Photodynamics: Is this a viable adjunctive periodontal therapy?

by

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Abstract

Objective: The aim of this study was to conduct a randomized *in vivo* clinical trial to assess the efficacy of a one time application of a photoactivated dye, methylene blue (PerioWave™) in treating chronic periodontal disease in humans.

Material and Methods: Thirty five periodontally maintained subjects with moderate to severe chronic periodontitis were randomly divided into a control group with nonsurgical periodontal debridement (NSPD) only versus an experimental group with NSPD and antimicrobial photodynamic therapy (APT). All teeth in both groups received periodontal treatment comprising of scaling, root planing, ultrasonics and local anaesthesia. The experimental group had the qualifying sites (pockets depths greater than 4.4 mm and bleeding on probing (BOP)) treated with the PerioWave™ system and compared to the control group. The primary endpoint was clinical attachment level (CAL) and secondary endpoints were pocket depth (PD) and BOP. The endpoints and plaque were evaluated at baseline, 6 and 12 weeks by a blinded examiner.

Results: This study showed PerioWave™ as a one time application of a cold diode laser with a wavelength of 670nm; a maximum power of 150mw; an average energy density of 20 J/cm²; a phenothiazine photosensitizer (methylene blue) in a concentration of 0.01% wt/vol; and at an optimal activation time of 60 seconds per mesiodistal surface had no statistical significance in the treated qualifying sites with regards to CAL (p=.69; p=.97) or PD (p=.14; p=.23) at 6 or 12 weeks after treatment *over* the NSPD group. However BOP did have a statistical significance over the control group at 6 weeks (p=.05) but not at 12 weeks (p=.47).
**Conclusions:** In patients with advanced chronic periodontitis, CAL and PD did not show improvement with one time application of the PerioWave™ photodynamic system over conventional NSPD. However, the lack of BOP did have a short time (6 weeks) reduction.
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Chapter 1: Literature Review

1.1 Overview of the Oral Cavity

1.1.1 Introduction

The human oral cavity is colonized by a large number of highly diverse bacteria existing in either a planktonic community or in a complex sessile community known as a biofilm (i.e. dental plaque) (Haffajee and Socransky 2006, Carranza 2006). While the majority of bacteria in these complex communities are nonpathogenic some bacteria are opportunistic pathogens and are associated with extraoral and intraoral diseases. For example, *Streptococcus oralis* can be isolated from supragingival plaque, mucosal surfaces, tongue and saliva but can induce endocarditis and extraoral abscesses (Wilson 2003). Plaque biofilm begins to develop immediately after a tooth surface is mechanically cleansed and increases both in amount and complexity over time (Wilson 2003). Presence of specific microorganisms in both supragingival and subgingival biofilms are associated with three common chronic intraoral inflammatory diseases; caries, gingivitis and periodontal diseases (Wilson 2003, Loesche and Grossman 2001).

1.1.2 Gingivitis

Gingivitis is an inflammatory disease that is associated with proliferation of local epithelial cells and loss of gingival connective tissues but is not associated with loss of connective tissue attachment (Page and Schroeder 1976). The most common form of gingivitis occurs due to the accumulation of biofilm on teeth. The undisrupted biofilm or “neglected” gingivitis is said to be the most common form of periodontal disease and is experienced by all individuals at some time (Loesche and Grossman 2001). Clinical
inflammation of the tissues is present and significant spontaneous or bleeding on probing is noted. Associated with this bleeding are a significant proportional increase of certain species like, *Actinomyces viscosus* and the appearance of *Campylobacter* and *Prevotella* species in the biofilm (Socransky and Hafajje 2006, Loesche and Grossman 2001). Increased bleeding may create a new niche which allows specific species to increase in number within the biofilm community (Loesche and Grossman 2001). Gingivitis is reversible by mechanically disrupting the biofilm which in turn reduces inflammation and allows for gingival healing (AAP 2005). Daily mechanical plaque control is essential for controlling this inflammation.

Presently researchers are unclear as to which sites with gingivitis can progress to periodontitis, in humans (Loesche and Grossman 2001). However, with development of chronic gingivitis there is a gradual shift to a Gram-negative anaerobic dominated biofilm that is consistent with what is found in periodontitis (AAP 2005, Loesche and Grossman 2001, Socransky and Haffajee 2006). Inadequate dental plaque control associated with poor oral hygiene (3 to 4 weeks) increases the potential to developing a more anaerobic flora and this may in some sites lead to periodontitis. In contrast, in animal models rapid biofilm (plaque) accumulation after placement of a foreign body such as silk ligature around teeth in either dogs or monkeys typically progresses to periodontitis (Schou et al. 1993, Loesche and Grossman 2001).
1.1.3 Periodontitis

Periodontitis is a chronic infectious inflammatory disease that affects the gingiva and is associated with loss of gingival and periodontal ligament connective tissue and alveolar bone (Page and Schroeder 1976). The pathogenesis of periodontal disease is multifactorial but the microflora associated with subgingival biofilm clearly plays a significant role in disease pathogenesis. The organisms associated with the periodontal diseases are primarily Gram-negative obligate anaerobes and capnophiles such as *Porphyromonas gingivalis, Prevotella intermedia, Fusobacterium nucleatum, Bacteroides forsythus, Actinobacillus actinomycetemcomitans* and various spirochetes (Wilson 2003, Suchett-Kaye et al. 2001, Loesche and Grossman 2001). The presence of these associated periodontopathogenic bacteria at threshold levels within the subgingival biofilms is strongly associated with tissue degradation. This tissue degradation is mediated directly and indirectly by bacterial expressed virulence factors. (Wilson 2003) For example, *P. gingivalis* secrete a number of potent proteases like collagenase that is directly involved in connective tissue degradation (Wilson 2003, Carranza 2006, Loesche and Grossman 2001). Another significant bacterial virulence factor that indirectly mediates tissue destruction is the expression of lipopolysaccharide (LPS) on the outer cell membrane of Gram negative microorganisms (Wilson 2003, Soukos et al. 1998, Loesche and Grossman 2001, Dahl et al. 1989, Carranza 2006). It is a key virulence factor within periodontal tissues because most periodontopathic microorganisms are Gram negative. Lipopolysaccharide induces inflammation by stimulating the expression of pro-inflammatory cytokines like interleukin-1, IL-6 and tumor necrosis factor-α (TNF-α) by resident and recruited cells and these proinflammatory cytokines induce host expression

Although the presence of biofilm-associated bacteria is required for disease, it is also understood that periodontal disease results from a complex interplay between biofilm associated bacteria, the host response (immune and genetic), and collectively is modified by variety of positive (e.g. brushing) and negative (e.g. smoking) behaviors factors (Page et al. 1997, AAP 2005).

The prevalence of periodontal disease increases with age and as more people are living longer and retaining more teeth, the number of people developing periodontal disease is likely to increase in the next decade (Loesche and Grossman 2001). Presently within the adult population 5% to 15% of any population suffers from severe generalized periodontitis, while about 80% have 'moderate periodontitis', with 10% of the population described as periodontally healthy (Heitz-Mayfield et al. 2002, AAP 2005). Practically it has been estimated by the National Institute of Cranio-Facial and Dental Research that 2 to 6 million people require professional periodontal care (Loesche and Grossman 2001, AAP 2005). To manage this disease it has been estimated in 1999 that periodontal and preventive procedures totaled $14.3 billion dollars, of which $4.4 billion dollars was spent on periodontal services to directly treat the disease (Brown 2002). In addition to the high cost of therapy it has become clear that chronic oral infections could serve as a reservoir of inflammatory mediators, LPS, and other bioactive molecules that might

To manage these diseases mechanical debridement (scaling and root planing via hand and power driven instruments) is the accepted gold standard (AAP 2005). These treatment modalities involve the removal or disruption of biofilm, its byproducts, and plaque-retentive calculus deposits from crown and root surfaces, and within the sulcus or pocket (O’Hehir 1999). This treatment approach reduces local inflammation and periodontal pocketing but the subgingival biofilm typically returns to baseline by 12 weeks (and Haffajee and Socransky 2006, AAP 2005). This primarily occurs for two reasons. First, ultrasonics and other mechanical debridement methods effectively disrupt biofilm, but they have no bactericidal activity and cannot eradicate all bacteria. Second, some periopathogens reside on and within soft tissues or have colonized areas that are non-accessible to mechanical disruption. These remaining microorganisms facilitate rapid recolonization of periodontal pockets and possibly further attachment loss. (Meisel and Kocher 2005). For these clinical situations a variety of disinfectants (e.g. chlorhexidine), or local and systemic antibiotics have been tested for their ability to kill periodontal disease-associated pathogens and improve clinical outcomes associated with periodontal debridement (Carranza 2006, Wilson 2003). Using these various strategies microbial colonization is reduced in the short term and clinical parameters are improved but these clinical improvements are typically small and short lived (Socransky and Hafajjee 1994, Carranza 2006).
1.1.4 Summary

Periodontal diseases are common chronic inflammatory conditions that affect a large proportion of the adult population. Although the disease is multifactorial the presence of a gram negative subgingival microflora is critical to the disease process and directly or indirectly mediates inflammation and loss of hard and soft connective tissues. Periodontal debridement effectively disrupts the plaque biofilm but this benefit is short lived and all patients are therefore required to continually present for maintenance therapy (debridement) every 12 weeks. For patients with limitation in physical ability, special needs patients, or patients with persistent local disease a variety of adjunctive pocket disinfection approaches have been tested. A growing concern over antibiotic overuse and the finding to date that improvement in clinical parameters are small and short lived when these agents are utilized has limited the general use of these adjuncts. However, there is a clear need to develop novel nonantibiotic approaches to eradicate subgingival microflora and to test these products in vivo to determine whether they are effective adjunctive approaches to use with periodontal debridement.
1.2 Clinical Parameters to Assess the Periodontium

1.2.1 Introduction

A diagnosis directly affects decisions in all phases of treatment, evaluating the outcome of therapy, or determining long-term prognosis. The diagnosis and prognosis of a patient’s periodontal health rely on evaluation of several clinical parameters. The goal of these parameters is to provide a reliable diagnostic process that aids in reducing the risk for further disease progression. These parameters indices include but are not limited to, clinical attachment level (CAL), probing pocket depth (PPD) and the presence of bleeding on probing (BOP). This section focuses on the potential measurement errors in assessing pocket depth, clinical attachment loss, and bleeding on probing using manual and controlled-pressed probe.

1.2.2 Clinical Parameters and Recognition of Periodontal Disease

The basic clinical measurements for assessing the periodontium, apart from gingival bleeding and radiographic assessment of bone loss, are clinical attachment loss (CAL) and probing depth (PD) (AAP, 2005).

1.2.2.1 Probing Depth (PD):

The distance from the gingival margin to the base of the probable crevice. It is a clinical approximation of the depth of a periodontal pocket or sulcus.

1.2.2.2 Clinical Attachment Level (CAL):

The distance from the cementoenamel junction (CEJ) to the base of the periodontal pocket or sulcus. It is a clinical approximation of the loss of connective tissue attachment from the root surface. This measurement is extremely useful in clinically monitoring
attachment level changes on a site-by-site basis from one visit to the next. Changes in clinical attachment level measurements are the most accurate method of assessing treatment outcomes and following long term periodontal stability. The gold standard of care requires the use of a periodontal probe to assess PD and CAL even though there is inherent measurement errors associated with its use (AAP 2005).

1.2.3 Operational Definitions (Measurement Terms)

When measuring an object specific criteria need to be established in order to 1) describe it and 2) to provide specifiable criteria for its application (Brunette 1996). In other words, a set of criteria or operational definitions i.e. validity, resolution, precision, accuracy and reliability, tell scientists what to look for or what to do in order to carry out the measurement, construction, or manipulation of variables. The above mentioned operational terms will be discussed with regards to CAL and PD when using a manual or pressured periodontal probe to assess periodontal health.

Validity:

Validity is the relationship between what a test is supposed to measure and what it actually does measure (Brunette 1996).

Resolution:

How fine a detail can be measured is known as the resolution of a measurement (Brunette 1996).

Precision:

The precision of a measurement refers to how close repeated measurements of the same quantity are to each other. A measurement is said to be precise if there is only a small
spread of numbers around the central tendency (average value) used. The standard deviation is often used as the criterion of the precision of a result (Brunette 1996).

Accuracy:
A measurement is accurate if its performance on average is close to the true value to be determined without bias. Therefore, a measurement is said to be accurate if the result, expressed as a range of possible values, includes the “true” value (Brunette 1996).

Reliability:
A measure is said to be reliable when the variation or random fluctuation due to errors in measurement is small (Brunette 1996).

The clinical diagnosis of most periodontal diseases requires sensitive, unbiased, reproducible and reliable measurements of gingival attachment. The widely used and best available chair side diagnostic tool for the clinical assessment of PD and CAL is the periodontal manual probe. Philtrom classified periodontal probes into 3 generations: first generation, non-pressure controlled with visual data recording (calibrated manual probe); second generation, pressure controlled with visual data recording; third generation, pressure controlled with direct computer data capture (Breen et al. 1999, Philstrom, 1992).

Measurements made with periodontal probes (manual or electronic) are subject to error for a wide variety of reasons. Variations in probing force, discrepancies in the diameter of the probe, inconsistent probe penetration with respect to the level of the connective tissue attachment in various disease states, lack of standardization of probe placement, improper angulation during insertion into pockets and pain felt by patient on probing can

1.2.4 First Generation Probe: Manual Periodontal Probes

Of the previously mentioned associated errors, the manufacturing error, lack of controlled probing force and low resolution are the three characteristics of a manual probe that contribute to significant measurement error.

1.2.4.1 Manual Probe Manufacturing Error

The periodontal probes mostly used today in practice and clinical research are slightly tapered metal cylinders with horizontal marks, with a rounded tip 0.4–0.5 mm in diameter. These types of probes have also been used in clinical trials to record pocket probing depth and clinical attachment level (Mombelli 2005). Manufacturing methods used to produce manual periodontal probes has improved in recent years, but probe-to-probe variations in calibration markings up to 0.5mm still occur (Winter 1979, Vander Zee et al. 1991). Winter (1979) studied 129 manual periodontal probes that he collected from different private practices. Out of 387 total measurements, only 130 were accurate. Time of manufacturing correlated to the probe’s accuracy. When testing the 7.0 mm marking half of the old Williams probes actually recorded 7.4-7.6mm while the range extended from 6.8 to 7.9mm. Newer Williams & Michigan probes showed a high degree of precision, but were not always accurate. Twelve years later in another study, Van der Zee et al. 1991 investigated the width of markings and accuracy of calibration in 7 different probe types. They used a stereomicroscope at a magnification of x40. The
authors found that the markings themselves varied from having no measurable width for engraved bands to a maximum width of 1.13mm for painted bands. Actually painted bands were found to have a mean width of 0.70mm. Irrespective of probe type, scribed (engraved) bands appeared to be the most accurate. Scribed grooves were twice as inaccurate.

As for the marking position on the probe tine, in very few cases was a marking exactly coincident with the manufacturer’s designated calibration to within 0.01mm. When all 7 probe sets were compared for accuracy of calibration, irrespective of type of marking, The “Ash/Dentsply Williams” probes appeared to be the most accurate set with a mean inaccuracy of only 0.06mm. In contrast, the “8-55C LM-Dental (COL-LM)” probes showed a range of 1.7-2.3 mm at the 2.0mm marking and a range of 7.8-8.5 mm at the 8.0mm marking making these probes imprecise but somewhat accurate since the true value is included in the measurements (Atassi et al. 1992).

1.2.4.2 Significance of Force on Manual Probing Accuracy

Periodontal probes fail to identify the apical termination of the junctional epithelium, or the coronal level of the connective tissue attachment (Badersten et al. 1984, Fowler C et al. 1982, Mombelli et al. 1997, Mombelli 2005). The error by which the probe misses these histological landmarks is variable and largely dependent on relative tissue inflammation and probing force (Mombelli 2005). These variables contribute to the discrepancy between clinical and histological measurements and ultimately compromises the accuracy of the probing depth measurements. When comparing probe-tip penetration
in treated and untreated periodontally involved teeth (buccal aspect of single rooted teeth with PD ≥6mm), Fowler et al. 1982, found a penetration of the probe into the connective tissue attachment (beyond the JE) of 0.45mm on average. When treated, the average penetration was 0.73mm short of the apical termination of the JE (Mombelli 2005). The difference of probe penetration between untreated and treated specimens was approximately 1.2mm. Thus the state of health of the tissues seems to strongly influence the precision (when comparing pre- and post-surgical readings of the same site) and the validity (mostly in diseased state).

The force applied influences measurement precision (Mombelli 2005). Clinicians exerted anywhere from 3.0-54.0N of force in the anterior region and up to 140.0N in the posterior region of the mouth (Hassell et al. 1973). In addition depth force graphs typically show a plateau with increasing probing force as small changes in probing force have a greater impact on reproducibility of probing depths when low forces are used (Mombelli 2005). Collectively the degree of force applied can dramatically affect probing validity and should be standardized.

**1.2.4.3 Precision and Resolution When Using a Manual Probe**

The ability to discern loss or gain of clinical attachment is limited by the resolution of the probe. Probing with a manual probe has a resolution of 1 mm. Thirty to forty percent of periodontal pockets that are re-probed with a manual probe after 1–3 weeks may show a positive or negative deviation in clinical attachment level (CAL) or probing depth (PD) of ±1 mm (Kaldahl et al. 1990, Mombelli 2005). The standard deviation of a single
measurement of an average periodontal pocket has been reported to be in the range of 0.8–1 mm (Badersten et al. 1984, Osborn et al. 1994). Consequently, in an existing periodontal defect, true changes in PD or CAL can hardly be discriminated from probing error unless the change is in excess of 2 mm (Mombelli 2005). Therefore, changes in pocket depth and attachment level in a clinical study would need to be at least 2 mm (i.e., two to three times the standard deviation) before the investigators can be confident that they are seeing real change rather than measurement error (Mombelli 2005).

1.2.5 Third Generation Pressure and Computer Controlled Periodontal Probes

The electronic probes provide a constant probing force that will limit some of the error associated with probing. In addition, electronic probes have the capacity for electronic data collection eliminating transcription errors. Last, electronic probes have higher resolution (finest increment the probe can be read to) than the manual probes.(Breen et al. 1999, Philstrom 1992). In contrast to a manual probe, the resolution of the electronic probe ranges from 0.1 to 0.2mm (Reddy et al. 1997). However resolution does not necessarily imply reproducibility or accuracy of the electronic probe. Reproducibility is how repeatable a measurement is and does not necessarily reflect accuracy (Brunette, 1996). A probe could record the same measurement every time; therefore being highly reproducible and can be totally inaccurate (i.e. fails to record correct measurement). The electronic pressure controlled probes appear to be more reproducible but the clinical accuracy of the measurements is largely unknown (Reddy et al. 1997). (Figure 1)
A study of the accuracy of a controlled force electronic probe and a manual probe was assessed in 15 patients scheduled for tooth extraction. The actual attachment level on the tooth was determined by staining the extracted tooth. It was found that the electronic probe significantly underestimated the clinical attachment level (-0.48 mm) whereas no significant difference was found between the manual clinical attachment level measurements and the actual attachment level. However, even with the error observed the authors concluded that the electronic probing depth measurements were clinically acceptable, reproducible and showed improved resolution (Hull et al. 1995).

Although not all studies have demonstrated significant improvements in probing reproducibility when using force controlled periodontal probes, standardization of probing force has been advocated because it reduces the possibility of operator bias. For example, clinicians probing with less force at time of post surgical evaluation. As previously stated, the probing force used by a clinician can significantly affect the validity of the reading as well as the accuracy. Therefore, in order to enhance the
reproducibility of pocket depth measurements the probing force has to be standardized and this is possible with an electronic controlled probe. Whilst the use of a high resolution electronic probe increases the sensitivity to detect disease activity it’s also at the expense of specificity (Jeffcoat and Reddy 1999).

1.2.6 Bleeding on Probing (BOP)

Bleeding on probing (BOP) evaluates bleeding after insertion of a probe to the base of the sulcus or pocket (Chase et al. 1999). BOP has been associated with inflammatory changes at the base of the sulcus or pocket and as a predictor of periodontal breakdown in retrospective and prospective clinical studies (Chaves et al. 1990, Badersten et al. 1985, Haffajee et al. 1983, Lang et al. 1986, Lang et al. 1991). Although the high degree of specificity of BOP makes it a good predictor of periodontal health (Chaves et al. 1990, Lang et al. 1991), the low sensitivity of BOP alone makes this parameter a poor predictor of periodontal destruction. The low sensitivity of BOP in clinical studies is indicated by the large number of false positives, or sites that were predicted to exhibit periodontal breakdown that did not. It has been suggested that utilization of standardized probing pressure might improve the sensitivity of BOP (Van der Velden and De Vries 1978, Polson et al. 1981).

Assessment of BOP during the maintenance phase of a patient is a poor prognosticator of attachment loss (AAP 2005). In one study (Lang et al. 1990, Mombelli 2005), 41 patients were monitored after periodontal therapy for bleeding on probing for 2 1/2 years in a maintenance program. The sensitivity of frequent bleeding to predict clinical attachment
loss >1 mm was only 29%, and the specificity 88%. On the other hand, continuous absence of bleeding on probing had a predictive value of 98% for stability.

Probing forces have been linearly associated with increase probing forces. Studies concluded tissue trauma due to probing with high force as a possible reason for bleeding in the absence of disease and thus a controlled probing force of .25N should not be exceeded (Mombelli 2005).

1.2.7 Summary
A manual calibrated dental probe in day to day clinical practice does not precisely measure the true level of the connective tissue attachment or gains in clinical attachment level or classify causes but can provide a reliable overall estimation of the progression or stability of an individual’s status. A key factor in adjusting for some measurement error is simply mastering the technique. To improve examiner reproducibility, accuracy and reliability clinician calibration was helpful (intra-inter) (Reddy et al. 1999, Magnusson 1996). However, errors associated with manual probe manufacturing, the impact that uncontrolled force has on probing accuracy and poor overall resolution does limit the usefulness of a manual probe for clinical trials where anticipated differences are likely to be small and therefore more difficult to detect. In contrast, controlled pressure probes should be implemented in clinical and epidemiological studies with well trained examiners (intra-inter; Kappa values). Use of third generation electronic probes with their controlled force and higher resolution raises the possibility that smaller burst of disease
activity may be detected where previously they had gone undetected when first generation manual probes were utilized (Goodson 1992).
1.3 Use of Adjunctive Chemotherapeutic Agents for the Management of Periodontal Disease

1.3.1 Introduction

Oral supragingival and subgingival microflora is clearly associated with onset and progression of periodontal diseases, however, several theories addressing the significance of biofilm (plaque) volume and significance of specific bacterial species have been put forth. Generally there doesn’t appear to be one single bacterial species that induces periodontal disease but presence of select organisms are strongly associated with disease onset and progression (Socransky and Haffajee 2005, Loesche and Grossman 2001). Regardless, present treatment approaches utilize nonsurgical periodontal debridement (NSPD) in the initial management of disease. The effectiveness of NSPD is unequivocal and initial use of this has been the gold standard of care. However, select patients or disease sites within a patient often do not adequately respond to NSPD even with additional surgery (Loesche and Grossman 2001). To deal with this clinical issue a myriad of adjunctive therapies have been developed to manage these patients or sites. In general these treatment modalities can be broadly divided into systemic administered antibiotics and local delivery of antiseptics or antibiotics. This section will review theories of disease pathogenesis, whether NSPD still sets the gold standard for the management of chronic periodontitis and reviews select currently available adjunctive approaches that are available to manage periodontal sites that fail to respond to conventional approaches.
1.3.2 Evidence of Bacterial Etiology

1.3.2.1 Nonspecific Plaque Hypothesis:

In the 1960’s the understanding of periodontal disease pathogenesis could best be summarized into a concept called the nonspecific plaque hypothesis. The core of this hypothesis is based on the principle that overgrowth of all bacterial species within bacterial biofilm triggers a host inflammatory response and it was the volume of plaque that was associated with disease onset and progression (Carranza 2006, Socransky and Haffajee 1994, 2006). In addition plaque was believed to be relatively similar from site to site and patient to patient (Socransky and Haffajee 1994). This was based in part on epidemiologic studies that had correlated patient’s age and biofilm volume with the severity of disease (Carranza 2006). The complexity of the oral microflora was shown in one classic study of chronic periodontitis. A total of 171 taxa representing 1900 isolates were identified (Moore et al. 1983). In this paper and a previous paper by Moore et al. 1982, which reviewed the bacteriology of severe periodontitis, no one bacterial species was identified as a causative agent of disease (Carranza 2006).

From a clinical perspective the nonspecific hypothesis states that removal or control of biofilm should effectively reduce disease (Loesche and Grossmann 2001, Carranza, 2006). However, this theory was called into question for two reasons. First, some individuals who presented for care may have accumulated large amounts of biofilm due to poor oral hygiene but did not always present with periodontal disease. Conversely, patients may have presented for care with excellent oral hygiene and minimal plaque accumulation but had significant periodontal pocketing and attachment loss (i.e.
aggressive periodontitis). Second, not all sites or teeth within a patient with disease exhibited the same apparent degree of tissue destruction even though the apparent amount of plaque was consistent throughout the mouth (Carranza 2006, Slot and Jorgensen 2002).

1.3.2.2 Specific Plaque Hypothesis

The specific plaque hypothesis states that specific bacterial species within plaque are pathogenic, and their specific presence and increase are associated with disease. In the past 2-3 decades, over 200 studies have compared the flora of diseased-associated plaques with the flora of healthy ones. A summary of these studies identified a limited number of mainly Gram-negative anaerobes, to be significantly associated with periodontal disease (Loesche and Grossman 2001). Specifically, plaque microorganisms from chronic periodontitis sites were often anaerobic (90%) and Gram negative (75%) (Polson et al. 1997). As well, disease sites consistently harbored elevated proportions of Spirochetes (Carranza 2006, Socransky and Haffajee 1994). The bacteria most often successfully cultured were *P. gingivalis, T. forsythia, P. intermedia, C. rectus, E. corrodens, F. nucleatum, A. actinomycetemcomitans, P. micros,* and *Treponema* and *eubacterium* species (Carranza 2006, Socransky and Haffajee 1994, Slots and Jorgens 2002, Loesche and Grossman 2001). The presence of these disease-associated bacteria and the expression of a myriad of virulence factors that they collectively express directly and indirectly mediates tissue destruction (Loesche and Grossman 2001, Carranza 2006, AAP 2005, Socransky and Haffajee 1994, Wilson 2003).
1.3.3 Evidence of a Host (Immune) Response

Bacteria within dental plaque biofilm have evolved strategies that favor their growth and survival within the oral cavity. The recalcitrant nature of dental plaque results in the constant release of virulence factors and antigens that the host recognizes as foreign and responds appropriately (Darveau et al. 1997). Collectively microorganisms associated with periodontal diseases secrete a wide variety of proteolytic and hydrolytic enzymes along with LPS and other outer membrane proteins (Holt and Bramanti 1991). Inflammation is induced when these virulence factors exceed a threshold. The initial host response to bacterial infections includes neutrophil, macrophage and lymphocyte recruitment. In chronic lesions activated antibody producing B cells are prominent as well (Loesche and Grossman 2001, AAP 2002, Kornman et al. 1997). Expressed bacterial virulence factors induce secretion of pro-inflammatory cytokines by resident epithelial, fibroblast and endothelial cells and recruited inflammatory cells (Kornman et al, 1997). Collectively the classic pro-inflammatory cytokines IL-6, TNF-α and IL-1 are expressed in conjunction with others like transforming growth factor β (TGF- β) and IL-3, -6, -7, -8, -10, -11, and -12 (AAP 2002, Kornman et al. 1997).

Periodontal tissue degradation is mediated directly and indirectly by bacterial expressed virulence factors (Wilson 2003). For example, *P. gingivalis* secrete a number of potent proteases like collagenase and gelatinase and these are directly involved in connective tissue degradation (Wilson 2003, Carranza 2006, Loesche and Grossman 2001, Holt and Bramanti 1991). Indirect tissue destruction is also mediated by bacterial virulence factors
like lipopolysaccharide (LPS) (Wilson 2003, Loesche and Grossman 2001). Lipopolysaccharide induces inflammation by stimulating the expression of pro-inflammatory cytokines like interleukin-1, IL-6 and tumor necrosis factor-α (TNF-α) by resident and recruited cells (i.e. fibroblast, epithelial, osteoclast, osteoblast) and these cytokines induce host expression of connective tissue degrading matrix metalloproteinases (proteases) and prostanoids (prostaglandin E₂, [EPG₂] (AAP 2002, Loesche and Grossman 2001).

### 1.3.4 Scaling and Root Planning: Still the Gold Standard?

Since the 1950’s, scaling and root planning or nonsurgical mechanical periodontal debridement (NSPD) has been the cornerstone of periodontal therapy and still remains the first approach to control periodontal infections (Ishikawa and Baehni 2004). In the 1960’s, the therapeutic endpoint focused on the sole removal of calculus and disruption of plaque (Carranza 2006). Today, NSPD therapy still involves the removal or disruption of bacterial plaque biofilm, its byproducts, and plaque-retentive calculus deposits from crown and root surfaces, and within the sulcus or pocket (O’Hehir 1999). However, the NSPD endpoint focuses now on the level of instrumentation required to reduce inflammation and return adjacent soft tissues to a biologically healthy state (Socransky and Haffajee, 1994, 2006, O’Hehir 1999, Caranza 2006).

The success of NSPD on reducing the clinical signs and symptoms of periodontal disease is unequivocal and well supported by systematic reviews. For example Hanes and Purvis 2003 determined that NSPD improved pocket depth by 1.45 mm and improved CAL by
0.89mm. Associated with this was also a significant reduction in bleeding on probing, gingival inflammation and plaque scores as well (Hanes and Purvis 2003). In addition NSPD when compared to surgery to manage 4–6mm pockets resulted in 0.4mm more attachment gain (weighted mean difference (WMD) 0.37 mm; 95% CI 0.49, 0.26) and 0.4mm less probing depth (WMD 0.35 mm; 95% CI 0.23, 0.47) at 12 months (Heitz-Mayfields et al. 2005). On average all systematic reviews reporting on the efficacy, effectiveness and efficiency of NSPD concluded patients with chronic periodontitis receiving subgingival debridement in conjunction with supragingival plaque control effectively reduced pocket depths and improved clinical attachment levels (Van der Weijden and Timmerman 2002, Heitz-Mayfields et al. 2005, Hanes and Purvis 2003, Bonito et al. 2005). However, presenting mean values of clinical indices at times is misleading and does not reflect variability that is found in individual sites.

1.3.4.1 Limitations of NSPD

Nonsurgical periodontal debridement aims at disrupting the associated bacterial biofilm, to suppress putative periodontal pathogens, and to arrest or slow the progression of the disease (Ehmke et al. 1990). Most patients can be managed with NSPD (Suvan 2005, Ishikawa and Baehni 2004) but unfortunately, 20% of the patients (Ishihara et al. 1992) do not respond favorably to conventional mechanical debridement alone (Ishikawa and Baehni 2004, Ehmke et al. 1990). A possible reason may be that NSPD does disrupt subgingival biofilm but remaining disease associated microorganisms may serve as reservoirs for reinfection and recolonization of the subgingival pocket (Ehmke et al. 1990, Bonito et al. 2005). The effectiveness of NSPD is also limited by deep pockets
(greater than 6mm), furcations, root concavities and microorganisms existing in dentinal tubules and within the soft and connective tissues. Collectively all of these factors make removal of all bacteria and their associated virulence factors by NSPD virtually impossible (Loesche and Grossman 2001, Slots and Jorgens 2002, AAP report 2001). This in conjunction with the fact that a modified host response due to non-microbial risk factors like smoking, stress, diabetes or genetic predisposing factors (e.g. Papillon-Lefevre syndrome) might explain in part why some treatment in select patients or sites do not respond favorably to conventional NSPD (AAP 2002).

Sites in which conventional therapy has failed may benefit from the use of novel adjunctive therapies to improve or maintain existing periodontal health (Slots and Jorgens 2002, Loesche and Grossman 2001, Worthington and Needleman 2005, AAP 2002). Key questions facing clinicians are whether NSPD accompanied by use of adjunctive treatment such as antimicrobial agents, improves patient outcomes over time more than mechanical debridement alone (Bonito 2005) and if so then what is the optimal agent(s), patients and/or treatment regime (Haffajee et al. 2006).

1.3.5 Chemotherapeutics Agents

A chemotherapeutic agent is a general term used to describe a chemical substance that provides a clinical therapeutic benefit (Slots and Jorgens 2002, Carranza 2006). This benefit may be derived through either antimicrobial or antiseptic actions or through an increase in the host’s resistance. Within the context of periodontal disease use of these adjuncts has been suggested in sites that continue to progress even with diligent home
care (refractory sites), possibly in patients with aggressive periodontitis or in patients with medical conditions that may predispose patients to periodontal disease (AAP-Position Paper 2004). Use of antibiotics or antiseptics may be broadly grouped into 2 categories. First, and most commonly, agents that are used in conjunction with periodontal debridement (adjunctive) and second, agents that are used in replacement of periodontal debridement (equivalence) (Hanes and Purvis 2003). However, due to limited diffusion of the agent into the biofilm, microorganism within biofilms are remarkably resistant to antibiotics and antiseptics. Therefore, mechanical disruption of plaque at the time of delivery of adjunctive therapies is highly recommended (Slots and Jorgensen, 2002). This section will primarily focus on the use of antibiotics (systemic and local delivery) and antiseptics adjunctively with periodontal debridement.

1.3.5.1 Chemotherapeutic: Adjunctive Systemic Antibiotics

An antibiotic is a naturally occurring or synthetic organic anti-infective agent that at low concentrations kills or inhibits the growth of selective microorganisms (Carranza 2006). The critical role that bacteria play in periodontal disease pathogenesis does open up the possibility that systemic antimicrobials may be therapeutically beneficial. Systemic delivery of antibiotics is advantageous because they deliver the antimicrobial at therapeutic concentrations in a simple and easy manner to all sites. In addition they effectively reduce the total bacterial load in the oral cavity. Conversely the disadvantages are the inability at times to achieve a sufficiently high concentration within gingival crevicular fluid, there is an increased risk of adverse reactions, increased selection of antibiotic resistant organisms and questionable patient compliance (AAP 2004, Jokovsky
et al. 2006). Regardless antibiotics prescribed should be i) specific for periodontal pathogens; ii) nonallergenic; iii) nontoxic; iv) substantive; v) activity should not be reduced by serum or exudate; vi) bactericidal rather than bacteriostatic; vii) compliance (simple); viii) and inexpensive (Carranza 2006).

Numerous systemically administered antibiotics for the management of periodontal disease have been tested either individually or in combinations (following table from AAP Position Paper 2004).

<table>
<thead>
<tr>
<th>Antibiotic</th>
<th>Adult Dose</th>
</tr>
</thead>
<tbody>
<tr>
<td>Metronidazole</td>
<td>500 mg/tid/8 days</td>
</tr>
<tr>
<td>Clindamycin</td>
<td>300 mg/tid/8 days</td>
</tr>
<tr>
<td>Doxycycline or minocycline</td>
<td>100-200mg/qd/21 days</td>
</tr>
<tr>
<td>Ciprofloxacin</td>
<td>500 mg/bid/8 days</td>
</tr>
<tr>
<td>Azithromycin</td>
<td>500 mg/qd/4-7 days</td>
</tr>
<tr>
<td>Metronidazole + amoxicillin</td>
<td>250mg/tid/8 days for each</td>
</tr>
<tr>
<td>Metronidazole + ciprofloxacin</td>
<td>500 mmg/bid/8 days for each</td>
</tr>
</tbody>
</table>


In general, the use of any antibiotic for the management of periodontal diseases should be based on selection criteria. Ideally, the causative microorganism(s) should be identified using either culture methods or molecular genetic techniques like DNA probes or PCR assays. In addition antibiotic-sensitivity testing should dictate which antibiotic(s) is prescribed (AAP 2004). The difficulty lies with the fact that not one microorganism has been identified as the etiologic microorganism(s) causing diseased (Socransky and Haffajee 1994, 2005, Loesche and Grossman 2001). This uncertainty makes selection of appropriate chemotherapeutic agents challenging. Unfortunately, as of today no one
antibiotic is clearly superior and antibiotic combinations are used at times to eliminate putative pathogens from recalcitrant periodontal pockets (Haffajee, 2006). One treatment approach summary is presented by Haffajee 2006:

"There are no ‘‘evidence-based’’ guidelines for the use of systemically administered antibiotics. For this reason, guidelines for antibiotic use will always remain that; guidelines. They provide starting points to make complex decisions. We (Haffajee et al.,2003) feel that antibiotics are useful in the treatment of aggressive forms of periodontal diseases, ‘‘refractory’’ periodontitis and in smokers. However, in the most common form of the disease, chronic periodontitis, which patients would benefit from systemically administered antibiotics and how would the decision to use antibiotics be made?"

Regardless of these treatment decision difficulties the use of systemically administered antibiotics has been extensively tested for their therapeutic efficacy. This review has focused on findings based on systematic results. Systematic reviews by Haffajee et al. 2003, 2007, Winkelhoff 2003, Herrera et al. 2002 and others have concluded the use of systemically administered adjunctive antibiotics with or without NSPD and/or surgery appeared to provide greater clinical improvement. All reviewers did note several different study characteristics that made direct comparisons difficult. When evaluating attachment levels of monotherapy (tetracycline, metranidozole, and amoxicillin) or combination of antibiotics, Haffajee et al. 2003, found a range of improvement from agent to agent but an average a 0.45mm attachment gain was noted. Improvements were consistent for both chronic and aggressive periodontitis, but aggressive subjects benefited more with duration of 6 months (Haffajee et al. 2003). Furthermore, the review suggests a gain in attachment is obtained in all pocket depths but optimal gains were noted in pocket depths ≥6mm.
While these reviews indicate, on average, antibiotics do contribute to a statistically significant improvement in periodontal clinical indices it is still unclear on the magnitude of the added benefit, the optimal dosage, the optimal agent(s) and the identification of the patients that would most benefit (Bonito et al. 2005, Haffejee et al. 2003).

1.3.5.2 Chemotherapeutics: Adjunctive Local Delivery Agents

Since periodontal disease sites are typically site specific the use of systemic antibiotics may be perceived as over treatment. Ideally targeting anti-infective agents at sufficiently high concentrations to specific disease sites is preferred. This problem or opportunity has prompted development of novel locally applied antiseptic and antibiotic delivery approaches (Hanes and Purvis 2003, Carranza 2006).

1.3.5.2.1 Localized Sustained-Released Antiseptic and Antibiotic Agents:

1.3.5.2.2 Chlorexidine Chips (Antiseptic Agent)

An antiseptic is typically applied topically or subgingivally to mucous membranes, wounds, or intact dermal surfaces to destroy microorganisms and inhibit their reproduction or metabolism. Since antiseptics, unlike antibiotics, are potentially toxic to both infectious agents and host cells, their application in humans is more limited to infected wounds, skin and mucosa (Slots and Jorgens 2002, Carranza 2006).

The PerioChip is composed of a biodegradable hydrolyzed gelatin matrix, cross-linked with glutaraldehyde, containing glycerin and water, into which 2.5 mg of chlorhexidine gluconate has been incorporated (Caranza 2006). After placement the chip begins to
dissolve and maintains a local chlorhexidine concentration in the gingival crevicular fluid (GCF) that is greater than 100ug/ml for at least 7 days (Carranza 2006). As the chip is biodegradable, a second appointment to remove the chip is not required. The meta-analysis by Hanes and Purvis 2003 indicated statistically significant adjunctive pocket depth (PD) reduction and clinical attachment level (CAL) gain compared to NSPD alone. The weighted mean difference for PD was 0.35mm and 0.16 for CAL with a percentage reduction for plaque scores (1.01% \( P< 0.05 \)), gingival inflammation scores (9.21%, \( P< 0.05 \)), and BOP (3.08%, \( P<0.05 \)).

1.3.5.2.3 Tetracycline Fibers (Antibiotic Agent)

The first product approved in the United States that was developed with the concept of local antibiotic delivery was the Tetracycline impregnated fibers. Each fiber is principally composed of an ethylene/vinyl acetate copolymer fiber that contains 12.7mg of Tetracycline/9 inches of fiber. This non-resorbable fiber is packed into the periodontal pocket for 10 days and delivers into the pocket sustained tetracycline release in concentrations exceeding 1300ug/ml. Although this method obtained statistical significance with most clinical parameters/indices, the common use of this approach was limited by the fiber placement which was associated with a significant learning curve and time consuming. Second, a subsequent appointment was required to remove the fiber. Last, oral candidiasis, occurred in some patients treated for 12 or more teeth (Carranza 2006). These side effects led to the development of novel tetracycline delivery approaches using gels, acrylic strips, collagen films or pastes. The effectiveness of these approaches was assessed in a systematic review (Bonito et al. 2005). Of the 16 studies
examining locally applied tetracycline preparations, four demonstrated statistically significant PD reductions: 0.93 mm at 12 weeks (combined with citric acid gel, \(P < 0.05\)), 0.73 mm at 24 weeks (\(P < 0.01\)), 0.67 mm at 26 weeks (\(P = 0.008\)), and 0.41 mm at 7 weeks (\(P = 0.047\)). The overall estimated PD reduction, 0.47 mm, was statistically significant favoring the adjunct treatment. Two studies showed a statistically significant gain in CAL associated with local tetracycline treatment compared to NSPD alone: 0.48 mm at 26 weeks (\(P < 0.05\)) and 0.15 mm at 26 weeks (\(P < 0.05\)). The overall effect size was a statistically significant 0.24 mm CAL gain (Bonito et al. 2005).

### 1.3.5.2.4 Doxycycline Gel (Antibiotic Agent)

A gel with 10% doxycycline that is delivered to a periodontal pocket using a syringe is approved by the FDA and is the only local delivery caring the approval of The American Dental Association. Two multi-centered clinical trials were reviewed and both found that after 9 months there was a statistically significant improvement in CAL, PD and BOP when compared to NSPD alone (carranza 2006). A reduction in CAL showed a gain of 0.4mm; reduction in PD was 0.6mm; and the reduction of BOP was 0.2 units greater than the control (Larsen 1991, Carranza 2006). In contrast, use of 10% doxycycline gel as a monotherapy without NSPD was associated with equivalent improvements in clinical attachment level and probing depth reduction (Garrett et al. 1997, Carranza 2006)

### 1.3.5.2.5 Minocycline Microspheres (Antibiotic Agent)

The final local delivery approach to be discussed is another FDA approved sustained-release approach using 2% minocycline encapsulated into biodegradable microspheres
with a gel carrier (Arestin®). Eight studies of locally applied minocycline examined in a systematic review were supportive of its use as an adjunct to NSPD. In four trials, experimental subjects experienced 1.0 mm more PD reduction at 12 weeks (for those with ≥7 mm initial PD, $P = 0.0001$), 0.77 mm and 1.10 mm more reduction at 65 weeks (respectively, for subjects with ≥5 mm and ≥7 mm initial PD, $P < 0.0001$ in both cases), 0.7 mm at 26 weeks ($P \leq 0.05$), 0.32 mm at 39 weeks ($P < 0.001$), and 0.3 mm at 12 weeks (for those with initial PD ≥5 mm, $P = 0.0018$). The mean effect size was a statistically significant 0.49 mm reduction in PD (Bonito et al, 2005). This approach used in conjunction with NSPD was associated with the most significant improvement (Bonito et al. 2005).

1.3.6 Summary

Chronic periodontitis is a chronic inflammatory disease that is challenging to treat due to the presence of an adherent biofilm community attached to a non-shedding tooth surface. Significant understanding on the microorganisms that reside in this biofilm and their association with disease has been made but no one specific microorganism can be said to cause disease. This has resulted in a treatment approach that follows the principle of a nonspecific plaque hypothesis and tries to remove as much of the plaque biofilm as possible using NSPD. This removes significant plaque and calculus but cannot remove all microorganisms due to the limitations of instrumentations, local tooth anatomy and the possibility of invasion of microorganisms into hard and soft tissues. In some select cases this is associated with residual inflammation and at times disease progression. To address these treatment challenges systemic or local adjunctive approaches have been
used or developed. Systematic reviews of localized sustained-released approaches that are used in conjunction with NSPD had overall modest further reductions in pocket depths of 0.25mm to 1.45mm in patients with initial pockets of ≥5mm. Statistically significant but minimal improvements in clinical attachment levels of 0.3mm to 0.50mm were also found (Hanes and Purvis 2003, Bonito et al. 2005, AAP 2006). Bleeding on probing, gingival inflammation and plaque scores were reduced by 48.37%, 40.14%, and 40.61%, respectively (Hanes and Purvis 2003). However, whether these statistically significant improvements are of clinical significance still requires further investigation. In addition, the limited benefit of currently available approaches does support the development of novel methods to manage this microorganism associated chronic inflammatory disease.
1.4 Photodynamics

1.4.1 Introduction

In the early 1900’s exposure of acridine dye to visible light was shown to be lethal to protozoa (*Paramecium caudatum*) (Meisel et al. 2005). In 1904 it was termed a photodynamic reaction due to the phenomenon’s ability to excite oxygen molecules, like singlet oxygen (McCaughan 1999). Singlet oxygen ($^1\text{O}_2$) is not a free radical but rather a highly reactive oxygen species that can be involved in generation of radicals (Pryor et al. 2006). More recently photodynamic therapies have been developed for the treatment of various malignancies. These include bladder, brain, breast, skin, gynecological, colorectal, thoracic, oral, and head and neck cancers (Macdonald and Dougherty 2001, McCaughan 1999, Maisch 2006).

The therapeutic use of photodynamics continues to expand for the management of various non-oncological diseases like macular degeneration, prevention of arterial restenosis, treatment of autoimmune disorders and epidermal/dermal pathologies (Jori et al. 2006). The terminology used for treatment changes from photodynamic therapy (PDT) associated with treating oncological diseases to photodynamic antimicrobial chemotherapy (PACT) or antimicrobial photodynamic therapy (APT) in treating localized bacteria, fungal, viral and yeast infections (Meisel and Kocher 2005, Stojiljkovic et al. 2001, Zeina et al. 2001). Most relevant for this dissertation is the potential use of photodynamics as an antimicrobial for the treatment of localized bacterial infections. This section will review the principles of photodynamics and discuss its potential use as an adjunct for the management of periodontal diseases.
1.4.2 General Overview of Photodynamics

Photodynamic therapies require two components. This includes a visible light source usually of a specific wavelength and a dye or photosensitizer (PS) that binds to the target cell and is activated by a light source (Hamblin et al. 2002, McCaughan 1999, Jori et al. 2006). The resulting reaction induces cell death due to the production of toxic oxygen species, like singlet oxygen and/or free radicals (Dahl et al. 1989). Light, photosensitizers and the resulting reaction (photochemistry) will be discussed independently.

1.4.3 Light Source for Photodynamics

1.4.3.1 Overview of Light

Visible light, listening to the radio or x-raying teeth all utilize different electromagnetic waves (electric and magnetic fields). These electromagnetic waves are also called "electromagnetic radiation" and the full range of wavelengths (and photon energies) is called the electromagnetic spectrum (McCaughan 1999). (Figure 2)

![Electromagnetic Spectrum](image)

**Figure 2** The Electromagnetic Spectrum. The electromagnetic spectrum covers a wide range of wavelengths and photon energies. A wavelength is inversely proportional to its energy or the shorter the wavelengths the higher the frequency and energy. (reprinted with permission www.mail.jsd.k12.ca.us/bf/bflibrary/projects.html)
Visible light or radiant energy encompasses a spectrum of wavelengths (390-760nm) with each color of the visible light spectrum being associated with specific wavelengths (nm), frequency (Hz) and photon energy (eV). (Figure 3) The light energy is measured in J/m² and is the product of time (seconds) and intensity, W/m² (Meisel and Kocher 2005).

**Figure 3** The Visible Light Spectrum. The visible light spectrum is part of the electromagnetic spectrum. Wavelengths are measured in nanometers (nm) and the longer the wavelength (nm), the shorter the frequency (Hz) and energy (eV) (reprinted with permission from Encyclopedia Britannica).

### 1.4.3.2 Light Sources

Historically, natural light was used to activate a photodynamic agent. With time conventional lamps were tested but had disadvantages because there were unwanted thermal effects, light intensity was too low to effectively activate the photosensitizer and there was difficulty controlling the light dose. Today, most commonly used photodynamic light source is a Light Amplification by the Stimulated Emission of Radiation or laser (Pervaiz and Olivo 2006). Unlike a standard light bulb or sunlight, the light emitted from a laser is monochromatic, that is, it is of one wavelength (color). The light waves that make up the laser energy beam (photons) are coherent (all the waves are aligned) and have little divergence (collimated) (Cobb 2006).
Lasers are named according to the active element(s) that are used to induce photons. They can be delivered as either a continuous wavelength (e.g. argon or diode lasers), or pulsed wavelengths (e.g. gold vapor laser, ruby laser, YAG or KTP lasers) (Cobb 2006). These laser light sources can be passed down an optical fiber and delivered directly to the target site using application specific illuminator tips (Pervaiz and Olivo 2005, Cobb 2006). (Figure 4)

**Figure 4** Illuminator Tip on a Cold Diode Laser. The laser light source can be passed down an optical fiber and delivered directly to the target site using application specific illuminator tip. The laser in the figure is a diode laser with an illuminator tip emitting a red beam of light.

### 1.4.3.3 Light Striking an Object

Light travels in a straight line until it strikes an object. The light may be reflected, refracted, transmitted, absorbed, or scattered (McCaughan 1999, Cobb 2006). These different possibilities are a result of the photons slowing down when they pass through different media. In the case of biological tissues, the optical properties of the tissues such as pigmentation, water content, mineral content and heat capacity can dictate to a certain degree the interaction with specific laser wavelengths. For example, CO\(_2\) laser (10,600nm) have a high absorption in water where as diode lasers (670-900nm) have lower absorption coefficient in water but are preferentially absorbed in pigmented tissues (Cobb 2006).
1.4.3.4 Light Penetration Through Tissue

When photons enter tissue a portion of the light energy will be reflected by the surface while the rest scatter in the tissue until they escape or are absorbed by endogenous or exogenous molecules (MacDonald and Dougherty 2001, McCaughan,1999). Light that does penetrate homogenous tissue is absorbed proportionally to tissue thickness. However, wavelength of light plays a critical role in the effectiveness by which light penetrates tissue. For example, the depth of light penetration is doubled from 4 to 8 mm when the wavelength increases from 500-600nm to 800 nm, respectively (Pervaiz and Olivo 2006). However, light at a wavelength near 800 nm transmits more deeply into tissue, but at this wavelength water molecules absorb the light energy and unwanted heat is generated (MacDonald and Dougherty 2001, Meisel and Kocher 2005, Cobb 2006, Wilson et al. 1984). The rate of attenuation (loss) of a light beam (photons) as it passes through tissue is called the ‘coefficient of extinction’ and is tissue specific and wavelength dependent (McCaughan 1999, Pervaiz and Olivo 2006). Light penetration depth of nearly 2.5 cm and having only 3-10% of the original light intensity can still produce biological effects such as free radicals which can initiate a cascade of events (Pervaiz and Olivo 2006, Dougherty and Marcus 1992).

In summary, there is a significant inter-relationship between the absorption of light as it passes through tissue and the depth of light penetration into tissues. The effectiveness of a light source for photodynamic treatment depends on spectral irradiance, tissue transmission and photosensitizer absorption (McCaughan 1999, Nilsson et al. 1995, MacDonald and Dougherty 2001, Moseley 1996). For example, when the photosensitizer
Photofrin is used, the laser is tuned to a wavelength of 630 nm. This wavelength is a compromise between porphyrin absorption and a requirement for transmission through tissue (Moseley 1996, Anderson and Parrish 1981). Conversely, a photosensitizer selected for treatment of bladder malignancies has an excitation wavelength of 510 nm. This was selected in order to reduce penetration depth and minimize damage to the underlying musculature (Moseley 1996, Stamp et al. 1990).

1.4.4 Photosensitizers (PS)

A photosensitive molecule is one which on activation by radiation or light causes another molecular component to react (McCaughan 1999, Meisel and Kocher 2005). In general there are three basic groups of PS, tricyclic dyes, tetrapyrroles and furocoumarins (Meisel and Kocher 2005). (Figure 5)

**Figure 5** Photosensitizer Compounds. More than 400 compounds are known to exhibit photosensitizing properties including dyes, drugs, cosmetics, chemical and many natural substances generally belong to these basic classic structures, Tricyclic dyes, Tetrapyrroles and Furocoumarins (Meisel and Kocher 2005, Santamaria and Prino 1972).
Upon absorption of light energy at the appropriate wavelength, a PS undergoes a transition from a low energy singlet ground state to a higher energy triplet state. (Figure 6) The process by which this high energy triplet state is generated is critical to the photodynamic reaction and involves the physics of electron spin configuration (Oleinick et al. 2001, Greer 2006, Pervaiz and Olivo 2006, Dougherty 1991, Laustriat 1986). Electron spin the nucleus but they also have an intrinsic magnetic field which induces a spinning effect on its axis (McCaughan 1999) (Figure 7). When all electrons spinning in one direction are equal to electrons spinning in the opposite direction i.e paired, the compound is referred to as a singlet state (Pryor et al. 2006, McCaughan 1999).

**Figure 6** Schematic Diagram of an Excited Electron. Excitation of an electron by energy, causing the electron to “jump” to another electron (energy) level (orbit) known as the excited state. [Reprinted with permission from Life: The Science of Biology, 4th Edition, by Sinauer Associates (www.sinauer.com)]

**Figure 7, A and B** Schematic View of Electron Spin. Electrons spin on an axis and orbit the nucleus. Here the schematic view of electrons spinning in a) an electron spinning counterclockwise or to the left and b) clockwise or right direction. Paired electrons can only occupy an orbital when the spins of the electrons are paired i.e. one spinning in each opposite direction. A singlet state is said to be paired as it has the same amount of electrons spinning in either direction (left or right).
Most molecules in their ground state do exist in this lower energy singlet state. When a quantum (photon) of energy from the light source is absorbed by the PS it may induce the spin of one of the electrons to reverse. These unpaired electrons can induce high energy and result in a highly reactive molecule that is now in a triplet state (excited state) (McCaughan 1999). Generation of a triplet state PS plays a critical first step in the photodynamic reaction (Laustriat 1986).

The ideal PS exhibits specific characteristics. They should; (i) be preferentially retained by the target cells/pathogens and not by surrounding healthy cells/tissues; (ii) they should absorb at the desired wavelength of light with a high extinction coefficient; (iii) they should be cytotoxic only upon photoactivation; (iv) they should have a high quantum yield of singlet oxygen or superoxide from the photochemical reaction; (v) they should be rapidly removed from the body with low systemic toxicity; and (vi) not generate tissue damage to neighboring healthy tissues/cells (Pervaiz and Olivo 2006, Jori et al. 2006).

1.4.5 Photochemical Reactions - Types I, II and III

Photochemistry is concerned with how light interacts with matter and initiates chemical reactions, or conversely, how chemical reactions cause light to be emitted. All photochemical reactions first start with the generation of high energy triplet state PS. However, postexcitation chemistry can potentially go one of three pathways and are classified as Type I, II and III reactions (Laustriat 1986, Girotti and Kriska 2004, McCaughan 1999). These types are broadly divided into photooxidation by radicals (Type I), photooxidation by singlet oxygen (Type II) and photoreactions not involving
oxygen (Type III) (Laustriat, 1986). The flux along these pathways is very much dependent on the concentrations of the substrate and/or molecular oxygen. For example, if the concentration of oxygen is high but the substrate concentration is low, the Type II reaction is favored (Laustriat 1986) (Figure 8).

Figure 8 A Schematic View of Photochemistry Reactions. The schematic view depicts a photosensitized reaction where a* is the excited PS. The (b) is the substrate. The notation hv is the incident photon energy required to excite the PS (modified from Laustriat 1986).

A Type I reaction is where photoxidation of a substrate generates radicals and then reactive oxygen species (ROS) like hydrogen peroxide and superoxide anions. Superoxide can subsequently react to form highly reactive hydroxyl radicals (OH') (Laustriat 1986, Foote 1984, Greer 2006, McDonald and Dougherty 2001). Alternatively, Type II reaction involves the transfer of energy of the triplet state PS to ground triplet state oxygen (\( ^3O_2 \)) generating singlet oxygen (\( ^1O_2 \)) (Foote 1984, Laustriat 1986).

The electronic ground state oxygen or triplet state (\( ^3O_2 \)) is very unique in that its biradical, or in other words it has two unpaired electrons with the same (parallel) spin. The triplet state is lower in energy than the singlet state because electrons with the same spin cannot occupy the same region of space. The singlet state of oxygen has electrons with opposite spin in the same region of space and with the electrons closer together,
repulsion between these electrons is greater and thus the singlet state of oxygen is higher in energy (Pryor et al. 2006). Thus, singlet oxygen ($^1\text{O}_2$) is produced by inverting the spin of one of the outermost electrons of the triplet state oxygen by the transfer of energy created by the excited triplet state photosensitizers (PS). (Figure 9)

![Diagram](image)

**Figure 9** A Schematic Representation of Singlet Oxygen eneration by a PS.

Singlet oxygen is highly reactive and can react with organic substrates like lipids, proteins and nucleic acids (Oleinick at al. 2001, McDonald and Dougherty 2001). Its half-life ranges from 10–100 ms in organic solvents. This short half life means that subsequent reactions occur within 10 nm from its point of generation (McDonalds and Dougherty 2001). However, mean diffusion distances of 100-200 nm have been described as well (Moan and Berg, 1991). In an aqueous environment singlet oxygen’s lifetime is said to be significantly reduced to approximately 2 ms and therefore shorter diffusion length of 0.02µm are likely to exist (Dougherty 1991, Laustriat 1986, Chondros et al. 2007). These data suggest that PS must be in close contact to target
microorganisms to ensure that generation of short lived highly reactive singlet oxygen molecules are positioned close to the target cell in order to exert its lethal effects on cell structures. (discussed below)

Tumor eradication and killing of bacteria has been successful due to the highly toxic and reactive singlet oxygen (Oleinick et al. 2001, Dahl et al. 1989). This reaction is of biological importance because they affect cell membranes by reaction with phospholipids and addition onto unsaturated acyl chains. In addition protein and enzymes are affected due to oxidation of tryptophan, histidine, cysteine and methionine amino acids and nucleic acids are affected due to photooxidation of guanosine (Laustriat 1986).

Last a Type III reaction is a unique PS reaction because it is oxygen independent. These reactions require either high concentration of the PS or a deaerated system, in order to bypass the reaction with oxygen. Under anaerobic systems radicals are generated and these can subsequently react (Laustriat 1986).

1.4.6 Antimicrobial Photodynamic Therapy (APT)

There is a growing concern about the overuse of antibiotics and the increase in antibiotic resistant strains of bacteria. This concern has fostered research into novel approaches to manage bacterial infections. APT is a novel method for eradication of bacteria and has been used to control non oral bacterial and fungal infections associated with wounds, psoriasis, acne vulgaris, Heliobacter associated stomach ulcers and fungal infections of
the skin (Maisch 2006). Within the context of microorganism the effectiveness of APT is dependent on the structural organization of the cell wall and membranes.

1.4.6.1 Structural Organization of Gram Positive and Negative Bacteria

The broad division of bacteria into Gram positive and negative microorganisms is based on the structural organization of bacterial cell walls and membranes (Figure 10). Gram-positive bacteria have a relatively thick 15 to 80 nm thick cell wall, which is composed largely of peptidoglycan (about 90%). Attached to this peptidoglycan is Teichoic acids, polysaccharides and peptidoglycolipids. The cell wall is attached to the plasma membrane. Conversely, Gram-negative bacteria have a very thin 5 to 10 nm thick peptidoglycan (20%) cell wall positioned between an outer and inner cell membrane. The outer cell membrane is about 7.5 to 10 nm thick and is anchored non-covalently to lipoproteins, which are covalently linked to the peptidoglycan cell wall. Lippolysaccharide which is anchored in the outer cell membrane through its lipid A moiety.

![Schematic View of Gram Positive and Gram Negative Bacteria](Willey Interscience and Jori et al. 2006)
1.4.6.2 Microbial Effects of APT on Bacterial Membrane

A photosensitizers (PS) should effectively co-localize to its target and to a large degree this is dependent on both the net charges of the PS agent and the targeted pathogen. Therefore the charge of the outer cell membrane/wall of bacterial, fungal, yeast or viruses is critical and will regulate the interaction of the PS with pathogens (Jori et al. 2006). Cationic PS is in general more efficient and effective at lower concentrations than neutral or anionic PS molecules because bacteria have a net negative charge (Dahl et al. 1987, 1989, Demidova and Hamblin 2005, Jori et al. 2006).

In addition a PS should also penetrate to the inner cell membrane of both Gram positive and negative bacteria to effectively kill the pathogens. Gram positive outer cell walls have a relatively open structure to the peptidoglycan cell wall and therefore the PS easily penetrates through the peptidoglycan cell layer and down to the cell membrane. Therefore generation of singlet oxygen due to a photochemical reaction and its subsequent reaction with the inner cell membrane are direct and do not involve secondary reactions (Dahl 1989, Jori et al. 2006, Soukos et al. 1998) (Figure 11). Killing of Gram positive organisms is accomplished more easily or at lower PS concentrations because of this more direct reaction of the PS with the cell membrane. In contrast Gram negative bacteria have an outer cell membrane that preferentially binds the cationic PS. In this situation generated singlet oxygen first reacts with components of the outer membrane but is further from the inner membrane. Secondary reaction products like peroxy radicals are generated and these in turn are toxic as well. For Gram negative microorganisms toxicity is the net effect of primary and secondary reaction products and requires higher
PS concentrations because of their unique cell wall structural organization (Dahl et al. 1987, 1989, Demidova and Hamblin 2005). (Figure 11)

![Diagram of bacterial membrane with singlet oxygen effects](image)

**Figure 11** Schematic View of Singlet Oxygen Effects on Bacterial Membrane. The arrows represent the singlet oxygen penetrating through the cell wall to reach the plasma membrane. The gram positive cell wall is easily penetrated and the singlet oxygen interacts directly with the plasma membrane. Gram negative toxicity from singlet oxygen is the net effect of primary (thin arrows) and secondary reaction (thick arrows) products.

Disruption of the inner cell membrane is a critical step in killing of bacteria by PS. Enzymes such as NADH, lactate or succinic dehydrogenase, are photo-inactivated by PS at are inactivated at a rate that parallels photo-induced cell death. Similarly, several outer membrane and plasma membrane proteins undergo extensive cross-linking by PS. Photosensitizers induce disruption of the membrane also results in loss of barrier function and disrupts ion balance and functioning of anabolic and catabolic pathways (Jori et al. 2006). Loss of barrier is also associated with a massive influx of the PS into the cytoplasm and subsequent photooxidation of intracellular proteins and DNA. However,
whether photooxidative modification of DNA plays a role in cell death is still debated (Jori et al. 2006, Lambrechts et al. 1991).

1.4.6.3 Use of APT in Periodontal Disease

Periodontal diseases can involve severe inflammation of the periodontium and chronic infections caused by a mixture of Gram-positive and Gram-negative bacteria growing as a biofilm (Jori et al. 2006). Biofilms that colonize tooth surfaces and epithelial cells lining the periodontal pocket (subgingival) are among the most complex biofilms that exist in nature (Soukos et al. 1998, Merchant et al. 1996). The presence of primarily Gram-negative obligate anaerobes, capnophiles and spirochetes at threshold levels within the subgingival biofilms, along with a wide range of host-compatible species for prolonged periods of time (Soukos et al. 1998) are strongly associated with tissue degradation (Wilson 2003, Soukos et al. 1998, AAP 2005, Socransky and Haffajee 1994, Jori et al. 2006). These subgingival biofilms exist in complexes (a matrix of polymeric material) that provide protection against antimicrobial agents and host defense mechanisms (Jori et al. 2006, Soukos et al. 1998, Millson et al. 1996).

Several studies have demonstrated that Gram-positive bacteria are susceptible to APT (Meisel and Kocher 2005, Soukos et al. 1998, 2005, Hamblin et al. 2002). On the other hand, Gram-negative bacteria have been resistant to APT action but have shown enhanced susceptibility to APT following the application of a PS with a cationic charge (Jori et al. 2006, Meisel and Kocher 2005, Soukos et al. 1998, 2005, Hamblin et al. 2002) like toluidine blue O and methylene blue (Soukos et al. 2005). Methylene blue has been
studied for decades (Meisel and Kocher 2005) and was proven to penetrate deeper in the plaque biofilm (Wood et al. 1999) and increase the killing rate (Meisel and Kocher 2005). Although most studies demonstrated a log reduction in bacteria, a confocal laser scanning micrograph (CLSM) of a biofilm after exposure to APT revealed that in some of the biofilm stacks, lethal photosensitization occurred predominantly in the outer layers of the stack leaving some of the innermost bacteria alive (O’Neil et al. 2002) which may allow for bacterial recolonization.

In vitro studies have established that several associated periodontopathogens in the subgingival biofilms like *P. gingivalis, Fusobacterium nucleatum, Staphylococcus sp.* are efficiently eradicated by photodynamic treatment, both in aqueous suspension and in biofilm (Jori et al. 2006, Wilson 2003). Moreover, *in vivo* animal studies showed that phenothiaziniums like toluidine blue-PDT can selectively kill *P. gingivalis* in the oral cavity and significantly decrease the level of alveolar bone loss in rats and dogs affected by periodontitis (Milanezi de Almeida et al. 2007, R. de Oliveira et al. 2007, Komerik et al. 2003, Hayek et al. 2005).

However, a number of investigations have demonstrated that bacteriocidal efficiency may be adversely affected by environmental factors such as the presence of saliva, serum and pH (Komerik and Wilson 2002, Komerik et al. 2003, Bhatti et al. 1997). Serum-derived gingival crevicular fluid (GCF) as well as blood may be confounders in treating periodontal pocket with APT. Matevski et al. 2003 identified the similarity between serum and blood kills was due to the presence of serum itself that provided protection to
P. gingivalis from photoactivated TBO. The protection could be due to the presence of light scattering/absorbing proteins (Wilson and Pratten 1995) or perhaps the serum component bind to P. gingivalis which in turn protects it from the activated PS (Matevski et al. 2003). Other explanations for the decreased effectiveness may be due to the proteins competing with the bacteria for PS molecules, thus decreasing the number of PS molecules available for binding (Bhatti et al. 1997). Furthermore, as previously mentioned singlet oxygen has a short half-life (Moan and Berg 1991) and may be quickly quenched by carotenoid pigments (Dahl et al. 1988).

The pH of the medium was also found to effect bacterial killing efficiency. Nitzan et al. 1987 determined PS and bacteria interactions are pH dependent. These changes are attributed to changes in membrane fluidity and the increased binding of the PS to the bacteria. Therefore, cationic sensitizers like methylene blue are more effective at neutral pH. Conversely, a higher pH (alkaline) may induce longer triplet state of the PS which may promote an increase in singlet oxygen half-life. (Nitzan et al. 1987, 1989, Matevski et al. 2003, Tuit and Kelly 1993).

An ideal treatment would target the pathogenic microorganisms and eradicate them while host non-pathogenic oral flora, cells and hard or soft connective tissues would not be damaged by the kinetics and efficiency of the photo-process (Jori et al. 2006). Komerik et al. 1998 and Soukos et al. 1998 demonstrated no evidence of damage to host tissues even when using light doses and PS concentrations at much higher than therapeutic concentrations.
1.4.6.4 Summary

The novel concept of APT to cause photodestruction of oral bacteria has been documented (Soukos et al. 1996, Wilson 2003, Jori et al. 2006) indicating that APT could be a useful alternative in eliminating periodontopathogenic bacteria. Therefore, in vivo randomized clinical trials could foster new concepts and establish clinical protocols for the management of periodontal diseases.
References:


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Chapter 2: Rationale, Hypothesis and Objectives

2.1 Rationale

Chronic periodontitis is challenging to treat due to the presence of an adherent biofilm community attached to a non-shedding tooth surface. Significant understanding on the microorganisms that are present within this biofilm has been made but the general treatment approach is based on the principle of non-surgical periodontal debridement (NSPD) to remove as much of the plaque biofilm as possible. This procedure removes significant plaque and calculus but removal of all microorganisms is not possible due to the limitations of instrumentations, local tooth anatomy and the possibility that microorganisms may invade into hard and soft tissues. In select sites this is associated with residual inflammation and at times disease progression. To target these sites of concern various local adjunctive approaches have been developed and tested. However, systematic reviews of localized sustained-released approaches that are used in conjunction with NSPD had overall modest further reductions in pocket depths of 0.25mm to 1.45mm in patients with initial pockets of ≥5mm. Statistically significant but minimal improvement in clinical attachment levels of 0.3mm to 0.50mm was also demonstrated and reductions of bleeding on probing, gingival inflammation and plaque scores were also achieved. (Hanes and Purvis 2003, Bonito et al. 2005, AAP 2006).

However, a growing concern over antibiotic overuse and the “empirical” based approaches has limited the use of these adjuncts. Thus there is a clear need to develop novel non-antibiotic approaches with the potential to eradicate the complex subgingival biofilm. The novel concept of photodynamic therapies to cause photodestruction of oral
bacterial biofilms has been documented indicating this approach may be a useful alternative for eliminating periodontopathogenic bacteria (Soukos et al. 1996, Wilson 2003, Pfitner et al. 2004, Jori et al. 2006). Therefore, it is important to test whether these products demonstrate effect, efficacy, effectiveness and efficiency using in vivo randomized clinical trials. This may result in novel approaches to treat periodontal diseases.

2.2 Hypothesis

A one time subgingival application of Periowave™ immediately after non surgical periodontal debridement (NSPD) will improve CAL and reduce PD and BOP more than NSPD alone.

2.3 Objectives

1) Primary objective is to conduct a randomized in vivo clinical trial to assess the efficacy of a one time application of a phoactivated dye, methylene blue (Periowave™) in treating periodontal disease.

2) The primary endpoint of the clinical trial is a significant gain in clinical attachment levels (CAL)

3) The secondary endpoints include a reduction in bleeding on probings (BOP) and pocket depths (PD).
References


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Chapter 3: Clinical Trial

3.1 Introduction

Periodontitis is a chronic inflammatory disease that is challenging to treat due to the presence of an adherent biofilm community attached to a non-shedding tooth surface (Wilson 2003). Presence of this plaque and its association with disease progression has resulted in a treatment approach that follows the principle of a nonspecific plaque hypothesis and removes as much of the biofilm as possible using non-surgical periodontal debridement (NSPD). This treatment approach removes significant biofilm and retentative factors, however, limitations of access due to instrument design and local tooth anatomy and the possibility of invasion of microorganisms into hard and soft tissues invariably leaves residual microorganisms which may induce inflammation and progression of disease (Mombelli 2005).

To address these treatment challenges a wide variety of systemic and/or local adjunctive antibiotic approaches have been used or developed (Haffajee 2006). Systematic reviews of localized sustained-released and systemic delivery of antibiotics that are used in conjunction with NSPD have demonstrated an overall further reduction in pocket depths and gains in clinical attachment levels. While these reviews indicate, on average, antibiotics do contribute to a statistically significant improvement in periodontal clinical indices; it is unclear whether the magnitude of the improvement warranted their widespread use. In additional there is still debate as to the optimal dosage, optimal agent(s) optimal treatment time, patient and site selection and the overwhelming concern of

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1 A version of this chapter will be submitted for publication.
antibiotic resistance have limited these treatment approaches (Bonito et al. 2005, Haffejee et al. 2003).

Photodynamics therapy in principal may provide an alternative to antibiotics. The first reported observations of photodynamic effectiveness in medicine referred to the inactivation of microorganisms more than 100 years ago (Meisel et al. 2005) and photodynamic therapy has become an established modality for the treatment of solid tumors and other accessible lesions through out the body, including the brain (Pervaiz and Olivo 2006).

The terminology used for treatment modalities changes from photodynamic therapy (PDT) associated with treating oncological diseases to photodynamic antimicrobial chemotherapy (PACT) or antimicrobial photodynamic therapy (APT) in treating localized bacteria, fungal, viral and yeast infections (Meisel and Kocher 2005, Stojiljkovic et al. 2001). Specifically to the oral environment APT has been shown to kill \textit{in vitro} a wide range of microorganisms found in the subgingival plaque microflora such as \textit{P. gingivalis, Fusobacterium nucleatum}, and \textit{Staphylococcus sp} (Komerik et al. 2000, Jori et al. 2006, Wilson 2003).

All photodynamics reactions require two components. This includes a visible light source usually of a specific wavelength and a dye or photosensitizer that binds to the target cells and is activated by the light source such as a laser (Hamblin et al. 2002, McCaughan 1999, Jori et al. 2006). The resulting reaction (photochemistry) induces cell
death due to the production of toxic oxygen species, like singlet oxygen and/or free radicals (Dahl et al. 1989).

The aim of this study was to test whether a one time application of a photodynamic agent (Periowave™) used adjunctively with NSPD increases clinical attachment levels (CAL) and reduces pocket depths (PD) and bleeding on probing (BOP) more than NSPD alone.

3.2 Material and Methods

3.2.1 Study Enrollment

The experiment protocol was reviewed and approved by the University of British Columbia Ethics Committee in Vancouver, Canada (Appendix A). Patients were treated sequentially following approval. All subjects were recruited from a private periodontal practice in North Vancouver, Canada. Periodontal maintenance ranged from 3 to 4 months with all subjects compliant for a minimal of two years (average years in maintenance therapy). Participation was not limited by race or gender and all subjects signed a consent form (Appendix A).

The inclusion criteria selected subjects with a minimum of 18 teeth and at least 4 measurement sites with pocket depth of greater or equal to 6mm that bleed when probed in at least two different quadrants of the mouth. Criteria for exclusion from the study were 1) the subject is pregnant or nursing or who plans to become pregnant in the next 4 months; 2) a significant liver disease by subject report; 3) an active malignancy of any type by subject report; 4) a chronic disease or diminished mental capacity that could
mitigate the ability to comply with the protocol; 5) any significant disease (either acute or chronic) or who is taking a medication with concomitant oral manifestations that in the opinion of the investigator would interfere with evaluation of safety or efficacy of PerioWave™; 6) an active periapical abscess or periodontal abscess or a history of acute necrotizing ulcerative gingivitis; 7) treated with antibiotics within the 1-month period prior to beginning the study or any systemic condition which requires antibiotic coverage for routine periodontal procedures; 8) a known allergy to Methylene Blue; 9) has glucose-6-phosphate dehydrogenase (G6PD) deficiency by subject report; 10) the subject currently uses anti-coagulant therapy at therapeutic doses; 11) the subject is currently using any photosensitizing medications; 12) the subject has participated in investigational treatment in the last 30 days or has the expectation for using a separate investigational treatment during the time of the study; 13) the site will not be of an implant; and 14) the subject has smoked tobacco products in the last year.

3.2.2 Study Design

This study was a three month randomized, control, single-blinded, parallel and single centre using a one time application of PerioWave™ as an adjunct to nonsurgical debridement (NSPD) in adults with chronic periodontitis. Training of the Florida Probe and photodynamic treatment (PerioWave™) was done prior to the study.

3.2.3 Recruitment of Subjects

The potential subjects were assessed at their previously scheduled (1 year prior) annual evaluation. All clinical parameters (complete odontogram) as well as the presence of
plaque were collected with the automated FloridaProbe® by the blinded examiner (periodontist). Upon completing the assessment, satisfying the criteria and signing the consent form, the subject was enrolled into the study. The subjects participated for up to 4 months with 5 visits in total.

3.2.4 Randomization and Randomization Codes

Upon entering the study the subjects were assigned to binders with an individual identification number. Each binder had a sealed envelop which randomized the treatment allocation. Forty-one subjects were randomized according to a computer generated pseudo-random code using the method of random permuted blocks to either photodysinfection (APT) or only scaling and root planing (SRP). The clinicians were not aware of the randomization sequence.

3.2.5 Blinding

This was a single examiner blinded trial. Blinding was achieved by using a blinded dental examiner (periodontist) qualified to take clinical measurements separate from the treating clinician (dental hygienist). The blinded examiner (periodontist) collected all the clinical measurements at baseline (screening visit) 6 and 12 weeks with the automated FloridaProbe® and was unaware of subject allocation. All NSPD was performed by the same treating clinician (periodontal dental hygienist) and was blinded to randomization until the completion of the subject’s instrumentation and opening of the individual’s sealed envelope.
3.2.6 Study Schedule

The subjects had a total of 5 visits (table). The first visit was the screening/baseline collection in which medical and dental information was collected. A comprehensive exam with the automated FloridaProbe® computer program by the blinded examiner recorded 1) all current and used medication within the past 30 days including any antibiotics in the last 6 months; 2) soft tissue exam, noting any hypertrophic gingival tissue; 3) missing teeth; 4) pocket probing depth (PD); 5) bleeding on probing (BOP) with/without exudate up to 20 seconds; 6) recession- measurement from CEJ to gingival margin; 7) occlusal stress, including fremitus; 8) mobility; 9) furcation involvement; 10) clinical attachment levels (CAL); 11) any known retained tooth roots, cysts or abnormalities; 12) concomitant medications; and 13) presence of plaque (P). Upon completing the exam, sites were identified for photodysinfection treatment (PerioWave™). A site was defined as a natural tooth with a periodontal pocket reading ≥5 mm and bleeding on probing. There were no patient preparations required by the study prior to treatment sessions.

Visit 2 entailed the NSPD of half the dentition with hand (a variety of curets and scalers) and ultrasonic (Denstply®) instrumentation with local anesthesia. All debridement was performed by the same clinician (dental hygienist) with no time restriction. The remaining dentition was completed in the same manner one week later at visit 3.

Visit 3 also included treating the qualifying sites with the photodysinfection treatment (PerioWave™). The subject’s corresponding sealed randomized envelop was opened
upon completion of instrumentation and revealed the two groups; NSPD and photodysinfection group (APT) and the control group (NSPD only). Of the APT group, the qualifying sites were treated with a cold diode laser with a wavelength of 670nm and a maximum power of 150mw/cm$^2$ together with a phenothiazine photosensitizer (methylene blue) in a concentration of 0.01mg/ml. The photosensitizer (PS) was applied via an irrigation cannula (diameter .5mm and side opening) to the base of the pocket and slowly instilled the solution while gently moving the cannula back and forth in a mesiodistal (facial or lingual) direction until the solution could be seen gently flowing over the free gingival margin. In sequence, the pocket was exposed to the laser light using the fiber optic applicator with an illuminator tip for one minute in the same mesiodistal direction while ensuring a slow gentle movement of the tip in both an apico-coronal and lateral direction without allowing the tip to rise above the gingival crest. Only one surface i.e. facial or lingual was irrigated then illuminated at a time.

Visit 4 and 5 were the 6 and 12 week re-evaluation exams respectively. The same complete exam as in visit 1 was performed by the same blinded examiner with the FloridaProbe® as well as any adverse events and concomitant medications were recorded. Subjects maintained their usual oral hygiene procedures throughout the course of the study.
Table 1 Study Design Schedule

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</tr>
<tr>
<td>Sign consent form</td>
<td>X</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Scaling &amp; Root Planing</td>
<td>X</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Randomized PerioWave™ treatment</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td>X</td>
</tr>
<tr>
<td>Record Adverse Events</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td>X</td>
</tr>
</tbody>
</table>

3.2.7 Statistical Analysis

The unit of analysis was a site (i.e. mesialfacial, facial, distalfacial, mesiallingual, lingual and distalllingual) with the primary endpoint of CAL and secondary endpoints, PD and BOP. Mean values and standard deviation (SD) were calculated for all analysis in conjunction with the independent T-test. For all statistical analysis, a significant level of 5% and 95% confidence interval were used. Absolute change evaluated any treatment differences between groups in the presence of any baseline differences. All analysis was in consultation with an independent PhD statistician and SPSS computer program.

3.2.8 Analysis Population

The primary analysis population included all randomized subjects who had a baseline value and at least one post-baseline measurement. The primary analysis of safety (AE’s) included all subjects who received randomized treatment and contributed post-
randomization follow-up data. Safety endpoints were based on treatment actually received.

3.2.9 Sub-Analysis

To allow for more informative trends, sub-analysis were carry out for i) plaque; ii) debdriment with immediate APT (PerioWave™) vs. one week post-debridement with APT (PerioWave™); and iii) light intensity output of the two diode-lasers units used in the study.

3.3 Results

3.3.1 Subject Summary

There were no adverse events reported or complications in either group and postoperative healing was uneventful in all cases. Forty-five subjects were assessed for eligibility. Table 2 details a flow chart of subjects. Forty-one subjects were randomized; 20 in the NSPD + APT group and 21 in the NSPD group. There were in total 6 subjects lost to follow-up. In the NSPD + APT group there were 4 subjects lost; 1 subject needed antibiotic coverage for unrelated dental treatment; 2 subjects’ analysis were not included due to the low light output (less than 80mw) of the treatment unit; and 1 subject had to leave the country (only baseline data collected with no treatment). The NSPD group had one subject lost due to antibiotics for unrelated dental treatment and one subject did not receive treatment (NSPD) due to prior antibiotic coverage needed for unrelated dental treatment (only baseline data collection). Thirty-five subjects completed the study; 19 in
the NSPD (N=224 sites; an average of 12 sites per subject) and 16 in the NSPD + APT group (N=134 sites; an average of 8 sites per subject) with a total of 358 sites analyzed.

Table 2 E-Flow Chart of Subjects

![E-Flow Chart of Subjects]

3.3.2 Qualifying Subjects’ Demographics
Table 3 details the baseline demographics of the 35 subjects who completed the study; 18 females and 17 males, between 24 and 65 years of age (mean age, 53.9±8.8) with a clinical diagnosis of chronic periodontitis were selected. The subjects’ medical history, two years prior to enrolling, presented with controlled states of diabetes and
cardiovascular disease with other physician monitored conditions. Table 3 also includes a summary of the periodontal treatment history two years prior to enrolment. The treatments included either one or combination of antibiotics or surgery. However, based on the exclusion criteria no subject received local or systemic antibiotics one month prior to enrollment in the study. Both groups had similar age and gender representation. The NSPD presented with more health conditions than the NSPD + APT group while the NSPD+APT group had more active periodontal treatment.

Table 3 Baseline Summary of Qualifying Subjects’ Demographics

<table>
<thead>
<tr>
<th></th>
<th>NSPD</th>
<th>NSPD + APT</th>
<th>Total (N)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Age (yr)</td>
<td>52.9±7.2</td>
<td>55.1±10.5</td>
<td>53.9±8.8</td>
</tr>
<tr>
<td>Gender:</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Female</td>
<td>n=10</td>
<td>n=8</td>
<td>18</td>
</tr>
<tr>
<td>Male</td>
<td>n=9</td>
<td>n=8</td>
<td>17</td>
</tr>
<tr>
<td>Health History:</td>
<td>(n)</td>
<td>(n)</td>
<td>(N)</td>
</tr>
<tr>
<td>Controlled Diabetes (Type 1)</td>
<td>1</td>
<td>0</td>
<td>1</td>
</tr>
<tr>
<td>Controlled Diabetes (Type 2)</td>
<td>2</td>
<td>0</td>
<td>2</td>
</tr>
<tr>
<td>Controlled Medicated</td>
<td>4</td>
<td>1</td>
<td>5</td>
</tr>
<tr>
<td>Blood Pressure</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Hormone treatment</td>
<td>2</td>
<td>5</td>
<td>7</td>
</tr>
<tr>
<td>Asthma</td>
<td>1</td>
<td>3</td>
<td>4</td>
</tr>
<tr>
<td>Other: osteoporosis, thyroid,</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>cholesterol, depression</td>
<td>4</td>
<td>5</td>
<td>9</td>
</tr>
<tr>
<td>Periodontal Tx. History:</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Systemic Antibiotics</td>
<td>1</td>
<td>4</td>
<td>5</td>
</tr>
<tr>
<td>(Amox + Metranidozole)</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Localized Antibiotics</td>
<td>1</td>
<td>0</td>
<td>1</td>
</tr>
<tr>
<td>Open Flap Debridement</td>
<td>1</td>
<td>2</td>
<td>3</td>
</tr>
</tbody>
</table>
3.3.3 Periodontal Status of Qualifying Sites (≥4.4mm and BOP)

3.3.3.1 Qualifying Site Distribution: Anterior and Posterior Teeth

The total sites analyzed (358 sites) were distributed within the dentition. Table 4 summarizes the sites distribution with molars having the majority of sites treated for both treatment groups. Overall the percentages of sites per category and between groups were similarly distributed.

Table 4 Summary of Qualifying Site Distribution

<table>
<thead>
<tr>
<th>Teeth</th>
<th>NSPD (N=224 sites)</th>
<th>NSPD + APT (N=134 sites)</th>
<th>Total (N=358 sites)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Anteriors</td>
<td>n=30</td>
<td>n=12</td>
<td>n=42</td>
</tr>
<tr>
<td></td>
<td>13.4%</td>
<td>9%</td>
<td>11.7%</td>
</tr>
<tr>
<td>Premolars</td>
<td>n=44</td>
<td>n=26</td>
<td>n=70</td>
</tr>
<tr>
<td></td>
<td>19.6%</td>
<td>19.4%</td>
<td>19.6%</td>
</tr>
<tr>
<td>Molars</td>
<td>n=150</td>
<td>n=96</td>
<td>n=246</td>
</tr>
<tr>
<td></td>
<td>67%</td>
<td>71.6%</td>
<td>68.7%</td>
</tr>
</tbody>
</table>

3.3.3.2 Qualifying Site Distribution: Plaque (P)

The FloridaProbe® default setting for evaluating the presence of plaque was at three facial sites (mesial, buccal, and distal) and one lingual site (straight lingual) for all teeth. Therefore 187 sites (4 sites versus 6 sites) were available for analysis. Table 5 indicates the plaque distribution among the 4 sites (MB, B, DB and L) in each group. The NSPD group had more plaque positives sites (N=94 sites) than the NSPD+APT (N=59 sites) but both groups total plaque accumulation at the mesial-facial and distal-facial sites accounted for 61.6% and 55.9% respectively.
Table 5 Summary of Qualifying Site Distribution of Baseline Plaque (P)

<table>
<thead>
<tr>
<th>Plaque Sites</th>
<th>NSPD N=94 sites</th>
<th>NSPD + APT N=59 sites</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>MF Facial DF Lingual</td>
<td>MF Facial DF Lingual</td>
</tr>
<tr>
<td>Facial</td>
<td>n=32     34% 8.5% 27.6%</td>
<td>n=8    8.5% 27.1% 6.4%</td>
</tr>
<tr>
<td>Lingual</td>
<td>N=26     N=16</td>
<td>N=5     n=17 n=2</td>
</tr>
<tr>
<td></td>
<td>27.6% 6.4% 27.1%</td>
<td>28.8% 8.5% 3.4%</td>
</tr>
</tbody>
</table>

3.3.3.3 Qualifying Site Distribution: Primary Endpoint, Clinical Attachment Level

The FloridaProbe® measures to 0.2mm but is preset to round 4.4, 4.6 and 4.8mm measurements to the whole number, 5.0mm and thus the sites qualifying at 5.0mm where possibly in a range from 4.4mm to 5.0mm. Therefore the data represented in this result section are representative of pocket depths (PD) and clinical attachment level (CAL) measurements of ≥ 4.4mm. The data was re-analyzed at a threshold of 5.0mm but this resulted in a decrease in sample size from 358 to 274 sites. This will be addressed later.

Based on the inclusion criteria advanced attachment loss for both groups were evident with the NSPD +APT group having more advanced CAL, 6.90±1.88mm. The NSPD group had more qualifying sites in 4-6mm range sites (59.8%) and fewer qualifying sites in the >6mm subgroups (38.4%) were as the NSPD +APT group had more sites in the >6mm (58.2%) and fewer sites in the 4-6mm range (40.3%). Differences in the means was also statistically significant (p<0.000). Table 6 summarizes the qualifying sites for CAL.
Table 6 Analysis of CAL in Qualifying Sites

<table>
<thead>
<tr>
<th>CAL</th>
<th>NSPD</th>
<th>NSPD + APT</th>
<th>P-value baseline</th>
<th>Total Sites</th>
</tr>
</thead>
<tbody>
<tr>
<td>n=224</td>
<td>6.14±1.49</td>
<td>6.90±1.88</td>
<td>.000</td>
<td>N= 358</td>
</tr>
<tr>
<td>Mean and SD</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>CAL range:</td>
<td>4-6mm</td>
<td>&gt; 6mm</td>
<td>4-6mm</td>
<td>&gt; 6mm</td>
</tr>
<tr>
<td>n=134</td>
<td>n=86</td>
<td>n=54</td>
<td>n=78</td>
<td></td>
</tr>
<tr>
<td>59.8%</td>
<td>38.4%</td>
<td>40.3%</td>
<td>58.2%</td>
<td></td>
</tr>
</tbody>
</table>

3.3.3.4 Qualifying Site Distribution: Secondary Endpoint, Pocket Depth

Qualifying sites when examined for PD were similarly distributed to CAL (Table 7). The NSPD+APT had deeper mean measurements than NSPD and similar number of sites in the greater than 6mm with a higher percentage of sites (56.7%) contributing to the experimental group. Differences of the means was also statistically significant (p<0.000).

Table 7 Analysis of PD in Qualifying Sites

<table>
<thead>
<tr>
<th>PD range:</th>
<th>NSPD</th>
<th>NSPD + APT</th>
<th>P-value baseline</th>
<th>Total Sites</th>
</tr>
</thead>
<tbody>
<tr>
<td>n=224</td>
<td>5.66±1.05</td>
<td>6.11±1.22</td>
<td>.000</td>
<td>N=358</td>
</tr>
<tr>
<td>Mean and SD</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>PD range:</td>
<td>4-6mm</td>
<td>&gt; 6mm</td>
<td>4-6mm</td>
<td>&gt; 6mm</td>
</tr>
<tr>
<td>n=162</td>
<td>n=62</td>
<td>n=58</td>
<td>n=76</td>
<td></td>
</tr>
<tr>
<td>72.3%</td>
<td>27.7%</td>
<td>43.3%</td>
<td>56.7%</td>
<td></td>
</tr>
</tbody>
</table>

3.3.4 Statistical Analysis of Qualifying Sites for all Endpoints

3.3.4.1 Primary Endpoint Analysis for Qualifying Sites: CAL

Table 8 summarizes the mean and standard deviation for both treatment groups at baseline, 6 weeks and 12 weeks. Both treatment groups were associated with a reduction in man CAL values at 6 and 12 weeks. These mean differences over time zero were statistically significant (p < .000) within and between each group at 6 and 12 weeks.
Baseline means were also statistically significant ($p<.000$) between groups. To control for differing baseline values on the outcomes, absolute change in CAL was subsequently examined. The absolute changes were not statistically significant at 6 weeks ($p=0.69$) or at 12 weeks ($p=0.97$) (Table 10).

**Table 8 Mean CAL Scores (± SD) at Baseline, 6 and 12 weeks**

<table>
<thead>
<tr>
<th>CAL</th>
<th>Baseline $\bar{x}$ ± SD</th>
<th>6 Weeks $\bar{x}$ ±SD</th>
<th>12 Weeks $\bar{x}$ ±SD</th>
</tr>
</thead>
<tbody>
<tr>
<td>NSPD</td>
<td>224</td>
<td>6.13±1.49</td>
<td>5.35±1.87</td>
</tr>
<tr>
<td>NSPD + APT</td>
<td>134</td>
<td>6.90±1.88</td>
<td>6.07±2.13</td>
</tr>
</tbody>
</table>

**Table 9 Mean CAL Difference ± SD, CI and $P$-values at 6 and 12 weeks**

<table>
<thead>
<tr>
<th>CAL</th>
<th>6 Weeks to Baseline</th>
<th>12 Weeks to Baseline</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>$d$ ±SD</td>
<td>$P$-value</td>
</tr>
<tr>
<td></td>
<td>Lower range</td>
<td>within Groups</td>
</tr>
<tr>
<td>NSPD + APT</td>
<td>.84±1.31</td>
<td>.000</td>
</tr>
<tr>
<td>NSPD</td>
<td>.78±1.11</td>
<td>.000</td>
</tr>
<tr>
<td>$P$-value bt. Groups</td>
<td>.001</td>
<td>-1.14 - -.29</td>
</tr>
</tbody>
</table>

Independent sample T-Test (2-tailed); Confidence Interval (CI) 95%
Table 10 Absolute CAL Change at 6 and 12 Weeks Between Groups

<table>
<thead>
<tr>
<th>CAL Absolut Difference</th>
<th>Mean Change CI Lower to Upper range</th>
<th>P-value</th>
</tr>
</thead>
<tbody>
<tr>
<td>At 6 weeks between Groups</td>
<td>-.05</td>
<td>-.31-.20</td>
</tr>
<tr>
<td>At 12 weeks between Groups</td>
<td>.01</td>
<td>-.27-.28</td>
</tr>
</tbody>
</table>

Paired Sample T-test (2-tailed); CI 95%

3.3.4.2 Secondary Endpoint Analysis for Qualifying Sites: PD

Mean pocket depth and standard deviation at baseline, 6 and 12 weeks are shown in Table 11. Both groups demonstrated a reduction in PD at 6 and 12 weeks over baseline. There was a statistically significant improvement in PD within the groups \((p< 0.000)\) at 6 and 12 weeks but none found between the groups (Table 12). The absolute mean change also confirmed no statistical significance at 6 weeks \((p=0.14)\) or at 12 weeks \((p=0.23)\) (Table 13).

Table 11 Mean PD Scores (± SD) at Baseline, 6 and 12 Weeks

<table>
<thead>
<tr>
<th></th>
<th>Baseline</th>
<th>6 Weeks</th>
<th>12 Weeks</th>
</tr>
</thead>
<tbody>
<tr>
<td>NSPD</td>
<td>224</td>
<td>5.66±1.05</td>
<td>4.87±1.46</td>
</tr>
<tr>
<td>NSPD + APT</td>
<td>134</td>
<td>6.11±1.22</td>
<td>5.13±1.62</td>
</tr>
</tbody>
</table>
Table 12 Mean PD Difference ± SD, CI and P-values at 6 and 12 Weeks

<table>
<thead>
<tr>
<th>PD</th>
<th>N</th>
<th>d±SD</th>
<th>6 Weeks CI lower-upper</th>
<th>P-value within groups</th>
<th>12 Weeks CI lower-upper</th>
<th>P-value within groups</th>
</tr>
</thead>
<tbody>
<tr>
<td>NSPD</td>
<td>224</td>
<td>.80±1.09</td>
<td>.65-.94</td>
<td>.000</td>
<td>.86±1.16</td>
<td>.70-1.01</td>
</tr>
<tr>
<td>NSPD + APT</td>
<td>134</td>
<td>.98±1.26</td>
<td>.77-1.20</td>
<td>.000</td>
<td>1.02±1.36</td>
<td>.79-1.25</td>
</tr>
</tbody>
</table>

P-value between groups

.11 -.59 -.06 .08 -.61 -.039

Independent sample T-Test (2-tailed); Confidence Interval (CI) 95%

Table 13 Absolute PD Change at 6 and 12 Weeks Between Groups

<table>
<thead>
<tr>
<th>PD</th>
<th>Mean Difference</th>
<th>CI Lower to Upper range</th>
<th>P-value</th>
</tr>
</thead>
<tbody>
<tr>
<td>At 6 weeks Between groups</td>
<td>-.19</td>
<td>-.43 -.06</td>
<td>.14</td>
</tr>
<tr>
<td>At 12 weeks Between Groups</td>
<td>-.16</td>
<td>-.43 .10</td>
<td>.23</td>
</tr>
</tbody>
</table>

Paired sample T-test (2-tailed); Confidence Interval (CI) 95%

Of the qualifying sites, pocket depth improvements by 1mm resulted with similar percentages for each group, 41.5% (n=93) control and 43.3% (n=58) experimental sites. Pocket depth improvements by 2 mm or more resulted with 13.4% (n=30) for the control group and 20.1% (n=27) for the experimental group. (no tables)

3.3.4.3 Secondary Endpoint Analysis for Qualifying Sites: BOP

Bleeding on probing (BOP) also a secondary endpoint was assessed; 1= no presence of BOP and; 2= presence of BOP. Due to the inclusion criteria, 100% of the qualifying sites
had BOP. Mean bleeding was reduced at 6 and 12 weeks when compared to the start. (Table 14) This reduction in BOP within groups was statistically significant at 6 week \((p<.000)\) and 12 weeks \((p<.000)\). Analysis between groups identified in a statistical significant difference at 6 weeks \((p<0.05)\) but none at 12 weeks \((p=0.47)\) (Table 15). Analysis of absolute change confirmed that treatment with NSPD+ APT when compared to NSPD alone significantly reduced BOP at 6 weeks \((p<0.05)\) and none at 12 weeks \((p=0.47)\) (Table 16).

Table 14 Mean BOP Scores (± SD) at Baseline, 6 and 12 Weeks (4.4mm)

<table>
<thead>
<tr>
<th>BOP</th>
<th>Baseline (\bar{x}) ± SD</th>
<th>6 Weeks (\bar{x}) ±SD</th>
<th>12 Weeks (\bar{x}) ±SD</th>
</tr>
</thead>
<tbody>
<tr>
<td>NSPD</td>
<td>224 2.00±0.0</td>
<td>1.24±.43</td>
<td>1.25±.43</td>
</tr>
<tr>
<td>NSPD + APT</td>
<td>134 2.0±0.0</td>
<td>1.34±.47</td>
<td>1.22±.41</td>
</tr>
</tbody>
</table>

Table 15 Mean BOP Difference ± SD, CI and \(P\)-values at 6 and 12 Weeks

<table>
<thead>
<tr>
<th>BOP</th>
<th>N</th>
<th>(d)±SD</th>
<th>6 Weeks CI Lower-upper</th>
<th>(P)-value within groups</th>
<th>12 Weeks CI Lower-upper</th>
<th>(P)-value within groups</th>
<th>(P)-value between groups</th>
</tr>
</thead>
<tbody>
<tr>
<td>NSPD</td>
<td>224</td>
<td>.76±.43</td>
<td>.70-.82</td>
<td>.000</td>
<td>.75±.43</td>
<td>.69-.81</td>
<td>.000</td>
</tr>
<tr>
<td>NSPD + APT</td>
<td>134</td>
<td>.66±.47</td>
<td>.58-.75</td>
<td>.000</td>
<td>.78±.41</td>
<td>.71-.85</td>
<td>.000</td>
</tr>
</tbody>
</table>

\(p\)-value between groups

Independent sample T-Test (2-tailed); Confidence Interval (CI) 95%
Table 16 Absolute BOP Change at 6 and 12 Weeks Between Groups

<table>
<thead>
<tr>
<th>BOP</th>
<th>Mean Difference</th>
<th>CI Lower-Upper range</th>
<th>P-value</th>
</tr>
</thead>
<tbody>
<tr>
<td>At 6 weeks between Groups</td>
<td>.09</td>
<td>-.00 - .19</td>
<td>.05</td>
</tr>
<tr>
<td>At 12 weeks between Groups</td>
<td>-.03</td>
<td>-.13 - .03</td>
<td>.47</td>
</tr>
</tbody>
</table>

Paired Sample T-test (2-tailed); Confidence Interval (CI) 95%

3.3.5 Statistical Sub-Analysis for Qualifying Sites

3.3.5.1 Statistical Sub-Analysis for Qualifying Sites: Plaque

Plaque was analyzed with the FloridaProbe® with 1= no plaque and 2= plaque present.

Table 17 reveals plaque means and standard deviation at baseline, 6 and 12 weeks for both groups with no statistical significance for baseline mean values (p=0.23). The mean plaque scores were reduced for both groups at 6 and 12 weeks (table 17) but there were no statistical significant difference within each group at 6 weeks but a reduction in plaque for the NSPD group was significant 12 weeks (p=.000) (Table 18). A three time points T-Test between groups resulted in no statistical significant differences at 6 weeks (p=.50) or 12 weeks (p=.60) (Table 18).

Table 17 Plaque Mean and SD at Baseline, 6 and 12 Weeks

<table>
<thead>
<tr>
<th>Plaque</th>
<th>Baseline $\bar{x} \pm SD$</th>
<th>6 Weeks $\bar{x} \pm SD$</th>
<th>12 Weeks $\bar{x} \pm SD$</th>
</tr>
</thead>
<tbody>
<tr>
<td>N</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>NSPD</td>
<td>94</td>
<td>1.77±.43</td>
<td>1.68±.47</td>
</tr>
<tr>
<td>NSPD + APT</td>
<td>59</td>
<td>1.67±.47</td>
<td>1.63±.49</td>
</tr>
</tbody>
</table>
Table 18: Plaque Mean Difference ± SD, CI and $P$-values at 6 and 12 Weeks

<table>
<thead>
<tr>
<th>Plaque</th>
<th>N</th>
<th>d±SD</th>
<th>6 Weeks CI Lower-upper</th>
<th>$P$-value within groups</th>
<th>d±SD</th>
<th>12 Weeks CI Lower-upper</th>
<th>$P$-value within groups</th>
</tr>
</thead>
<tbody>
<tr>
<td>NSPD</td>
<td>94</td>
<td>.09±.63</td>
<td>-.04-.21</td>
<td>.20</td>
<td>.23±.61</td>
<td>.11-.36</td>
<td>.000</td>
</tr>
<tr>
<td>NSPD + APT</td>
<td>59</td>
<td>.05±.51</td>
<td>-.08-.18</td>
<td>.44</td>
<td>.10±.52</td>
<td>-.03-.24</td>
<td>.14</td>
</tr>
<tr>
<td><em>P</em>-value between groups</td>
<td>.50</td>
<td>-09-.11</td>
<td>.60</td>
<td>-.12-.06</td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

Independent sample T-Test (2-tailed); Confidence Interval (CI) 95%

3.3.5.2 Statistical Sub-Analysis of sites: Debdriment with Immediate Application of PerioWave® vs. One Week Post-Debridement and The Application of PerioWave®

The 358 sites were further separated into two group; sites that had the application of PerioWave™ immediately following debridement (*group A*) versus sites having the application of PerioWave™ one week post-debridement (*group B*). Sites were analyzed to determine if the immediate application of the agent in the presence of excess serum and blood from debridement would reduce the agent’s effectiveness. Table 19 reveals the 134 sites analyzed, N=71 sites for group A and N=63 sites for group B, for clinical attachment levels and probing depths. Independent sample T-test concluded no statistical significance between groups for either CAL at 6 (p=.38) or 12 (p=.09) weeks or for PD at 6 (p=.87) and 12 weeks (p=.20). (Table 19).
Table 19 Sub-Analysis for Sites: Debriment with Immediate Application of PerioWave™ vs. One Week Post-Debridement and the Application of PerioWave™

<table>
<thead>
<tr>
<th>Parameters</th>
<th>N</th>
<th>Baseline</th>
<th>6 Weeks</th>
<th>12 Weeks</th>
</tr>
</thead>
<tbody>
<tr>
<td>CAL</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Bleeding</td>
<td>71</td>
<td>6.86±2.00</td>
<td>5.91±2.06</td>
<td>5.79±2.07</td>
</tr>
<tr>
<td>Non-bleeding</td>
<td>63</td>
<td>6.95±1.74</td>
<td>6.23±2.20</td>
<td>6.42±2.16</td>
</tr>
<tr>
<td>Mean Difference</td>
<td></td>
<td>-0.09</td>
<td>-0.32</td>
<td>-0.633</td>
</tr>
<tr>
<td>P-values</td>
<td></td>
<td>0.77</td>
<td>0.38</td>
<td>0.09</td>
</tr>
<tr>
<td>PD</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Bleeding</td>
<td>71</td>
<td>6.17±1.26</td>
<td>5.11±1.65</td>
<td>4.92±1.57</td>
</tr>
<tr>
<td>Non-bleeding</td>
<td>63</td>
<td>6.05±1.16</td>
<td>5.16±1.59</td>
<td>5.29±1.73</td>
</tr>
<tr>
<td>Mean Difference</td>
<td></td>
<td>0.12</td>
<td>-0.05</td>
<td>-0.37</td>
</tr>
<tr>
<td>P-values</td>
<td></td>
<td>NS(0.58)</td>
<td>NS(0.87)</td>
<td>NS(0.20)</td>
</tr>
</tbody>
</table>

Independent sample T-Test (2-tailed); Confidence Interval (CI) 95%

3.3.5.3 Statistical Sub-Analysis of Sites: Light Output of Units

The study started with a unit which when tested halfway through the subjects was found to be producing only a minimal amount of light output, 80mW. The faulty first unit (Unit 1) and handles treated 32 sites. As previously mentioned in the subjects’ summary section, 2 subjects were excluded from the data due to the minimal light output. The second unit (Unit 2) and new handles completed the 21 subjects with 102 sites. An analysis between the two units at baseline, 6 and 12 weeks for CAL, PD and BOP was done to determine if there were any possible differences. Table 20 illustrates no statistical difference with the clinical parameters between the two light groups at any given time line.
Table 20 Sub-Analysis Between the Two Light Output Units for Baseline, 6 and 12 Weeks for CAL, PD and BOP

<table>
<thead>
<tr>
<th>Parameters</th>
<th>N</th>
<th>Baseline</th>
<th>6 Weeks</th>
<th>12 Weeks</th>
</tr>
</thead>
<tbody>
<tr>
<td>CAL</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Unit 1</td>
<td>32</td>
<td>6.72±1.58</td>
<td>5.99±2.32</td>
<td>6.11±2.23</td>
</tr>
<tr>
<td>Unit 2</td>
<td>102</td>
<td>6.96±1.96</td>
<td>6.09±2.08</td>
<td>6.08±2.10</td>
</tr>
<tr>
<td>Mean Difference</td>
<td>-0.23</td>
<td>-0.10</td>
<td>0.03</td>
<td></td>
</tr>
<tr>
<td>P- values</td>
<td>0.54</td>
<td>0.81</td>
<td>0.94</td>
<td></td>
</tr>
<tr>
<td>PD</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Unit 1</td>
<td>32</td>
<td>5.99±1.15</td>
<td>5.06±1.52</td>
<td>5.11±1.61</td>
</tr>
<tr>
<td>Unit 2</td>
<td>102</td>
<td>6.15±1.25</td>
<td>5.15±1.65</td>
<td>5.09±1.68</td>
</tr>
<tr>
<td>Mean Difference</td>
<td>-0.16</td>
<td>-0.10</td>
<td>0.02</td>
<td></td>
</tr>
<tr>
<td>P- values</td>
<td>0.53</td>
<td>0.77</td>
<td>0.96</td>
<td></td>
</tr>
<tr>
<td>BOP</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Unit 1</td>
<td>32</td>
<td>**</td>
<td>1.34±0.48</td>
<td>1.19±0.40</td>
</tr>
<tr>
<td>Unit 2</td>
<td>102</td>
<td>**</td>
<td>1.33±0.47</td>
<td>1.23±0.42</td>
</tr>
<tr>
<td>Mean Difference</td>
<td>**</td>
<td>0.01</td>
<td>-0.04</td>
<td></td>
</tr>
<tr>
<td>P- values</td>
<td>**</td>
<td>0.91</td>
<td>0.65</td>
<td></td>
</tr>
</tbody>
</table>

Independent sample T-Test (2-tailed); Confidence Interval (CI) 95%. *BOP at baseline 100%.

3.3.6 Additional Analysis of Data at 5.0mm (No Tables)

The default setting of the FloridaProbe® data rounded CAL and PD measurement from 4.4, 4.6, and 4.8 to 5.0mm. Based on the initial study design we re-examined all the variables sites ≥ 5.0mm. This reduced N=358 to an N=274 sites however there were still no difference in any of the analyses done. There was no statistical significance differences between the NSPD group and NSPD + APT group for either CAL (p=.94, p=.57) and PD (p=.27 p=.16) at 6 or 12 weeks respectively.
3.4 Conclusion (Discussion)

3.4.1 Effectiveness of Current Treatment Modalities

The effect of nonsurgical periodontal debridement (NSPD) on the subgingival microflora has been summarized in reviews (Haffajee and Socransky 1994, Haffajee et al. 1997). NSPD remains the gold standard in maintaining chronic periodontal sites by reducing and shifting the microbial load to a more biological compatible microflora and this improves clinical parameters. Unfortunately these improvements are short lived (approximately 12 weeks) due to repopulation of the biofilm matrix by periopathogens which may result in inflammation and/or loss of attachment and alveolar bone (Carranza 2006).

Furthermore, NSPD requires a certain level of skill and time, has limited access in challenging areas (deep pockets and furcations) and the potential for unnecessary removal of root substance. Collectively total subgingival pocket disinfection is difficult. Another difficulty lies with the fact that no one microorganism has been identified as the etiologic microorganism(s) causing disease (Socransky and Haffajee 1994, 2005, Loesche and Grossman 2001). This uncertainty coupled with the limitations of NSPD makes treating chronic periodontal sites challenging.

The adjunctive use of either systemic or localized antibiotics has been extensively tested for their therapeutic efficacy. Systematic reviews by Haffajee et al. 2003, 2007, Winkelhoff 2003, Herrera et al. 2002 and others have concluded the use of systemically administered adjunctive antibiotics with or without NSPD and/or surgery appeared to provide greater clinical improvement (0.45mm attachment gain). While these reviews
indicate, on average, antibiotics do contribute to a statistically significant improvement in periodontal clinical indices it is still unclear on the magnitude of the added benefit, the optimal dosage, the optimal agent(s), the frequency of application, the identification of the patients that would most benefit, patient compliance and the increasing concern of antibiotic overuse and resistance (Bonito et al. 2005, Haffejee et al. 2003).

In summary new therapies developed to help patients with periodontal diseases ideally should be more effective than current treatment modalities or at least deliver similar outcomes while enhancing clinician and patient acceptance and/or compliance.

3.4.2 Antimicrobial Photodynamic Therapy (APT)

The above mentioned concerns have fostered research into novel approaches to manage bacterial infections. APT has been extensively investigated in vitro with a lesser extend in vivo for the eradication of oral bacteria. In vitro research established that several associated periodontopathogens in the subgingival biofilms like P. gingivalis, Fusobacterium nucleatum, Staphylococcus sp. are efficiently eradicated by photodynamic treatment, both in aqueous suspension and in biofilm (Jori et al. 2006, Wilson 2003).

APT utilizes visible light at a certain wavelength to activate a selective tissue-binding photosensitizing dye agent (PS). The antimicrobial activity of the PS is mediated by singlet oxygen which, because of its high chemical reactivity, can have a lethal effect on both the bacterial and biofilm (Konopka and Goslinski 2007), making APT an attractive treatment modality for periodontal diseases.
3.4.3 APT Potential Confounding Factors

The primary goal of APT is to produce adequate levels of singlet oxygen to induce cell death (Dahl et al. 1989, Jori et al. 2006). Investigators have evaluated potential APT mechanism and confounding factors such as, but not limited to, the i)PS; ii) light source (power, fluence rate); iii) the presence of blood and serum, and iv) pH and saliva that may inhibit or diminish the bacteriocidal efficiency of APT (Komerik and Wilson 2002, Komerik et al. 2003, Bhatti et al. 1997).

3.4.3.1 Photosensitizer (PS)

The PS compound selection is crucial to achieve a successful photochemical reaction. Its imperative the chosen PS for treatment is retained by target cells (bacteria in this case), absorbs at the desired wavelength of light with a high extinction coefficient and high quantum yield of singlet oxygen (Pervaiz and Olivo 2006, Jori et al. 2006).

The structural characteristics of phenothiazinum-based photosensitizers (PhBPs) like MB and derivatives allow them to be highly suited for APT by exhibiting significant singlet oxygen yields (0.40) and absorbing at therapeutic wavelengths (620-700nm) (Wainwright 2007). Chan and Lai (2003) in vitro revealed that oral microorganisms achieved bacterial kills with a 665nm diode laser, at 100 mW with 60 seconds irradiance while incorporated with 0.01% wt/vol methylene blue (MB) PS. Furthermore the cationic phenothiazine has been proven to penetrate deeper in the plaque biofilm (Wood et al. 1999) and increase the killing rate (Meisel and Kocher 2005). Although most studies demonstrated a log reduction in bacteria, a confocal laser scanning micrograph (CLSM) of a biofilm after
exposure to phototoxic mechanism revealed that in some of the biofilm stacks, lethal photosensitization occurred predominantly in the outer layers of the stack leaving some of the innermost bacteria alive (O’Neil et al. 2002) which may allow for bacterial recolonization such as, *Aggregatibacter actinomycetemcomitans* (A.a.) and *P. gingivalis* which are known to invade host tissue cells and elude the effects of NSPD (Carranza 2006).

Loebel et al. 2007 *in vivo* retreated qualifying sites (chronic periodontitis) with a sequential application of APT without NSPD at 6 weeks in the experimental group. At 12 weeks, the experimental group had achieved statistical difference in pocket depth reductions over the control. Further studies are needed to aid in elucidating appropriate protocols.

**3.4.3.2 Laser Light Dose-Dependent**

The effectiveness of a light source for APT depends but is not limited to, spectral irradiance, tissue transmission and photosensitizer absorption (McCaughan 1999, Nilsson et al. 1995, MacDonald and Dogherty 2001, Moseley 1996). Chan and Lai (2003) demonstrated *in vitro* that both wavelength and energy density are important factors in achieving optimal bacterial kill. As previously mentioned, the most-effective combination was that of a 665-diode laser at 100 mW at 60 seconds (energy density 21.2 J/cm²) irradiance.
In the present study a sub-analysis was added to assess the light intensity (irradiance) of two 670nm-diode laser units (PerioWave™) used. The two units had default light settings with a minimum of 80 mW to a maximum output of 150mWcm² and neither had laser spectral stability (nm) or output stability (mW) readings. The first light intensity testing (mid-way of trial) the laser unit revealed below acceptable levels (28 and 29 mWcm²). At that time, the second unit was introduced and completed the study. The sub-analysis did not indicate a statistical significant difference between the two units but it should be noted that both units were unable to indicate the numerical light intensity during application leaving the clinician to assume correct light intensity was being obtained. A possible explanation for the lack of statistical difference in the sub-analysis is having too small of a sample size to detect a difference (Table 20).

Since the present study, new laser units provided by PerioWave™ are now self-calibrated with higher light intensity ranges (max: 220mWcm²). Anderson et al. 2007 compared the laser light intensities at 150mW and 220mW and concluded 220mW was statistically significant in improving CAL (p=0.002) and PD (p=0.01) over NSPD. Albeit, the clinician is still not aware of the numerical light intensity during application, the spectral stability or the output power stability which are crucial from a research perspective.

### 3.4.3.3 Blood and Serum

Serum-derived from gingival creviccular fluid (GCF) as well as blood may be confounders in treating periodontal pocket with APT. Matevski et al. 2003 *in vitro* identified the similarity between serum and blood kills was due to the presence of serum itself that
provided protection to \textit{P. gingivalis} from toluidine blue (TBO). The protection could be due to the presence of light scattering/absorbing proteins (Wilson and Pratten 1995) or perhaps the serum component bind to \textit{P. gingivalis} which in turn reduce the bacteriocidal effectiveness of the PS (Matevski et al. 2003). Other explanations for the decreased effectiveness may be due to the proteins competing with the bacteria for PS molecules, thus decreasing the number of PS molecules available for binding (Bhatti et al. 1997).

The sub-analysis in the present study evaluated the presence of blood as a potential confounder. The results concluded no statistical significance differences between sites treated with APT directly after debridement versus sites treated with APT one week post-debridement. This may be elucidated by the quantity (heavy versus light) of blood present. The subjects in this study albeit advanced chronic sites, were well maintained with low levels of bleeding which may not have been enough to affect the data. Komierick and Wilson (2002) \textit{in vitro} found an interrelation between higher concentrations (50\%) of blood serum and lower levels of gram negative bacterial killing but quickly returned to high levels of bacterial killing upon serum washout.

3.4.3.4 Saliva and pH

\textit{In vitro} studies PS and bacteria interactions were found to be pH dependent by Nitzan et al. 1987. The dependency was elucidated by changes in membrane fluidity and the increased binding of the PS to the bacteria. Cationic sensitizers like methylene blue (MB) in an aqueous solution, are more effective at neutral pH as MB triplets are excited state bases (pH 7.5). Conversely, a higher pH (8.0) may induce longer triplet state of other
types of PS which may promote an increase in singlet oxygen half-life (Nitzan et al. 1987, 1989, Matevski et al. 2003, Tuit and Kelly 1993).

Although the present study did not analyze the pH levels, Forscher et al. 1954 determined the mean pH of periodontal pockets in chronic periodontitis to be 7.09 ± 0.07 with no significant variation related to the depth of the pocket. Therefore saliva and pH levels in the present study should not have posed as confounders.

Saliva was also found to influence certain PS activity. The more acidic saliva in patients diagnosed with mucositis was found to affect the PS toluidine blue (TBO) in treating oral lesions with photodynamic therapy. The addition of a pre-mild alkaline rinse was found to increase the pH levels in saliva and allowed for a more effective PS (Komerick and Wilson 2002).

3.4.4 In Vivo Studies of APT

In vivo animal studies are showing phenothiazines like toluidine blue can eradicate oral pathogens from the oral cavity, reduce inflammation and significantly decrease the level of alveolar bone loss in rat and dog periodontal disease models (Milanezi de Almeida et al. 2007, Komerik et al. 2003, Hayek et al. 2005).

In vivo human study by Braun et al. 2008 evaluated twenty patients with untreated chronic periodontitis. All teeth received periodontal treatment comprising scaling and root planning using a split-mouth design. Two quadrants (test group) were additionally treated
with aPDT (APT). Sulcus fluid flow rate (SFFR) and bleeding on probing (BOP) were assessed at baseline, 1 week and 3 months after treatment. Relative attachment level (RAL), probing depths (PDs) and gingival recession (GR) were evaluated at baseline and 3 months after treatment. The results reported values for RAL, PD, SFFR and BOP decreased significantly 3 months after treatment in the control group (median delta RAL: -0.35 mm, interquartile range: 0.21 mm), with a higher impact on the sites treated with adjunctive aPDT (APT) (median delta RAL: -0.67 mm, inter-quartile range: 0.36 mm, p<0.05). GR increased 3 months after treatment with and without adjunctive aPDT (p<0.05), with no difference between the groups.

The subjects in the Braun study were untreated chronic sites with a split-mouth design. In contrast, the sites in our study were not untreated but rather long term periodontally maintained. These significant differences do make it difficult to make direct comparisons and may explain apparent differences in treatment effect. The qualifying sites in the Braun study, were irrigated with the PS (no mention of concentration or type) employing a blunt cannula and after 3 minute residence time, the pockets were rinsed with an unknown specified time or amount of water to remove excess PS. The additional irrigation in the pockets could have aided with reducing the bacteria levels in those sites versus those only receiving NSPD. All the sites (6) of the 4 teeth (first premolar and 1 first molar of 2 quadrants) were treated without a given rational. An indication of teeth distribution would also have aided the reader to know which teeth/sites presented with significant disease. Furthermore, there wasn’t any mention of relative plaque levels,
whether improvement was noted or having any bearing on the result. Plaque levels in the present study revealed no statistical difference and no bearing on the results. (Table 18) It would have aided if the teeth that improved were noted as different teeth can heal differently (Karlsson et al. 2008). Of the 358 sites evaluated in the present study, 68.7% (n=246) were from molars with 67% (n=150) and 71.6% (n=96) from the control and experimental group respectively. The high percentage of molars in the present study may have negatively impacted pocket reduction and gain in clinical attachment.

In another in vivo human study, Andersen et al. 2007 assessed 33 patients with chronic periodontitis. The clinical effect of a photodisinfection treatment was evaluated over a 12 weeks period. All subjects were randomly treated in one of three study groups with either photodisinfection alone (Group 1); SRP alone (Group 2); or with SRP and photodisinfection combined (Group 3). Clinical variables such as BOP, PPD and CAL were assessed after three, six and 12 weeks following therapy. The BOP results revealed a significant improvement in all treatment groups, but no statistical significant differences between the groups. At the 12 weeks follow-up statistical significant improvements were reported both for the ΔPPD (group 2: 0.74 ± 0.43 m vs. group 3: 1.11 ± 0.53 mm, \( P <0.05 \)) and ΔCAL (group 2: 0.36 ± 0.35 mm vs. group 3: 0.86 ± 0.61 mm, \( P <0.02 \)). Karlson et al. 2008 noted plaque controls levels during the active periodontal therapy and follow-up were not reported, no power calculation for the sample size was reported in that study. Although that study indicates randomization it appears subjects were randomly assigned rather than true randomization. Neither the patients nor the clinician were blinded to the outcomes. In addition Karlson et al. 2008 notes the authors only
reported the mean PPD reduction and mean CAL change. It would have been of interest, if the results had been analyzed with respect to initial PPD and characteristics of the sites i.e., anteriors and posterior teeth and with furcation (Karlsson et al. 2008). Furthermore the use of only hand instrumentation versus hand and ultrasonic instrumentation, as in the present study, might have influenced their study outcomes. Leon and Vogel 1997 found that ultrasonics in furcations can effectively reduce bacteria and keep levels at a healthy level longer than hand instrumentation.

The results of the present study demonstrated PerioWave™ as a one time application of a cold diode laser with a wavelength of 670nm; a maximum power of 150mW; an average energy density of 20 J/cm²; a phenothiazine photosensitizer (methylene blue) in a concentration of 0.01% wt/vol; and at an optimal activation time of 60 seconds per mesiodistal surface had no statistical significance in the treated qualifying sites with regards to CAL or PD at 6 or 12 weeks after treatment over the NSPD group. These clinical results compare well with those of a recent controlled clinical study by Chondros et al. 2007, evaluating the effects of photodynamics on non-surgical periodontal therapy in chronic periodontitis patients on maintenance programs at 3 and 6 months. Those results failed to demonstrate any statistically significant differences between the two treatments in the clinical parameters investigated, CAL and PD. However BOP did have a statistical significance over the control group in the present study at 6 weeks but not at 12 weeks. Chondros et al. 2007 also resulted with a significant reduction of BOP at 3 months but not at 6 months post-treatment. Plaque scores for either study had no statistical significance and thus had no bearing on BOP results.
The resulting decrease in BOP for all *in vivo* studies raises the question as to whether sequential applications of APT in the long term could improve clinical parameters and if so, when would *that* therapeutic window be. A three month clinical trial albeit adequate to evaluate a reduction in periopathogens, may be inadequate to assess CAL in such advanced chronic sites. And although pocket depth resulted in no statistical difference a trend was examined in the data as a ±2 mm percent improvement of control (13.4%) and experimental (20.1%) sites. There is a possibility that conjoint use of photodynamics may have had an adjunctive effect. Further research with long term, larger sample size and sequential applications are required to confirm these possibilities

When analyzing photodynamic therapy (PDT) in tumors the subject-based is the unit of observation (analysis) as PDT destroys the diseased tissue (tumor) and the subject responds to treatment. In contrast, APT is a site-specific treatment of local infections. Localized lesions with a site-based etiology like many periodontal diseases require site-specific periodontal risk assessments and diagnostic outcome measures (Persson 2005). There are both advantages and disadvantages for either type (site or subject) of observation (Tu et al. 2004) in research and perhaps it’s not “either” but rather a combined unit of observation that would allow the data to be evaluated at the level of the tooth, site and subject simultaneously. Future intervention studies should aim for this *joint* unit of observation.

In summary an ideal phototoxic/antimicrobial treatment like APT needs to target the microorganisms and eradicate them while other oral environment structures, such as
collagen, oral flora, and epithelial cells would not be damaged by the photo-process. Komerik et al. 1998 and Soukos et al. 1998 demonstrated no evidence of damage to host tissues. The observation that the post-operative healing was uneventful in all cases throughout the study does indicate that NSPD with the PerioWave™ system is well tolerated.

The novel concept of antimicrobial photodynamics of oral bacteria has been documented using in vitro and in vivo animal studies. However results cannot always be directly extrapolated to human disease (Calderon 2000). Within its limits, our in vivo human study showed the additional application of a single episode of phototoxic antimicrobial to NSPD alone in advanced maintained chronic sites failed to result in additional improvement in terms of CAL and PD, but resulted in a significant reduction of bleeding scores at 6 weeks over the NSPD group with chronic periodontitis. Sites that lack BOP are said to have achieved disease stability (Kornman and Wilson 2003) which is crucial for long term maintenance of chronic periodontal sites.

In conclusion, there are few human RCT and of those conducted the varying study design (split-mouth to full mouth), laser power (660 to 667nm), power density (20 to 75J/cm²), PS utilized (chloride phenothiazines to chlorin e6), concentration (0.01mg/ml to 15mg/ml) and irradiance time (60 seconds to 5 hours) makes extrapolation challenging. However, APT has sound scientific merit and warrants further investigation. Additional human in vivo independent RCT as well as interdisciplinary research requiring input from physics, engineering, and the dental profession are needed to develop APT models for
new concepts, applications and establish clinical protocols for the management of localized microbial infections.

3.4.5 Future Work

Further *in vivo* photodynamic antimicrobial clinical trials are needed to further enhance guidelines and protocols in order to aid the clinician treatment plan and implement it as a modality of care.

After evaluating the literature and completing this clinical trial, the following points should be incorporated into future RCTs; i) the sample populations are of the same periodontal classification; ii) equally randomized samples prior to treatment; iii) the laser unit displays the laser spectral stability (nm) readings with every activation; iv) the laser unit displays the output stability (mW) readings with every activation; v) quantitative measurements of bleeding from BOP and debridement prior to the PS application vi) additional study arms to compare APT alone to NSPD alone to NSPD and APT in the same disease classification as well as sequential PS application at 12 weeks for a year; vii) microbiological pre and post sampling to determine the bacterial counts are indeed being affected by APT *in vivo (human)* as well as to identify when a pocket becomes “anaerobic” which may aid in determining the “ideal” time of treatment; viii) would also include laser light alone as an arm to assess if low laser light influences healing; and ix) use multilevel modelling analysis to incorporate the tooth, the site and the subject simultaneously.
References:


Demidova T, Hamblin M. Effect of cell-photosensitizer binding and cell density on microbial photoinactivation. Antimicrobial agents and chemotherapy. 2329-2335,2005.


Loesche WJ, Gossman NS. Periodontal Disease as a specific, albeit Chronic, Infection: Diagnosis and Treatment. Clinical Microbiology Reviews, 14; 727-752, 2001.


Appendix A

Medical Device License
Appendix B

UBC Research Ethics Board Human Ethics Certificate of Approval

Certificate of Expedited Approval: Amendment
Clinical Research Ethics Board Official Notification

Principal Investigator: Putini, E.E.
Department: Oral Biological & Medical Sci
Institution(s) Where Research Will Be Carried Out: Other
Co-Investigators: Hatzimanolakis, Penny, Oral Biological & Medical Sci; Lezzi, Sonia, Oral Biological & Medical Sci
Sponsoring Agencies: Ondine Biopharma Corporation

Title: A Randomized Clinical Trial Using Photodynamic Disinfection Adjunctively with Scaling & Root Planing in Treating Chronic Periodontitis

Approval Date: 06-03-15
Term: Years 1
Amendment: Subject Consent Form version date 16 March 2006
Amendment Approved: 21 March 2006

Certification:
In respect of clinical trials:
1. The membership of this Research Ethics Board complies with the membership requirements for Research Ethics Boards defined in Division 5 of the Food and Drug Regulations.
2. The Research Ethics Board carries out its functions in a manner consistent with Good Clinical Practices.
3. This Research Ethics Board has reviewed and approved the clinical trial protocol and informed consent form for the trial which is to be conducted by the qualified investigator named above at the specified clinical trial site. This approval and the views of this Research Ethics Board have been documented in writing.

The amendment(s) for the above-named project has been reviewed by the Chair of the University of British Columbia Clinical Research Ethics Board and the accompanying documentation was found to be acceptable on ethical grounds for research involving human subjects.

The CREB approval period for this amendment expires on the one year anniversary date of the CREB approval for the entire study.

Approval of the Clinical Research Ethics Board by one of:
Dr. Gail Bollwein, Chair
RESEARCH SUBJECT INFORMATION AND CONSENT FORM

**TITLE:** A Randomized Clinical Trial Using Photodynamic Disinfection Adjunctively with Scaling & Root Planing in Treating Chronic Periodontitis

**Principal investigator:** Dr. Edward E. Putnins  
The University of British Columbia  
Faculty of Dentistry  
Vancouver, B.C., Canada  
(604) 822-1734

**Co-investigators:** Ms. Penny Hatzimanolakis RDH & Dr. Sonia Leziy (Dip Perio)  
XX Esplanade, Suite XX  
North Vancouver, B.C.  
(604) XXX-XXXX

**Sponsor:** Dave Hammond CCRC, CCRA  
Ondine Research Laboratories  
Redmond, Washington  
United States  
(425) XXX-XXXX

**PROTOCOL NO.:** ORL-0605-5 (Rev B)

**Emergency (24 hours) Telephone Numbers:** (778) XXX-XXXX or (604) XXX-XXXX

**Introduction**  
You are being invited to take part in this research study to evaluate a product called Periowave, because you have periodontal (gum) disease. Periodontal disease is a serious infection that, if left untreated, can lead to tooth loss. The word *periodontal* literally means “around the tooth”. Periodontal disease is a chronic bacterial infection that affects the gums and bone supporting the teeth. Periodontal disease can affect one tooth or many teeth. It begins when bacteria in plaque (the sticky colorless film that constantly forms on your teeth) causes the gums to become inflamed. With time,
plaque can spread and grow below the gum line and stimulate inflammation. This results in tissue and bone break-down and destruction. Gums separate from the teeth, forming pockets (spaces between the teeth and gums) that become infected. If the disease progresses, the pockets deepen and more destruction occurs which can lead to tooth loss.

The Periowave system is composed of a very low concentration solution of a common dye called methylene blue. Washing the tissues with this dye allows the dye to attach itself to the wall of the bacteria. A low power laser is then used to expose the tissues to concentrated light. The light causes a reaction in the dye that produces extra oxygen molecules, which are toxic to bacteria. In the presence of this high level of oxygen, the bacterial wall is expected to rupture and be destroyed. The laser light is similar to what is commonly seen in laser pointer devices. The study solution and device have been approved by Health Canada.

**Your participation is voluntary**

Your participation is entirely voluntary, so it is up to you to decide whether or not to take part in this study. Before you decide, it is important for you to understand what the research involves. This consent form will tell you about the study, why the research is being done, what will happen to you during the study, and the possible benefits, risks and discomforts.

If you wish to participate, you will be asked to sign this form. If you do decide to take part in this study, you are still free to withdraw at any time and without giving any reasons for your decision.

If you do not wish to participate, you do not have to provide any reason for your decision not to participate nor will you lose the benefit of any dental care to which you are entitled or are presently receiving.

Please take the time to read the following information carefully and to discuss it with your family, friends, and doctor before you decide.

**Who is conducting the study?**

The study is being funded by Ondine Research Laboratories. The Investigators have received financial compensation from Ondine for enrolling subjects into this study. All Investigators are UBC faculty members in the Faculty of Dentistry. You are entitled to request any details concerning this compensation from the Investigators.
**Background**

The main cause of periodontal disease is bacterial plaque. However, factors like smoking, genetics, stress, pregnancy, puberty, clenching, grinding teeth, poor nutrition and other systemic conditions can also influence its progression.

The first step in treating periodontal disease is usually non-surgical and most cost-effective. This is accomplished through scaling and root planing (a careful cleaning of the root surfaces to remove plaque and calculus (tartar) from deep periodontal pockets and to smooth the tooth root to remove bacterial toxins). Depending on the case, treatment may be followed by adjunctive therapy such as antibiotics and/or antimicrobials.

Over the past few years, researchers have investigated ways to eliminate bacteria without using antibiotics. The Periowave approach lessens the risk of the bacteria becoming resistant to antibiotics as well as possible allergic reactions people may have to antibiotics. A recent study using this product was conducted as a pilot study on humans and treated areas showed more gum attachment to the tooth (230% more at 6 weeks) and less areas of bleeding (144% less at 6 weeks) when the new Periowave treatment approach was compared to scaling alone.

**Purpose of the study**

The purpose of this study is to determine the effectiveness of the product Periowave as an adjunct (in addition) to scaling and root planing in treating chronic periodontal disease in human adults.

Currently, there are no approved products available in Canada that work in this way.

**Who can participate in the study**

A patient who meets all of the following criteria is eligible for the study:

1. The subject is capable of giving informed consent.
2. The subject is willing to sign a consent form.
3. The subject is an adult male or female greater than 18 years of age.
4. The subject is diagnosed with chronic periodontitis.
5. The subject has greater than 18 fully erupted teeth.
6. The subject has at least 4 measurement sites with a pocket depth of 6-9 mm that bleed when probed in at least two quadrants of the mouth.
7. The subject is willing and able to return for treatment and evaluation procedures scheduled throughout the course of this clinical study.

**Who should not participate in the study?**

A patient who has any of the following conditions is ineligible for the study:

1. The subject is pregnant or nursing or plans to become pregnant in the next 4 months.
2. The subject has significant liver disease by subject report.
3. The subject has an active malignancy (cancer) of any type by subject report.
4. The subject has chronic disease or diminished mental capacity that would mitigate the ability to comply with the protocol.
5. The subject has any significant disease (either acute or chronic) or is taking a medication with concomitant oral manifestations (related mouth symptoms) that in the opinion of the investigator would interfere with evaluation of safety or efficacy of PERIOWAVE®.
6. The subject has an active periapical (tooth) abscess or periodontal (gum) abscess or a history of acute necrotizing ulcerative gingivitis (painful canker-like gums).
7. The subject has been treated with antibiotics within the 1-month period prior to beginning the study or any systemic condition which requires antibiotic coverage for routine periodontal procedures (e.g. heart conditions, joint replacements, etc.) by report of the subject.
8. The subject has a known allergy to Methylene Blue.
9. The subject has glucose-6-phosphate dehydrogenase (G6PD) deficiency by subject report.
10. The subject currently uses anti-coagulant therapy at therapeutic doses.
11. The subject is currently using any photosensitizing medications.
12. The subject has participated in investigational treatment in the last 30 days or has the expectation for using a separate investigational treatment during the time of the study.
13. The subject has smoked tobacco products in the last year.

**What does the study involve?**

Subjects will participate for up to 12 weeks. Prior to any trial related procedure, the patient will be given the consent document, given a chance to ask questions and sign the Informed Consent Form. At each visit the patient will be questioned about any
changes in their health status. In addition, they will receive an oral examination to evaluate any changes from baseline.

A) Visit 1  Screening visit (-1 to -16 days before treatment)

At this visit the following procedures will be performed and documented by the blinded Examiner:
- Inclusion / exclusion
- Medical/dental history and demographics
- Concomitant medications
- Record all current medications and those used within the past 30 days. Document any antibiotic use in the last 6 months.
- Complete Documented Oral Examination:
  - Soft tissue exam – note any hypertrophic (enlarged) gingival tissue
  - Missing teeth
  - Pocket Probing Depth
  - Bleeding on Probing with/without exudates (pus)
  - Measurement from the cemental enamel junction (CEJ: where the root surface and the white part of the tooth meets) to gingival (gum) margin
  - Abutment (supporting) teeth for removable or fixed prosthesis
  - Occlusal Stress – Fremitus (a vibration felt on teeth when biting)
  - Teeth with +2 or +3 mobility
  - Furcation (bone loss between roots of some teeth) involvement
  - Selection of PerioWave® treatment sites
  - Any known retained tooth roots, cysts or abnormalities

1. Selection of PerioWave® Treatment Sites
A site is defined as natural tooth with a periodontal pocket reading greater than 5 mm and showing bleeding on probing at the screening visit.

2. Randomized (distributed by chance into a certain study group)
You will be randomized (i.e. nor you or the investigator will determine which study group you are assigned to) PerioWave® group or the non-PerioWave group. This next portion may be done within 0-3 days after the completion of the second Scaling and Root Planing visit. Those subjects randomized to the PerioWave® group will have all qualifying sites treated at this visit. All subjects will have the following information collected: adverse (unexpected) events; concomitant medications (medication being taken); Record any changes in medication.

3. Blinded
This is a single blinded study, meaning the clinician who does the evaluation at the first, fifth and sixth appointment will not know who has received the product Periowave. The clinician who will provide the treatment will know.

4. Follow-up phone call
After the first visit, a researcher will follow-up via telephone to answer any questions the subject may have about the study.

B) Visit 2  Scaling and Root Planing (Thorough cleaning of your teeth with dental instruments)

The first half-mouth SRP (scaling and root planing) is to be performed. Freezing may be needed.

C) Visit 3  Scaling and Root Planing (plus PerioWave® Treatment for the subject group)
The final half-mouth SRP (scaling and root planing) is to be performed. Freezing may be needed. Periowave product is a photodynamic (laser and solution activated) disinfection system provided by Ondine Biopharma (sponsor of the study). After scaling and root planing the infected area (under the gums), the researcher will irrigate (flush) the infected (diseased) area with a solution called methylene blue with a syringe (plunger). The researcher will then introduce the low intensity laser light directly into the affected sites using a fiber optic probe for 1 minute per site.

D) Visit 4  Follow-up Visit 6-weeks (+/- 4 days)
Blinded Examiner evaluation will include the following:

  o  Complete Oral Charting with the Florida Probe
  o  Concomitant medication (medication being taken) and any changes
  o  Adverse (unexpected) Events

E) Visit 5  Study Termination Visit 12-weeks (+/- 8 days)
Blinded Examiner evaluation will include the following:
  o  Complete Oral Examination with the Florida Probe
  o  Concomitant medication (medication being taken) and any changes
  o  Adverse (unexpected) Events

If you decide to join this study: Specific Summary of Procedures

Your time required for this study will be approximately 6.5 hours for a 12 week interval. Once all treatment is completed, you will receive standard care.
If you agree to take part in this study, the procedures and visits you can expect will include the following:

**First Appointment:** evaluation: 1 hr

**Phone contact:** phone call by one of the investigators to review the consent form and answer any of your questions

**Second appointment:** 1.5 hrs to scale and root plane one-half of your mouth. Freezing may be required.

**Third appointment:** 1.5 hrs to scale and root plane the remainder of the mouth. If you have been randomly selected to have the Product, then the researcher will apply the product Periowave to certain areas of your mouth. Freezing may be required. Periowave product is a photodynamic (laser and solution activated) disinfection system provided by Ondine Biopharma (sponsor of the study). After scaling and root planing the infected area (under the gums), the researcher will irrigate (flush) the infected (diseased) area with a solution called methylene blue with a syringe (plunger). The researcher will then introduce the laser light directly into the affected sites using a fiber optic probe for 1 minute per site.

**Fourth appointment:** 6 weeks re-evaluation after your last scaling and root planing session. Your mouth will be re-evaluated (same procedure as 1st appointment). Approximately 1 hr

**Fifth appointment:** 12 weeks re-evaluation after your last scaling and root planing session. Your mouth will be re-evaluated. Approximately 1 hr

**What are my responsibilities?**

It is very important that you do not tell the evaluator who is the same evaluator (1st, 5th, 6th visits) which treatment you are receiving until the clinical trial is completely over.

You must notify the clinicians of any changes in your medical condition, if you become pregnant, are taking antibiotics, or suffer from any adverse (unexpected) reactions as soon as possible.

**What are the possible harms and side effects of participating?**
The potential risks to you in having scaling and root planing with or without PERIOWAVE® include:

- Swelling of the gum tissues
- Redness of the gum tissues
- Bleeding small amounts from the gum tissues
- Short-term staining of the gums
- Emergence of canker sores
- Infection of the gums
- Localized pain, usually not for more than twenty-four hours
- There are standard risks to dental anesthetics should your study dentist choose to use them.

The potential risks of PERIOWAVE® include:

- Staining of the gums (temporary)

**Your periodontal condition may not improve or may worsen while participating in the study. There may be additional risks that are unknown at this time.**

**What are the benefits of participating in this study?**

No one knows whether or not you will benefit from this study. There may or may not be direct benefits to you from taking part in this study. We hope that the information learned from this study can be used in the future to benefit other people with similar disease.

**What are the alternatives to the study treatment?**

There are standard therapeutic procedures and treatments available for the treatment of dental disease that are available to you, such as scaling and root planing outside of the study, antibiotics, and surgery. Your study dentist has discussed these possibilities with you and has clarified that in the event that you do not voluntarily decide to participate in this study, the study dentist will continue to treat you with one of these conventional approaches to dental care. You do not need to participate in this study to receive treatment for your particular periodontal problem.

**What if new information becomes available that may affect my decision to participate?**

Any new findings developed during the study, that might change your decision to be in or continue in this study, will be provided to you.
What happens if I decide to withdraw my consent to participate?

Your participation in this research is entirely voluntary. You may withdraw from this study at any time. If you decide to enter the study and to withdraw at any time in the future, there will be no penalty or loss of benefits to which you are otherwise entitled, and your future medical and dental care will not be affected.

What happens if something goes wrong?

Treatment will be provided at no cost to you for a research-related injury. The term “research-related injury” means physical injury caused by the product or procedures required by the study that are different from the medical treatment you would have received if you had not participated in the study. By signing this consent form in no way limits your legal rights against the sponsor, investigators, or anyone else.

Can I be asked to leave the study?

You may decide to leave the study at any time. Your study dentist may also decide to remove you from the study at any time without your consent if you fail to follow instructions, for taking antibiotics, for medical reasons, or for other reasons. There is also the possibility that the study could be stopped by the sponsor, Ondine Research Laboratories, before your participation is complete. If you leave the study for any reason, you will be asked to return to the study dentist to complete final study activities and the follow-up visits if possible. If you choose to enter the study and then decide to withdraw at a later date, all data collected about you during your enrollment in the study will be retained for analysis. By law, this data cannot be destroyed.

After the study is finished

You may not be able to receive the study treatment after your participation in the study is completed. There are several possible reasons for this, some of which include: the treatment may not turn out to be effective or safe; the study doctor may not feel it is the best option for you; you may decide it is too expensive and insurance coverage may not be available.

What will the study cost me?

There will not be a fee for any examinations or treatments you receive during this study. If you have insurance coverage, you cannot submit a claim.

Reimbursement:
Your reimbursement for travel expenses, time, lost wages and parking will amount to $50 per visit with 5 visits in total for a maximum of $250.00 during the study.

**Confidentiality**

Your confidentiality will be respected. No information that discloses your identity will be released or published without your specific consent to the disclosure. However, research records and medical records identifying you may be inspected in the presence of the investigator or his or her designate by representatives of Ondine, Health Canada, U.S. Food and Drug Administration, and the UBC Research Ethics Board for the purpose of monitoring the research. However, no records which identify you by name or initials will be allowed to leave the Investigators’ offices.

**Who do I contact if I have questions about the study during my participation?**

If you have any questions or desire further information about this study before or during participation, you can contact the Co-investigator, Ms. Penny Hatzimanolakis @ (778) XXX-XXXX or Director of Clinical Trial, Dave Hammond @ (425) XXX-XXXX.

**Who do I contact if I have any questions or concerns about my rights as a subject during the study?**

If you have any concerns about your rights as a research subject and/or your experiences while participating in this study, contact the “Research Subject Information Line” at the University of British Columbia Office of Research Services at (604) 822-8598.
SUBJECT CONSENT TO PARTICIPATE:

☐ I understand that all of the information collected will be kept confidential and that the result will only be used for scientific objectives.

☐ I understand that my participation in this study is voluntary and I am completely free to refuse to participate or withdraw from this study at any time without changing in any way the quality of care that I will receive.

☐ I understand that there is no guarantee that this product will provide any benefits to my periodontal health.

☐ The study sponsor, Ondine Research Laboratories, may stop the study at any time. If this happens, I will no longer receive the study procedure or planned examinations.

☐ I have read the information in this consent form and the risks described (or it has been read to me).

☐ I have had a chance to ask questions. All of my questions have been answered.

☐ I voluntarily agree to participate in this study.

☐ By signing this consent form, I have not waived any of my legal rights which I otherwise would have as a subject in a research study.

Printed name of subject                           Signature of subject                           Date

Printed name of witness                           Signature of witness                           Date

Printed name of investigator                      Signature of investigator                      Date