilized, because the same disk was analyzed before and after laser treatment.

Surface roughness

A profilometer (Pneumo Form TalySurf Series 2, Taylor Hobson, Leicester, England) was used to measure the surface roughness of the titanium alloy disks. This machine operates by slowly tracing a 2 micron-diameter diamond tip across the surface of a specimen and recording the heights and depths of the surface. The amplitude/height over a 3x3 mm area was analyzed in the middle of the specimen. By rastering the line scans, software (TalyMap Gold 4.1, Taylor Hobson) produced a 3-dimensional...
reconstruction of the surface of the disk, and made calculations of Sa and Sdr. Due to the potentially destructive nature of this test, it was only conducted on disks after other tests had been performed. The parameter Sa is the arithmetic average of the 3D surface roughness value, and is calculated using the following formula:

\[
Sa = \frac{1}{MN} \sum_{k=0}^{M-1} \sum_{l=0}^{N-1} |z(xk, vl)|
\]  

(1)

where M and N represent the dimensions of the area.

The roughness parameter Sdr, which represents the ratio of the surface area of the actual surface (textured surface) compared to the surface area of a flat plane of equal dimensions (cross sectional area), and is presented as a percentage.

\[
Sdr = \frac{(textured \ surface \ area - cross \ sectional \ area)}{cross \ sectional \ area} \times 100\%
\]

(2)

Paired two-tailed Student’s t-tests were used to compare the roughness parameters of the disks before and after treatment.

**Temperature Measurement**

Temperature measurements were made using K-type thermocouple wire whose ends were formed into a thermocouple junction using a spot welder. One disk was prepared to receive a thermocouple at the surface of the disk by drilling through the outer perimeter of the disk and placing the thermocouple on the interior aspect of the hole, immediately subjacent to the disk surface without perforating the surface. Once in place, the thermocouple allowed for precise temperature measurement at the surface of the
Another thermocouple was placed on the underside of the disk to allow for temperature measurement at the bottom side. The output of the thermocouple was connected to an internal A/D converter (TCIC-USB-ENC E204460, Omega, Stamford, CT, USA) (Figure 9) and directed to the USB port of a personal computer. A custom made user interface program was written (LabVIEW, National Instruments, Austin, TX, USA) where on-screen imaging of real time temperature measurements with respect to time were displayed. The data were also recorded digitally, and then entered into a spreadsheet program (EXCEL 2010, Microsoft), where the time-based temperature profiles were recorded. Temperature data were recorded using the different power output levels of the laser (0W, 0.5W, 1W, 2W), each of which was conducted with and without air/water irrigation. In addition, a trial was completed where the laser was applied without irrigation for the first half of the run, and irrigation was turned on for the second half. Exposure time was controlled at 120 seconds.
Figure 9. Picture of the thermocouple attached to the disk, with the laser handpiece in place. It can be confirmed in this image that the thermocouple was placed immediately subjacent to the disk surface without perforation.

Temperature data were visually analyzed (n=1) to observe trends.

**Biofilm Analysis**

Details of the biofilm analysis are available in Appendix A. After laser treatment, the biofilm-containing disks were subjected to live/dead staining, then imaged using a confocal microscope; the fluorescence values were then quantified using software (ImageJ). Due to the large amount of background fluorescence, it was decided to remove some of the background “noise” in the images. After several trial calculations,
where 50%, 90%, 95%, 99%, and 99.9% of the background noise was removed, the optimal level of background elimination was determined to be 99%. Utilizing controls having no bacteria allowed for removing noise attributed only to the disk or from errors in imaging. Therefore, 99% background reduction was utilized for both the PI- and SYTO 9-stained images.

Because the confocal analysis data did not demonstrate a normal distribution, tests for non-parametric statistics were utilized. The Kruskal-Wallis test was performed to identify non-equivalence of the treatment groups, and the Jonckheere-Terpstra test was used to evaluate evidence for a monotonic increase in percent of dead bacteria present as laser power was increased.

**Statistical Analysis**

Student’s paired t-tests, one-way and two-way ANOVA, Kruskal-Wallis, and Jonckheere-Terpstra analyses were all utilized as detailed previously. These tests were conducted with statistical software (SigmaPlot v11.0, Systat Software, Berlin, Germany).
RESULTS

Implant Surface Analysis

Roughness

The Sa and Sdr results are reported in Tables 1 and 2 and Figures 10 and 11. There were no statistically significant different changes observed between any of the laser power treatment groups for Sa analysis (p-values reported in Table 1, based on Student’s paired two-tail t test), indicating that there was no effect of laser power on measured micro-roughness. A statistically significant change in Sdr was noted at the 0W and 2W power levels, indicating that both the negative control and the highest laser power had an impact on the measured Sdr. There was also a significant change in Sdr when all disks were pooled together (p=0.0001, based on Student’s paired two-tail t test), indicating that a common element between all treatment groups had more of an impact on Sdr than did the laser treatment.

Table 1. The results (mean +/- SD, n=5) of the profilometer readings before and after laser treatment. A two-way ANOVA analysis follows, indicating that laser power did not significantly affect the Sa or Sdr, but that Sdr changed significantly after treatment. (DF = degrees of freedom, SS = sum of the squared deviations, MS = mean square, F = F-test, P = p-value)

<table>
<thead>
<tr>
<th>Power (W)</th>
<th>Sa (um) before</th>
<th>Sa (um) after</th>
<th>Sdr (%) before</th>
<th>Sdr (%) after</th>
</tr>
</thead>
<tbody>
<tr>
<td>0</td>
<td>1.71 ± 1.27</td>
<td>1.47 ± 0.56</td>
<td>7.03 ± 2.38</td>
<td>4.33 ± 1.06</td>
</tr>
<tr>
<td>1</td>
<td>1.32 ± 0.65</td>
<td>1.84 ± 1.10</td>
<td>8.06 ± 1.65</td>
<td>5.84 ± 1.42</td>
</tr>
<tr>
<td>1.5</td>
<td>1.33 ± 0.68</td>
<td>2.06 ± 2.06</td>
<td>7.02 ± 1.03</td>
<td>5.68 ± 3.43</td>
</tr>
<tr>
<td>2</td>
<td>1.99 ± 1.40</td>
<td>1.70 ± 0.93</td>
<td>7.84 ± 2.19</td>
<td>4.46 ± 1.35</td>
</tr>
<tr>
<td>Source of Variation</td>
<td>DF</td>
<td>SS</td>
<td>MS</td>
<td>F</td>
</tr>
<tr>
<td>---------------------</td>
<td>----</td>
<td>-------</td>
<td>------</td>
<td>------</td>
</tr>
<tr>
<td>POWER</td>
<td>3</td>
<td>9.015</td>
<td>3.005</td>
<td>0.789</td>
</tr>
<tr>
<td>BEF/AFT</td>
<td>1</td>
<td>63.234</td>
<td>63.234</td>
<td>16.594</td>
</tr>
<tr>
<td>POWER x BEF/AFT</td>
<td>3</td>
<td>5.935</td>
<td>1.978</td>
<td>0.519</td>
</tr>
<tr>
<td>Residual</td>
<td>36</td>
<td>137.185</td>
<td>3.811</td>
<td></td>
</tr>
<tr>
<td>Total</td>
<td>43</td>
<td>218.949</td>
<td>5.092</td>
<td></td>
</tr>
</tbody>
</table>

Table 2. Mean +/- SD (n=22) of the Sa and Sdr difference measurements from pooled data for before and after laser treatment.

<table>
<thead>
<tr>
<th>Laser Power (W)</th>
<th>Change in Sa (um)</th>
<th>Change in Sdr (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>0</td>
<td></td>
<td></td>
</tr>
<tr>
<td>0.5</td>
<td></td>
<td></td>
</tr>
<tr>
<td>1</td>
<td></td>
<td></td>
</tr>
<tr>
<td>1.5</td>
<td></td>
<td></td>
</tr>
<tr>
<td>2</td>
<td></td>
<td></td>
</tr>
<tr>
<td>2.5</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

Figure 10. The mean +/- SD (n=5) of the Sa and Sdr difference values from before and after laser treatment.
Contact Angle

Contact angle results are presented in Tables 3 and 4 and Figures 12 and 13. There was no statistically significant difference noted among any of the groups, indicating that laser treatment up to 2.0W had no measurable effect on contact angle. However, it can be noted that a statistically significant change in contact angle can be noted when all treatment groups were pooled, similar to what was seen for Sdr.

Table 3. Contact angle (mean +/- SD, n=5) measured before and after treatment. A two-way ANOVA follows, confirming that the total treatment had an impact on contact angle, whereas laser power had no significant effect.

<table>
<thead>
<tr>
<th>Power (W)</th>
<th>Contact Angle (degrees)</th>
<th>before</th>
<th>after</th>
</tr>
</thead>
<tbody>
<tr>
<td>0</td>
<td>120 ± 7.5</td>
<td>85 ± 4.4</td>
<td></td>
</tr>
<tr>
<td>1</td>
<td>118 ± 1.7</td>
<td>82 ± 8.1</td>
<td></td>
</tr>
<tr>
<td>1.5</td>
<td>120 ± 1.5</td>
<td>84 ± 4.9</td>
<td></td>
</tr>
<tr>
<td>2</td>
<td>116 ± 7.5</td>
<td>79 ± 5.8</td>
<td></td>
</tr>
</tbody>
</table>
Table 4. Pooled results (mean +/- SD, n=22) of contact angle from before and after laser treatment.

<table>
<thead>
<tr>
<th>Source of Variation</th>
<th>DF</th>
<th>SS</th>
<th>MS</th>
<th>F</th>
<th>P</th>
</tr>
</thead>
<tbody>
<tr>
<td>WATTAGE</td>
<td>3</td>
<td>161.821</td>
<td>53.94</td>
<td>1.619</td>
<td>0.202</td>
</tr>
<tr>
<td>BEF/AFT</td>
<td>1</td>
<td>14320.07</td>
<td>14320.07</td>
<td>429.85</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>WATTAGE x BEF/AFT</td>
<td>3</td>
<td>4.228</td>
<td>1.409</td>
<td>0.0423</td>
<td>0.988</td>
</tr>
<tr>
<td>Residual</td>
<td>36</td>
<td>1199.308</td>
<td>33.314</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Total</td>
<td>43</td>
<td>15804.82</td>
<td>367.554</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

Figure 12. No difference in contact angle (mean +/- SD, n=5) from before and after laser treatment, with all power groups combined, demonstrating no effect of the laser on contact angle.

Table 4. Pooled results (mean +/- SD, n=22) of contact angle from before and after laser treatment.

<table>
<thead>
<tr>
<th>Source of Variation</th>
<th>Before</th>
<th>After</th>
<th>p-value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Contact Angle</td>
<td>118 ± 1.17</td>
<td>82.5 ± 1.26</td>
<td>0.0001</td>
</tr>
</tbody>
</table>
Figure 13. The contact angle (mean +/- SD, n=22) as measured before treatment, and after treatment.

**Biofilm Analysis**

The percentages of dead bacteria of each treatment group were calculated and are presented in Figures 14 and 15. Figure 15 represents the mean +/- standard error for the total pixels of dead and live bacteria, as determined by the confocal imaging and software analysis of the images. Figure 14 shows the percent dead bacteria as determined by dividing the number of PI-positive pixels into the number of STY09-positive pixels.
Figure 14. Representation of the percent dead bacteria (mean +/- SD, n=7) at each laser power level. A highly significant quadratic correlation can be noted for percent dead bacteria based on the laser power output.

Figure 15. Representation of the amounts (mean +/- SD, n=7) of total (SYTO 9-positive) and dead (PI-positive) pixels at each power level.
Based on the Kruskal-Wallis analysis, it was confirmed that the different laser powers resulted in differing percentages of dead bacteria (alpha value 0.05, p=.0002). A Jonckheere-Terpstra test was then conducted to determine the evidence of monotonic increase in percentage of dead bacteria as the laser power is increased. This test confirmed that statistically significantly increasing percentages of dead bacteria were found as the output wattage was increased (alpha value 0.05, p=0.0002).

Temperature Measurements

The results of the temperature trials are presented in Figures 16-21. There was a marked increase in temperature rise as laser power was increased, indicating that more energy was delivered to the surface with increasing power. However, when irrigation was used along with the laser (as per manufacturer’s instructions), there was no discernible rise in temperature. Minor temperature fluctuations can be observed, coincident with the laser tip passing over the disk. This finding also provides proof of the repeatability and precision of the moving stage. Figure 20 represents temperature readings from the bottom and top of one of the trials, indicating that the disk diffuses heat well from the top to the bottom. Figure 21 demonstrates the peak temperatures measured during the trials.
Figure 16. The temperature readings using 0W laser output, and air/water irrigation.

Figure 17. The temperature readings using 0.5W laser output, with and without irrigation.
Figure 18. The temperature readings using 1.0W laser output, with and without irrigation.

Figure 19. The temperature readings using 2.0W laser output, with and without irrigation, and with irrigation turned on halfway through the laser application.
Figure 20. No difference in temperature readings measured from the top and bottom of the disk.

Figure 21. Peak temperature readings based on laser power and irrigation.
DISCUSSION

Surface Analysis

Roughness

It was hypothesized that, as laser power was increased above 1.0W, there would be a dose-dependent effect on the surface roughness parameters, as measured by Sa and Sdr. As illustrated in Figure 10, the Sa value did not significantly change from before to after treatment, when all groups were combined (p-value: 0.59). However, the Sdr did change from before to after treatment, and the difference was statistically significant (p-value=0.0001). As seen in Figure 10, there was a trend for the Sa to change the most at the 1.0W and 1.5W treatment groups, and the Sdr changed the least for the 1.0W and 1.5W groups. These findings can be explained by examining the 0W treatment group. At no laser output (see Table 1), the Sdr had a mean change of 2.86 percentage points. As the power was increased, the Sdr value changed less dramatically, and had a greater difference for the 2.0W group. These trends indicate that the biofilm has an impact on Sdr measurement, more so than on the Sa, but also indicates that the effect of the biofilm on Sdr value is negated using moderate laser power levels. It should be noted that none of these measurements are statistically significant from each other (due to the large variation among measurements), but they indicate trends in the data. Because Sdr is a measurement of surface texture, it is likely that bacterial byproducts, either lysed cell remnants or waste products of the biofilm, are filling the pores of the disk surface. In other words, leftover bacterial remnants are smoothing out the valleys seen on the surface. An SEM image (Figure 22, unpublished data) from another study
demonstrated that bacterial remnants could be seen after laser treatment at 1.5W with the radial firing tip used in the present study.

Figure 22. After laser treatment at 1.5W with the RFPT-5 on the Er,Cr:YSGG, bacterial remnants can be observed in the complex microstructure of the disk surface.

Sa is an effective tool for evaluating the overall texture of a surface. However, it is insensitive in differentiating surfaces with complex features, e.g. peaks vs valleys. Sdr is a more effective tool for evaluating intricate surfaces, because it can distinguish between complex surface features. Sdr is also more sensitive to texture amplitude as well as spacing. For these reasons, Sdr may be a more effective tool for evaluating these complex implant surfaces.

The effect of Er,Cr:YSGG laser treatment on the surface of microtextured implants has been examined previously. SEM images show that, at high laser power output levels, damage to the implant surface, as depicted by melting and flattening of the surface topography, could be readily observed \(^{58}\). Although that study utilized a straight-firing tip, it still demonstrates the potential of this laser to damage titanium
surfaces. The Er,Cr:YSGG laser, at 1.5W output with a straight-firing tip, significantly impacted the Sa parameter of an SLA-treated titanium implant surface, changing the value from 1.99 to 3.37 microns\textsuperscript{59}. However, microscopic images of the treated surface indicated that there was a lack of overlap between the treated and non-treated areas of the surface. The present study utilized a moving stage to ensure that there was repeated overlapping of the laser to the disk surface. Also, the previously mentioned studies utilized a straight-firing tip oriented perpendicularly to the surface, delivering a large wattage directly to the implant surface. In the present study, a radially-firing tip, which delivers significantly less direct energy to the surface, was utilized as a more clinically applicable scenario in the treatment of peri-implantitis.

**Contact Angle**

As with the roughness measurements, it was hypothesized that the contact angle (wettability), as measured using the dynamic contact angle, would be significantly increased, as laser power output increased above 1.0W. Wettability is an important attribute of implant surfaces, because it can directly affect the adhesion of cells to the surface of the disk\textsuperscript{40}. On the other hand, hydrophilicity is considered a secondary factor in the viability and attachment of osteoblasts to titanium surfaces\textsuperscript{60}. Regardless of the effect of wettability on the success of a dental implant, it is clear that a significant change in contact angle after laser therapy is an indicator that there has been a change in the surface of the implant, possibly indicating damage. Lowering of the contact angle indicates a higher surface energy is being created. Such associations are made when
the roughness or porosity of the substrate increases. However, if bacterial remnants were being trapped in the pores of the rough SLA implant surface, then the presence of these hydrophilic organic components would also render the surface more wettable, and result in a lowering of the contact angle, which was seen in the present study.

As illustrated in Figure 13, there was a statistically significant decrease in mean contact angle of the disk surface from before biofilm growth to after laser treatment. However, as seen in Figure 12, this change was independent of the power of the laser applied to the disk. The change in contact angle in the control group (0W) was nearly identical to that of the 2W treatment group. This result indicates that laser treatment has no impact on the wettability of the SLA disks, but that some other portion of the experiment significantly changed the hydrophilicity. The post-treatment surface was more hydrophilic, which might be explained by incomplete cleansing of the disks before analysis; bacterial byproducts may not have been completely removed from the intricate and complex surface textures even after sonication in ethanol.

An in vitro study indicates the Er,Cr:YSGG laser did not negatively affect osteoblast attachment to an SLA surface, and in fact, may have improved the cell affinity \(^6\). However, no studies have examined the effect of this specific laser and its unique ring-firing tip on the contact angle or wettability of implant surfaces.

**Biofilm Analysis**

It was hypothesized that increasing the power output of the Er,Cr:YSGG laser would cause dose-dependent response in the amount of dead bacteria, as measured by
live/dead staining analysis and confocal microscopy. The results of the biofilm analysis, illustrated in Figures 14 and 15, indicate that the amounts of dead bacteria are not increasing as the laser power is increased. Rather, the numbers of total bacteria appear to be decreasing as laser power is increased. This decrease has the effect of increasing the percent of dead bacteria associated with the laser power application. Based on the Jonckheere-Terpstra statistical analysis, there is a statistically significant, monotonic increase in percent dead bacteria as the laser power is increased. Even though the percentage of dead bacteria seemed modest, it is known from periodontitis models that it is not necessary to remove all the biofilm in order to improve clinical parameters.

As stated previously, there are four methods by which lasers can kill bacteria: photothermal, photoablative, photochemical, and photomechanical. The Er,Cr:YSGG laser creates microexplosions in water, thus creating a photomechanical effect and destroying bacteria by creating microexplosions within the cell membrane. In an in vitro experiment, the Er,Cr:YSGG laser, at 2W output using a radial firing tip, was as effective as 5% NaOCl at disinfecting a root canal system. Given the results seen in Figures 14 and 15, it can be hypothesized that the laser is interfering with the attachment of bacteria to the disk surface, and is therefore removing live bacteria from the surface. However, a more likely explanation is that the laser is lysing the cells, and as the nucleic acids are spilled onto the remaining biofilm and disk surface, they are being washed away by the irrigation. This effect would result in a decreasing amount of GFP-positive cells, while not increasing the amount of PI-positive cells; this trend is exactly what is demonstrated in Figure 15.
Upon examining the individual results of the biofilm analysis, it is clear that there is a wide variation in the amounts of bacteria (both dead and alive) from disk to disk. However, the percentage of dead bacteria on each disk remains relatively consistent, accounting for the rather large error bars in Figure 15 and the comparatively small error bars in Figure 14. As an example, disk A has 10 dead bacteria and 100 total bacteria, while disk B has 20 dead bacteria and 200 total bacteria; the numbers of bacteria varies widely while the percentage of dead bacteria for both disks is 10%. This can be explained by daily variations in bacteria viability or growth, while the laser treatment has a predictable effect on the percentage of dead bacteria.

A previous in vitro study utilized atomic force microscopy to evaluate the effect of Er,Cr:YSGG laser with a radial firing tip on E. faecalis samples. It was concluded that the laser caused changes in cell roughness and that clear signs of cell lysis were observed, indicating that the laser caused ablation and physical disruption of cell membranes. Other research examined the ability of the Er,Cr:YSGG laser, using a radial firing tip, to eliminate plaque biofilms that were grown on intraoral splints. The results of this study indicated a dose-dependent response of the residual plaque biofilm after laser treatment, indicating that increasing the power level of the laser resulted in more extensive biofilm removal.

Temperature

Figures 16-21 illustrate the results of the temperature measurement. Without irrigation, the surface temperature of SLA disks rose in a dose-dependent manner with
respect to the power of the laser. At a power level of 0.5W, the surface temperature, as measured by a thermocouple immediately subjacent to the disk surface, rose about 5°C. When the laser power was increased to 1.0W, the temperature rose approximately 10°C. Finally, at a power level of 2.0W, the surface temperature rose 20°C. Using this trend, a correlation of near surface temperature rise of 10°C per applied Watt for 120 seconds can be established. However, when irrigation was used in conjunction with the laser, the surface temperature stayed approximately at the temperature of the water irrigant, without significantly rising. This result indicates that the laser certainly applies heat to the surface of the SLA disks, but that the overall effect on the surface temperature is negligible when irrigation is used appropriately. Because the greatest volume component in bacteria is water, and this laser has been shown to specifically interact with water molecules, it may be possible with sufficient irrigation to kill bacteria, but not significantly raise the implant metal temperature to levels causing significant effects.

A previous study examined the change in temperature at the apex of an implant, when an Er,Cr:YSGG laser at 1.5W with a straight-firing tip was applied to the coronal portion of the implant \(^{57}\). The findings indicated that neither the laser nor water irrigation had an overall cooling effect on the apical portion of the implant. The current study differed in design, in that the surface temperature of the disk was examined both with and without irrigation, allowing analysis of the immediate effect of the laser on the surface. The current experimental design also allowed for separation of the effects of the laser and of the irrigation on the implant surface temperature. In addition, the analysis, where the irrigation was activated halfway through the laser application,
indicated that the irrigant had a rapid cooling effect on surface temperature. Temperature readings from the bottom and the top of the disk were indistinguishable, indicating that the disk diffuses heat well, up to a distance of at least 2mm (the thickness of the disk).

The discovery that the surface temperature rises without irrigation is significant in that it could explain the method of bacterial ablation and potential damage to an implant surface or the surrounding bone. Heat is known to kill bacteria, a method that is commonly utilized in the sterilization of dental instruments. Also, extreme heat could damage an implant surface, negatively affecting any attempts at bone regeneration or defect resolution. Another potential sequela is that the surrounding bone could be negatively affected by generation of heat, as bone has been shown to exhibit necrosis with temperature increases. In a rabbit model, with a baseline body temperature of 38°C, a temperature increase to 47°C for only 1 minute resulted in reduced bone regeneration \(^66\). If the temperature in a human patient were increased by 20°C, as seen in the experimental group of the present study, an adverse effect on bone healing would be expected. Clinically, when water irrigation is used appropriately and reaches the intended target, there should not be any significant temperature rise of the surrounding tissues. However, if the tissues interfere with the irrigant reaching the target, e.g. in a non-surgical treatment of a peri-implant defect where the tip is 7 mm subgingival, then a rise in the temperature of the peri-implant tissues would be expected. This is significant in that, due to the demonstrated thermal diffusivity of the disks, temperature rise could be propagated along an entire implant, interfering with osseointegration and osteoblast viability, not only at the defect site, but also all along the length of the implant.
Clinical Implications

This study is significant in that it is the first investigation to assess surface changes of an SLA surface after irradiation by an Er,Cr:YSGG laser through a *P. gingivalis* lawn. The clinical implications are that a similar situation would be expected in a peri-implantitis or mucositis lesion, where an implant surface is covered by a biofilm while being irradiated by the laser. Therefore, it is reasonable to expect that similar results would be expected in terms of effects on the biofilm and effects on the surface characteristics if the RFPT-5 laser tip is utilized with the Er,Cr:YSGG laser at these power settings. Based on the results of the current study, bacterial ablation can be expected at power levels as low as 1.0W and increasing ablation as power is increased up to 2.0W. Also, no quantifiable effects of increasing laser power can be observed on surface micro-roughness or wettability, providing evidence that the Er,Cr:YSGG laser does not affect these characteristics at power levels up to 2.0W. It is important to note that there is a step-down of the power setting of the laser which actually reaches the implant surface, using the radial firing tip. This was previously discussed in Figure 5. In addition, the effects of this same lateral power level should be investigated on the sulcular gingival tissue against which the laser is also delivered.

Study Limitations

There are several limitations of the current study, which must be kept in mind when interpreting the results. First of all, this was an *in vitro* experiment, and the effects of using this same laser on humans with *in vivo* implants may be highly different. Saliva
contamination, dissipation of heat through implant components and surrounding tissues, and absorption of the laser may all behave differently in the oral cavity than on the benchtop used to model a clinical scenario. In addition, the biofilm that accumulates on implant surfaces is vastly different than the monoculture of *P. gingivalis* that was utilized in the current study. The *in vivo* biofilm contains hundreds of different bacteria, including streptococci, fusobacteria, spirochetes, and even fungi and viruses. *P. gingivalis* was chosen for this experiment, based on its establishment as a keystone periodontal pathogen. It is worth noting that different bacterial species may react differently to the laser application, and interactions of different species may allow the bacteria to be more or less resilient to ablation. However, if the main action of this laser is interaction with water, then it is expected that its output would have a lysing action on whatever bacteria are exposed.

As well as utilizing a single bacterial species, the current study only examined a single commercially available implant surface treatment. Different implant surfaces may react differently to the Er,Cr:YSGG laser. In a previous study, TPS-coated implants were found to be unaffected by this type laser at power output values up to 6W, while HA-coated implants showed almost complete ablation of the coating \(^{67}\). This finding could mean that clinical recommendations for peri-implantitis therapy may include different power settings or application times, depending on the particular implant surface that is present.

The present study also utilized a highly controlled distance of the laser tip to the target surface, and tightly controlled overlap of the laser path as it was drawn across the disk surface. While these controls are valuable in an *in vitro* experiment to obtain
reproducible testing conditions, it is not reasonable to expect that these circumstances can be duplicated in a clinical setting. For example, when the laser tip is applied to a peri-implant defect, the tip will be nearly in contact with the threads of the implant, but will be at some distance from the spaces between the threads, where the majority of contamination is located. For this reason, more studies on the impact of tip distance on both biofilm and the implant surface should be conducted. In addition, it is likely that the irrigant would not penetrate the peri-implant defect during a clinical situation as it does in vitro. Therefore, it is possible that the temperature of the surrounding tissues could be raised briefly until the irrigation makes its way through the defect. Such a temperature rise may significantly affect the vitality of perimplant tissues, including bone.

An advantage of the current study is that it utilized a keystone periodontal pathogen on a widely used, commercially available implant surface. The laser was then applied through the bacteria to the implant surface. This set up is unique among previously published studies, which examined either the effect of the laser on bacteria or the effect on an implant surface alone, but not in combination. The current study provides more clinically relevant results on the effect of the laser treatment to a contaminated implant site. However, further studies should be conducted using more complex biofilms that involve other periodontal bacteria as well as other implant surfaces in order to draw broader conclusions on the effect of the Er,Cr:YSGG laser in a clinical scenario.
Future Studies

Future research should focus on utilizing implants with different types of surface treatments, including the SLA, to generate a controlled study for comparison between the surfaces. Also, a more complex biofilm that includes more than a monoculture of *P. gingivalis* should be grown on the disks. This design would allow for a more translatable experiment that would have greater clinical implications. Finally, *in vivo* studies should be performed to verify the *in vitro* results in a clinical setting.

CONCLUSIONS

Within the limitations imposed by the conditions of this study, the following conclusions may be made:

1. The SLA surface micro-roughness and wettability do not measurably change as a result of increasing power using an Er,Cr:YSGG laser,

2. The exposed implant surface temperature increases in a dose-dependent manner using an Er,Cr:YSGG laser when no irrigation is used, but there is no measurable increase when the prescribed irrigation is utilized, and

3. *P. gingivalis* grown on an SLA-treated dental implant surface is ablated in a dose-dependent manner as increasing power levels of an Er,Cr:YSGG laser are used.
Appendix A

Flowchart of Pilot Study

An outline of the pilot study is seen in Figure 23.

Figure 23: Pilot study to determine effects of laser exposure time and power setting on bacterial elimination from SLA-treated titanium disks

Selection of titanium disks

Implant disks having a commercially available surface modification (sand-blasted, large grit, acid-etched, SLA) were supplied by a manufacturer (Dentium, Yong-In, South Korea). This SLA surface is widely used by multiple implant manufacturers, and represents a significant portion of implants currently placed in dental practices.
Biofilm growth

P. gingivalis strains were maintained anaerobically (10% H₂, 10% CO₂, and 80% N₂) in an anaerobic system glove box (model 1025/1029, Thermo Scientific, Waltham, MA, USA) at 37°C in a specialized broth (Difco anaerobe broth MIC 1, BBL Microbiology Systems, Cockeysville, MD, USA). This medium contains the following nutrients: pancreatic digest of casein, peptic digest of animal tissue, papa in digest of soybean meal, dextrose, yeast extract, sodium chloride, dipotassium phosphate, hemin, L-cystine, and Tris ⁶⁸. Bacterial specimens for growth on the titanium disks were obtained during their peak phase from the bacteria stocks. The bacterial suspension was washed 2 times in PBS, and P. gingivalis was re-suspended to an OD value of 0.11 at 660 nm, which was previously determined to be equal to 5 x 10⁷ CFU ⁶⁹. A total of 2x10⁸ cells were placed into wells containing the titanium disks. The disks were incubated in the anaerobic chamber mentioned above for 48 hours, then laser treatment was applied.

Laser exposure of disks

The Er,Cr:YSGG laser (Waterlase iPlus, Biolase, Irvine, CA) was applied to the disks utilizing a custom-made, computer-controlled, moving platform, having a programmable X- and Y-axis speeds, allowing laser application to be applied consistently and evenly to the entire disk surface, utilizing a raster-like movement. Laser energy was applied to the disc surface using a radial firing perio tip (RFPT5, Biolase), with the tip held 0.5mm from the surface, using air and water settings of 50%, 50%. The laser pulse frequency was set to 30 Hz, and the exposure time and power output were varied to include all combinations of time (30s, 60s, 120s) and power (0W, 0.5W, 1.0W, 2.0W). These
combinations were conducted once each (n=1), and specimens were analyzed to determine the effects of the combinations. The combinations of exposure duration and laser power which were deemed to be most effective at ablating bacteria without changing the surface were re-tested with more replicates in the full study. The pilot study was conducted to conserve disks so that a higher number of replicates could be performed in the full study. Exposure of bacteria-laden discs to an autoclave cycle (121 °C for 15 minutes) was used as a positive control to cause 100% bacteria killing.

**SEM analysis**

The surfaces of the titanium disks were treated according to standard protocols for scanning electron microscopy and were observed both before and after laser treatment. A magnification of 5000X at a working distance of 8.5 mm at 10.0 kV was used to visualize topographical changes to the surface of the titanium disks as a result of laser exposure (Model XL30 FEG, Philips, FEI Company, Eindhoven, The Netherlands).

**Crystallinity composition**

An x-ray diffraction (XRD) machine (Miniflex, Rigaku, Tokyo, Japan) was used to analyze the surface crystalline composition of the specimens. This machine operates by measuring the diffraction of x-rays when reflected off the surface of a sample, and provides data that can be used to compare to known samples in order to determine the crystalline composition and content of the specimen. The copper source produced x-rays at a wavelength of 1.54184 Å, The scans were conducted in steps of 0.01 degrees at 0.50 degrees per minute. The miniflex machine used a fixed 30kV at 15mA electron
beam generator. The data was compared to spectra of known titanium alloy compositions from a library; the XRD spectra of the samples were also compared to one another to determine the effect of laser treatment on the samples.

Biofilm analysis

A live/dead staining kit (LIVE/DEAD BacLight Bacterial Viability and Counting Kit L34856, Life Technologies, Grand Island, NY) was used to stain the samples. The protocol followed the manufacturers’ instructions, with the exception that re-suspension of the bacterial pellet was performed using 200 uL of media instead of 1000 uL, to generate a higher concentration of bacteria for the analysis. Both components use nucleic acid stains: propidium iodide (PI, Figure 24) leaves a red stain on dead bacteria having damaged cell membranes, and SYTO™ 9 (Figure 25) leaves a green stain on all bacteria.

![Figure 24](image_url)

*Figure 24*
Fluorescence Excitation (blue)/Emission (red) spectra of propidium iodide bound to DNA of cells with damaged cell membranes.
Figure 25
Fluorescence Excitation (blue)/Emission (red) spectra of SYTO® 9 green fluorescent nucleic acid stain bound to DNA of cells with intact cell membranes.

Samples were examined using confocal microscopy (LSM 780 Multiphoton Confocal Microscope, Zeiss, Oberkochen, Germany) at the GRU Imaging Core facility. Images were evaluated using a color-quantifying software (ImageJ, National Institutes of Health, Bethesda, MD, USA) to assess the fractions of live and dead cells.

**Pilot Study Results**

Because of the low number of available titanium disks for testing, only a single disk was used for each treatment group in the pilot study. The results were analyzed to observe trends resulting from different combinations of output power settings and exposure durations with respect to their potential for modifying surface characteristics of the implant, as well as their ability to kill bacteria. An optimal combination of parameters would provide no surface modification, yet would demonstrate significant bacterial killing. The time/power combinations that were identified as most useful were then re-tested in the main experiment.
Specific aim #1 – Surface Characteristics

This specific aim tested the hypothesis that surface roughness, surface wettability, and composition of the titanium alloy will be significantly altered at power levels over 1.0W, but will not be altered at a power level of less than 1.0W.

Surface Analyses

X-ray Diffraction

The XRD analysis was unable to show an effect on the crystallinity or composition of the disk, based on the treatment group. Therefore, this parameter was not included in the full experimental protocol. Samples of the XRD data are presented in Figures 26 and 27. Although the plots of the diffraction events are nearly indistinguishable, Figure 26 shows the data from a 0W control, while Figure 27 shows data from a 2W treatment group.
Figure 26. Sample data from XRD analysis. Various peaks correspond to different crystalline structures found within the sample. This sample represents data from a 0W control specimen.
Figure 27. Sample data from XRD analysis. This sample represents data from a 2W test specimen.

**SEM Analysis**

The SEM images obtained before and after laser treatment appeared to show a dose-dependent response of laser power on qualitative surface changes. As laser power increased, there appeared to be more melting and elimination of surface topography characteristics. Representative SEM images from each laser power are seen in Figures 28, 29, 30, and 31. In Figure 28A, 28B, and 28C, the untreated, intact disk surface is visualized. Qualitative changes to the surface are seen in Figures 30A
and 30B as flattening or melting of the surface topography. In Figure 31C, melting of the surface peaks and granulation debris are present.

Figure 28. (A) 0W for 30 sec, (B) 0W for 60 sec, (C) 0W for 120 sec

Figure 29. (A) 0.5W for 30 sec, (B) 0.5W for 60 sec, (C) 0.5W for 120 sec

Figure 30. (A) 1W for 30 sec, (B) 1W for 60 sec, (C) 1W for 120 sec

Figure 31. (A) 2W for 30 sec, (B) 2W for 60 sec, (C) 2W for 120 sec
Based on the results of the pilot study, and upon current clinical recommendations from the laser manufacturer, it was decided to perform a more in-depth analysis (henceforth termed the main experiment) using power settings of 0W, 1.0W, 1.5W, and 2.0W, all of which are performed for a duration of 120 seconds.

**Specific aim #2 – Biofilm Analysis**

This specific aim tested the hypothesis that bacterial load will be significantly reduced after irradiation by the Er,Cr:YSGG laser, in a dose-dependent manner, demonstrating greater bacterial elimination with increasing power levels of the laser.

*Bacterial Elimination*

In an attempt to increase statistical power, 4 confocal images were exposed for each disk. Laser exposure demonstrated a dose-dependent, inverse relationship between power output and viable bacterial cells. The region of interest for laser power appeared to lie between 1.0 - 2.0 W (Figure 32). Increasing laser exposure time (no matter the power setting) did not have a statistically significant impact on either live or dead bacteria.
Figure 32. Number of pixels stained for dead bacteria by laser power and duration of exposure. There was no impact of increasing duration on the amount of dead bacteria pixels.
REFERENCES


30. Froum SJ, Froum SH, Rosen PS. Successful management of peri-implantitis with a regenerative approach: a consecutive series of 51 treated implants with 3- to


